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İSTANBUL, TURKEY *13-17 SEPTEMBER 2015*

ONE VISION FOR A SUSTAINABLE

ANIMAL HEALTH, HUMAN HEALTH AND WELFARE

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KEYNOTE PRESENTATIONS

A Global View on Safety of Foods of Animal Origin

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Animals are primary sources of foodborne illnesses, not only being associated with foods of animal origin, but also with foods of plant origin. Animal feces is the major source of pathogen contamination be it through direct contact with meat, poultry and fish and seafood during production and processing or through indirect contact in irrigation water and soil amendments applied to food crops. Wildlife is yet another important vector of foodborne pathogens in animal- and plant food. From a global perspective, aquaculture is the fastest growing form of food protein in the world, with Asia accounting for 89% of global aquaculture production. Raw animal manure is a major source of nutrients for aquaculture ponds, contributing as a direct source of foodborne pathogen contamination. A major public health concern of international importance is the development of antimicrobial-resistant microbes from the use of antibiotics, especially those that are critically important for human use, in animal production. Although many countries are adopting methods to mitigate the use of such antibiotics in animal production, this topic is receiving minimal attention in many developing countries where untreatable bacterial infections are becoming commonplace. Use of critically important antibiotics in aquaculture production is of special concern. Animals are important contributors of pathogens in the global food supply.

Climate Changes, Food Security, Global Health, and Veterinary Epidemiology: Challenge of the 21st Century

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Climate change and human population growth raise the justifiable concern that the 21st century will be different to past centuries. These concerns emphasize the urgency with which the challenges of food security and global health must be addressed. During the last five decades, the discipline of veterinary epidemiology has been a major contributor for the efficiency of livestock production systems, combating animal diseases including zoonoses, animal well fare, wildlife conservation research, and animal health policy development among others. Tools and approaches used on both micro veterinary epidemiology through herd health programs and macro veterinary epidemiology through public service institutions have shaped veterinary medicine and public health for better society and economic benefits for humanity. In collaboration with a broad range of disciplines and policy makers, veterinary epidemiology has a significant role to play in addressing the forthcoming concerns in global food security, climate changes, and global health with the potential to help in solving some of these complicated issues. The challenges, however, are more frightening and complex than anything this discipline has faced in its history.
The aim of this presentation is to highlight issues within these three inter-related topics (Climate Changes, Food Security, and Global Health) in which veterinary epidemiology can play a major role. The presentation will include the following sections:

• Global statistics of the trends of associated common factors to Climate Changes, Food Security, and Global Health with emphasis on the common inter-related factors;
• Demonstrations of the value of veterinary epidemiology to the above associated factors with real examples from the past;
• Values of application of epidemiological concepts to understand a complex system associated with the Climate Changes, Food Security, and Global Health;
• Proposed future direction of veterinary epidemiology within the framework of understanding a complex system;
• Requirements for the new direction of veterinary epidemiology with options for policy making process including funding.

Welfare of Working Equids - Supporting Vets to Play Their Part

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The global population of horses, donkeys and mules is estimated to be around 113 million (FAO stats); of these approximately 100 million are in the developing world workingin traction and transport to support the livelihoods of their owners and the wider communities. These working equine animals support an estimated 300 million people globally. They may work domestically carrying water and firewood for the home and transporting livestock feed and manure on farms. They are used throughout farming systems from ploughing and sowing to harvesting and threshing, as well as the transport of agricultural and livestock products towards markets. Children travel to school on them and the sick and women in labour are often carried to clinics by a working equine animal. Pastoralists will rely on their working equids to move their households. Commercially working equids may be used to earn an income for their owner through being used as taxis, rented out to small businesses, transporting goods for payment and in industries such as tourism and construction. Some construction industries such as brick kilns or mining represent some of the most hazardous working environments for both working animals and for the people working there with poor welfare outcomes for both. Working equids contribute to almost every aspect of household life and help alleviate poverty and assure food security. However, they are often owned by marginalised communities and perceived as low status animals. This leads to their invisibility to policy makers and health care providers and, although they live and work along traditional livestock, they are usually excluded from animal health initiatives such as vaccination programmes. Challenges to vets providing effective health and welfare services to working equids include: poorly resourced government vets, not available or accessible to the working animal owning communities and private vets with little motivation to work in the rural areas or with marginalised communities.Veterinary training also varies considerably and, in many countries, vets receive less than adequate training in animal welfare and practical equine subjects. The delivery of animal health services involves other actors from traditional healers, community based animal health workers and other paravets, not forgetting ancillary services such as farriers and harness makers. Aside from providing treatment and prevention services to working equids, vets should play a role in building the capacity of, mentoring and supervising theseparaveterinary and ancillary service providers. As an organisation committed to improving the welfare of working equids the Brooke started by employing and training its own vets but now works to strengthen existing service providers, as well as engaging with communities to support them in making welfare improvements for their animals. Strengthening existing service providers entails developing and supporting the skills, attitudes and resources of vets in the field. Practical skills training and developing training resources (often technology based such as e modules and online wikis) are important. Long term mentoring helps to develop confidence and motivation, exchange visits and peer learning and certification all play key rolesin changing attitudes, as does facilitating linkages between local animal health service providers and the equine owning communities. Supporting local credit and insurance schemes, drug revolving funds, drug purchasing groups and providing business skills training can all assist with ensuring local providers have appropriate resources to deliver effective services. At a higher level advocating for animal welfare and equine medicine training in teaching institutions, for a stronger veterinary infrastructure and for better availability of appropriate equine drugs are also part of the support vets require to ensure working equine welfare is well catered for within local animal health services.

Role of OIE Animal Welfare Working Group in Delopement of Standards

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Animal welfare has been identified as a strategic priority for the OIE since 2001.The OIE Animal Welfare Working Group was inaugurated at the 70th General Session of the OIE in May 2002 and the first recommendations of the Working Group were adopted one year later. Since May 2005, the World Assembly of OIE Delegates (representing the 180 Member Countries) has adopted eleven animal welfare standards the latest being Dairy Cattle Production Systems,in the OIE Terrestrial Code and four in theOIE Aquatic Animal Health Code. Three OIE Global Conference on Animal Welfare have now been held (in 2004,2008 and 2012) They provide a valuable forum for all interested stakeholders to share their experiences, learn from others experiences, and identify needs for and barriers to effective implementation of OIE animal welfare standards. A draft globalOIE animal welfare strategy has beendeveloped and reviewed by the Code Commission. The draft chapter on the welfare of working equids has been reviewed by the ad hoc group and new text on the electrical stunning of chickens is envisaged taking into account discussions on this subject at the last general session. The first draft of guidelines on disaster management and risk reduction in relation to animal health and welfare and veterinary public health werecirculated with the February 2015 Code Commission report.The next standard to be developed will be on animal welfare and pig production systems. The 4th OIE Global Conference will be held in Mexico in December 2016.

Future Animal Welfare Challenges

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Significant challenges in animal welfare will continue into the next decade. This presentation considers issues that will impact the largest number of animals, where public pressure is likely to be directed or may emerge in the future. These challenges include rising vulnerability to natural disasters, the increased demand for nutritious food to feed an expanding human population, and the provision of basic animal health care. In addition, there are challenges of meeting the needs of the growing number of companion animals kept in varying social and economic contexts. Underlying factors relating to poverty and to affluence, to level of understanding, to beliefs and to the options availablewill be explored. How these factors affect human behaviour, including the choices consumers and animal owners make that determine the ultimate welfare of animals in society, are highlighted. Ensuring animals have good welfarehas direct social and economic benefits, it is a public good, and satisfies moral imperatives in using animals for food, work or as companions. Animal welfare challenges are interrelated with other global challenges. Hence, the need for a cross disciplinary understanding and a structured approach in analysing them in order for humane sustainable optionsto be identified. The veterinary profession plays a critical role in making sense of the contrasting and, at times, conflicting priorities and in effectively communicating theseto owners, consumers and to society in general so that all their decisions fully consider the needs of animals. There are increasing opportunities for veterinarians to influence policy and to act on behalf of animals outside of their traditional clinical role. This has been supported by advances in veterinary, welfare and social science, information and communication technology, societal expectation, and the veterinary profession'saspiration to bean advocates for animals that is based on compassion, use of evidence and a pragmatic approach. In this era of globalization, future animal welfare challenges could be addressed more effectively and equitably by strong, international professional cooperation and collaboration, resulting in benefits for society, the profession and animals. The audience will be given the opportunity to vote on several of the issues discussed.

Future Animal Welfare Challenges- Asia Pacific Examples

Natasha Lee
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Animal welfare challenges from a veterinary perspective can be summarised in a 6 point "A-frame":
1. Academe: ensuring veterinarians are educated in animal welfare science and can make decisions based on scientific evidence
2. Accountability: adhering to professional ethics and maintaining basic veterinary standards (e.g. anaesthetics, analgesic, and asepsis for surgery)
3. Access & affordability: veterinary care for animals in need
4. Authority: veterinarians' influence on legislation and governance
5. Attitude: view point of vets and the public towards animals
6. Advocacy: veterinarians standing up for animals

Many veterinarians are becoming more aware about animal welfare science and its importance for the animals and the community. This is evident as many veterinary schools now have animal welfare as a compulsory subject within the curriculum such as in Indonesia or the Philippines. Many veterinary associations are also offering continuing education opportunities in animal welfare, such as in this conference. World Animal Protection have also carried out, for the first time, an online animal welfare course for Asian vets in 2014, and a second course is currently being conducted. Animal welfare issues and solutions are not all equal, so scientific assessment and evidence-based solutions can be applied according to local needs, as veterinarians become more knowledgeable in this scientific field. For example, 'catch-neuter-release' (CNR) as a mean of dog population control may not be applicable where drugs are unavailable or in communities where dogs are not tolerated, so other means of control may be necessary such as public education and reducing abandonment of owned dogs. Attitudes towards animal welfare are also improving and this can be seen through improvements in veterinary standards, such as the increase use of analgesia for animals undergoing routine surgeries such as castration. Several veterinary associations had made efforts to develop standards of veterinary services such as the development of animal hospital standards in Thailand. In countries where the veterinary profession are relatively young, there are new veterinary legislations and veterinary bodies being formed such as in China with the formation of the Chinese Veterinary Medical Association in 2009. Veterinarians have a lot of influence on animal-related legislations, not only through direct influence by holding positions of authority, but also as a collective voice to raise concerns related to animals. One successful example in Asia Pacific is the Regional Animal Welfare Strategy (RAWS), led by the World Animal Health Organisation (OIE), where veterinarians in the region agree on a strategy to improve animals' lives. This has impact on several countries where new animal welfare legislations have been drafted, national animal welfare strategies developed and many activities has been carried out. One of the most important roles for veterinarians is to become a leading advocate for the voiceless animals. As vets in the Asia Pacific region become more aware and competent in animal welfare, vets can start to speak up for the welfare of animals such as recommending euthanasia to end the suffering of animals, improve humane slaughter techniques, reduce the use of painful farming practices such as castration without analgesia, or as simple as educating the public on responsible pet ownership. This presentation will further showcase animal welfare examples in Asia Pacific according to those 6 "A-frame" points, and ends with a case study of a comprehensive dog population management programme in Sri Lanka.

A Brief Historical Overview of Turkey's Animal Welfare Legislation

Abdullah Özen
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There were some significant legal regulations which aimed to protect and improve animal welfare in Turkish history, but there were no separate animal welfare laws until 2004. Although the Law for the Protection of Animals (5199) had been accepted on June 24, 2004 and put into effect on its date of publication, no progress had been made until 2010 except the establishment of animal ethics committees. Article 9 of the law governs the establishment of ethics committees. As secondary legislation, the Regulation on Operating Procedures and Principles of Animal Research Ethics Committees was issued in 2006. The regulatory principles of these committees are mainly aligned with the EU's regulations. Within the framework of adhering to the EU, the regulation of the welfare and protection of animals during transport and on the farm had been made under Law No. 5996 on Veterinary Services, Plant Health, Food and Feed in 2010. This law applies to all farm animals. After going in effect, two new secondary regulations were issued on December, 2011: The Regulation on the Welfare of Farm Animals and, The Regulation on the Welfare and Protection of Animals during Transport. But at the end of 2014, a new The Regulation on the Welfare of Farm Animals was issued and the former one replaced. All this legislative activity reflected similar EU's regulations on the welfare of animals during transport and on the farm, yet there was no subject-specific slaughtering and species-specific legislation forthcoming. In this presentation, animal welfare legislation will be discussed and analyzed.

Flock / Herd and Reproduction Management: Concepts and Applications

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Control of reproduction improves management of sheep / goats and increases their productivity. There are various applications for reproductive control in small ruminants. The first main applicationis the synchronisationof oestrous cycles and the 'out-of-season' breeding of adultfemale animals. Another important application is the inductionof the onset of puberty of ewe-lambs or doelings and that way the acceleration of the onset of their reproductive activity. Other applications, performed less often, include the induction ofincreased ovulations, the multipleovulation andembryo transfer programmes (MOET), the control of parturition, the improvement of reproductive ability of male animals for 'out-of-season' breeding andthe induction of lactation. This paper summarises concepts behind reproductive control and discusses clinical application of these management interventions. 2. Synchronisations of oestrous cycles and 'out-of-season' breeding of adult ewes/does. The concept behind synchronisation of oestrous cycles is to induce females to lamb in groups. The concept behind 'out-of-season' breeding of ewes/does is to achieve parturitions outside the usual period, i.e., to overtake the hinderance of natural seasonality of small ruminants, which can act as limiting factor in sheep/goat production. Benefits of these reproductive management approaches are (i) improvement of the general health management in a farm, e.g., vaccinations/anthelmintic treatments of females, weaning of newborns in groups, (ii) births of lambs/kids in seasons with high market prices, (iii) increased ovulation rate and hencelambing/kidding rate, (iv) around-the-year milk production (in dairy farms), (v) increased use of fixed-time artificial insemination, (vi) induction of multiple ovulations during embryo transfer programmes and (vii) acceleration of genetic improvement programs. Successful application of reproductive control methods depends upon (i) selection of the appropriate method, (ii) estimation of potential costs, (iii) evaluation of the farm's infrastructure, (iv) human resources in the farm. Methods usually employed are (i) so-called 'natural' methods (e.g., presence of male animals adjoining the females ['male effect'], improved nutrition), which include a small degree of intervention, but also have a limited efficacy and (ii) hormonal techniques. Hormonal techniques simulate natural endocrinological patterns in female sheep/goats. For example, progestagen administration is used to mimic the luteal phase of animals. In cases of 'out-of-season' breeding, administration of exogenous hormones leading to ovulation (e.g., equine chorionic gonadotrophin) is necessary, although various other schemes involving other hormonal pathways (e.g., administration of GnRH) have also been proposed. Melatonin, which signals the induction of reproductive season in small ruminants, is also administered for achievement of 'out-of-season' breeding. The most commonly employed hormonal schemes for synchronizations of oestrous cycles and 'out-of-season' breeding of ewes/does are summarised in Table 1, although various combinations of these have also been reported. Reproductive performance of ewe-lambs or doelings is smaller than that of adult females; also, it varies a lot among individuals. The above limit the reproductive potential of young animals. If a ewe-lamb or doeling can conceive, bear and give birth to lambs or kids, then potential forhigher profitability and lifetime reproductive performance is increased. Additional advantages include (i) increase in total number of lambs/kids born per farm per year, hence higher income, (ii) early selection tool for adult animal replacement, (iii) more progeny born on farm, thereby increasing selection pressure for replacements and (iv) reduction in the generation interval through the selection of progeny born to the replacement animals. Methods usually employed are the same as in mature animals, i.e. (i) 'natural' methods and (ii) hormonal techniques. Nevertheless, factors affecting reproductive efficacy when applying such techniques in ewe-lambs or doelings should be considered; these include age of animals, timing of onset of puberty in relation to season, genetic line or breed, bodyweight and daily growth, body condition score, nutrition and male effect.

Increased number of ovulations can be achieved by (i) increased energy administration for at least 35 (ewes) or 43 (does) days before start of the mating period, (ii) genetic selection for increased numbers of ovulations, (iii) injection of equine chorionic gonadotrophin at (ewes) or two days before (does) removal of progestogen sponges in doses varying from 100-300 i.u. depending on species, breed, latitude, season or (iv) immunisation against hormones limiting ovulations (e.g., anti-androstenedione). Protocols for multiple ovulation (superovulation) and embryo transfer are used (i) to accelerate genetic gain by shortening the generation interval, (ii) to spread the selected animal genetics and (iii) for ex situ preservation of endangered breeds. Hormonal treatment consists of progestagen administration in combination with high doses of gonadotrophins for stimulation of follicular growth. However, ovarian response to exogenous gonadotrophins remains highly variable. Factors responsible forthe high variability constrain multiple ovulations and embryo transfer (MOET) protocols; they are classified as extrinsic (depending on the protocol of treatment used for multiple ovulation) or intrinsic (as a result of the species, breed, age, nutrition, reproductive and lactational status of donors). Control of parturition can be achieved if expected date of lambing/kidding is known with some accuracy; it contributes to improved management around parturition day and immediately post-partum. Various protocols have been proposed; corticosteroids, cloprostenol, oestradiol and prostaglandin F2a are the main hormones employed in those. Improvement of reproductive ability of male animals for 'out-of-season' breeding is achieved by subcutaneous administration of melatonin implants. It is important for achievement of increased reproductive efficacy in cases of reproductive manipulation. Reproductive management techniques are useful tools forimproving profitability of small ruminant farms. Mostly, reproductive management allows ewes and does to breed throughout a year for supply of markets on a year-round basis. Also, reproductive management of ewe-lambs and doelings can increase lifetime performance of the animals. Of course, selection of the appropriate intervention must be based in evaluation of benefits and constraints of each method. The appropriate nutrition are the 'sine qua non' of success of reproductive management, when used 'natural methods' or hormonal treatment. By studying interactions between reproductive physiology, nutrition, health (diseases prevention), genetics, epigeneticsand environment, it is feasible to improve management, productivity and profitability in small ruminant farms.

Table 1. Summary of commonly employed hormonal schemes for synchronisations of oestrous cycles and 'out-of-season' breeding of ewes/does

Intravaginal administration of progestogen sponges for 6-14 (ewes) or 17 (does) days	Synchronisations of oestrous cycles within the reproductive period
Intravaginal administration of progestogen sponges for 6-14 (ewes) or 17 (does) days, followed by injection of equine chorionic gonadotrophin at (ewes) or two days before (does) removal of progestogen sponges in doses varying from 300-800 i.u. depending on species, breed, latitude, season	Synchronisations of oestrous cycles and induction of oestrus outside the reproductive period
Injection of prostaglandin $F_{2\alpha}$ or synthetic analogue, repeated after 7-9 (ewes) or 11 (does) days in doses varying depending on pharmaceutical used	Synchronisations of oestrous cycles within the reproductive period
Melatonin implant subcutaneous administration	Acceleration of the onset of reproductive period

Abomasal Emptying in Calves - Effects of Different Protein Sources in Milk Replacers

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Abomasal emptying rate in calves is influenced by a number of factors like the composition of the diet, pH, osmolarity and energy density of the abomasal content, abomasal volume and pressure, curd formation, age of the animal and concurrent diseases. Further the frequency of suckling and the volume is of vital importance for abomasal pH and emptying rate. Increased or decreased abomasal emptying rates may result in indigestions, decreased body condition or clinical disease. Abomasal emptying can be measured in calves using different techniques like absorption tests duodenal cannula, ultrasonography or szintigraphy. Invasive techniques or methods requiring high personal or financial input are reserved for research. However, ultrasonography as practical, non-invasive, and rapid method for assessing abomasal volume, location, and emptying rate in calves can be considereda clinically useful diagnostic tool in field practice. Because abomasalhypomotility has been associated with hypocalcaemia, endotoxemia, alkalemia, hyperinsulinemia, and hyperglycemia the current focus in treating adult cattle and calves suspected to have abomasalhypomotility is correcting acid-base, electrolyte, and metabolic abnormalities, combating the effects of endotoxemia, and eliminating gram-negative bacterial infections. Additionally there are reports about the effects of prokinetic drugs (erythromycin, metoclopramide, parasympathomeimetics) in calves. Especially erythromycin offers a high potential to accelerate abomasal emptying in calves. One of the major factors determining abomasal emptying rate is the composition of the diet. Research has been done on the effect of different oral electrolyte solutions, milk and milk replacer on abomasal emptying ratein calves. Since feeding the saleable whole milk to calves is not cost effective a number of dairy farms substitute this as quickly as possible with milk replacer. The major protein component of milk replacers has typically been milk-based skim milk or whey proteins but with the increasing demand for these proteins by the food industry their cost has increased resulting in partial substitution by plant proteins derived from wheat, soy or potato. Such proteins have different effects on abomasal emptying rate and digestion in general. It could be demonstrated the wheat protein in milk replacers increased abomasal emptying rate which might contribute in pathogenesis of diarrhea.

One Health: The Theory and Practice of Integrated Health Approaches

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One Health (OH) has evolved into a multi-national "movement" that is informing and inspiring us to rethink our veterinary and country centric approaches to improving health. The theoretical foundations of OH are embedded in humankind's interdependence with animals and our shared ecosystems. OH today moves us more and more into integrative and systems approaches to improving health delivery for humans and animals, and it extends our reach to social-ecological systems thinking. This means we must reach out across disciplines and across traditional and formal leadership groups to facilitate dialogue with multiple stakeholders, communities, professionals and governments, becoming an iterative and measured transdisciplinary process. We are fortunate now that valuable scientific publications and books are documenting our OH progress. Practical examples abound, such as applying OH to the control of brucellosis, bovine tuberculosis, rabies, leptospirosis, trypanosomiasis, as well as animal welfare, wildlife conservation, plant health, food safety and security, nutrition and non-communicable diseases such as obesity to name a few (1) Global disasters such as SARS and avian influenza created development of One Health as "Securitization" making pandemic preparedness a theoretical foundation of OH in the early 2000s. The Global Health Governance of High Path Avian Influenza stimulated international level coordination of animal and human health, and in 2010 the OIE/WHO/FAO/World Bank published a OH global joint strategic framework that is being translated into national and sub-national policies worldwide. Currently antimicrobial resistance concerns are unifying global OH approaches from farm to consumer. Like our sister "movements" of Ecosystems Health and Global Health, our methods and approaches must capture its "added value" in order to effect sustainable change at multiple levels from communities, to nations and beyond boundaries.We need to demonstrate that OH contributes to lessen time to detect the infectious agent. Through economic analyses of the cumulative health effects of a zoonotic disease we demonstrate a savings to communities and governments. When animal, public, social services and environmental health units share resources and communicate better we must quantify economic savings, such as the rabies efforts in Chad (1). In order to actualize the future of OH, our own institutions must see not only the economic benefits, but also cultural and ethical valuation of this approach. An important value proposition is that health, and therefore OH, is a public good that should be integrated into public health, social, political, ecological and global health systems (1). The future of OH resides in our ability to take integrative approaches to research, teaching and practice. This means we need to engage, from the beginning of creating the OH conceptual framework, all relevant actors. We as veterinarians have been trained in comparative veterinary sciences, but we need to go beyond our comfort zone. Academia can play a crucial role developing "transdisciplinary transmitters" - experienced OH researchers and educators who can collaborate across disciplines, have expertise in leadership and partnership building that is based on field research, and are also experts within their respective disciplines. Some tools available to academia as well as governments include collocating or clustering multiple disciplines and sector offices; developing joint training and cross discipline classes; creating mentorship programs that link individuals from different disciplines; provide additional training in partnership building, communication, and negotiation skills; and purposefully developing advocates and specialists who span disciplines, motivating interest and urgency to apply OH transdisciplinary approaches.OH must be resilient, sustainable and produce positive outcomes that are measurable, increase cost effectiveness, mitigate harmful or unintended outcomes, and build collaborative OH capacity locally and beyond. We are already well along this journey.

References

1. One Health Then Theory and Practice of Integrated Health Approaches, Ed. J. Zinsstag, et. al., CABI Publishers, 2015

The tripartite organizations (FAO, OIE and WHO) and WVA will make presentations on their experiences of implementing One Health practices either jointly or through public-private partnerships.

Biosecurity and Hygiene towards Healthy Poultry

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The main ways to control any infectious disease are to prevent the introduction in an area supposed to be free from the infectious agent by regular biosecurity and monitoring and in case of the presence of the infection in one area measures should be taken to prevent the spread to other places. For that measures such as biosecurity, movement restriction, treatment, vaccination and in some cases eradication can be applied. Eradication policy and killing of animals for disease control purposes are commonly applied in cases of suspicion or confirmed outbreaks with a considerable public health and/or economic impact. These infectious agents can be introduced and spread in poultry farms by different routes. It occurs either by the vertical and/or horizontal route. At early days of age several vertically transmitted infections such as salmonella, E. coli, leucosis, Mycoplasmas, avian encephalomyelitis and others can be introduce into the farm. Those and other infectious agents can also be transmitted horizontally (laterally) by direct contact between infected and non-infected susceptible birds, and through indirect contact with contaminated feed, water, equipment, environment and dust through ingestion or inhalation. Biosecurity is a set of management practices to prevent and /or to reduce the potential for the introduction, and spread of infectious agents onto and between farms. Since the flock performance is directly linked to good biosecurity measures, the biosecurity of farms has to be upgraded to a much higher level than that for disease control. However, a high level of biosecurity at all times and at all production levels is difficult and expensive to maintain. Biosecurity und hygiene plans should be adopted for each farm. Farm owners, managers and workers should be involved in the design and implementation of the biosecurity plan. The aim is to create an environment where poultry are protected from carriers of infectious agents. Controlling the movement of people, animals, equipment, and vehicles in and out of the farm and within the farm area is essential. Visitors are one of the major vectors and carriers of infectious agents. Visitors include supervisors, veterinarians, vaccination crews, catching crews, electricians, feed truck drivers, and other similar. Visitors can transmit diseases from one farm to another via dust on hands, hair, and clothing. Ask visitors to register by name including the date and time of their visit and make visitors wear boots and coveralls. Install properly managed spray stations for use on traffic entering and leaving the farm. Make sure that all go through the wash station before and after leaving the farm. Make sure that all visitors use footbaths when entering the farm and moving from one area of the farm to another. Vehicles onto the site have to be restricted. The wheels and underside of necessary vehicles entering the farm (feed trucks) should be sprayed with disinfectant. Do not allow the driver to leave the truck or wander around the farm. In general, other animal species must never be present on the farm. Employees should not have, or work with, other domestic animal species at home. Since also household pets constitute a serious hazard, dogs and cats should not be present in the barns.

Is the World Heading for a Post Antibiotic Era?

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We live now in the "Age of Bacteria" Our planet has always been in the "Age of Bacteria" every since the first fossil-bacteria, of course-were entombed in rocks more than 3 billion years ago" Stephen Jay Gould. The real question is, are humans losing almost eight decades war against infectious microbes? or is this" The End of of Golden Age Therapeutic Revolution" what are the real answers of these questions?… First workshop on "Livestock Production in the Post Antibiotic Era" will be held Uppsala the Global Challenges University Alliance on 1-3 December 2015. Do we really need to develop strategies for livestock production in the post antibiotic era? Are some pessimistic academicians, politicians, some scientific writers, international organizations or NGOs creating apocalyptic doomsday scenarios. For example, as the chief medical officer of the United Kingdom Prof. Dame sally Davies said, "Soon we will live in a world where infection is so dangerous that anyone with even minor symptoms would be locked in confinement until they recover or die". The anxiety of certain individual and organizations about antibiotic resistance phenomenon is not new at all. The "end of antibiotics" was envisioned almost from beginning. First warning came from discoverer of first antibiotic penicillin, Sir Alexander Fleming. Accepting the 1945 Nobel Prize in Medicine, he said" …. There is the danger that the ignorant man may easily under dose himself and by exposing his microbes ton on-lethal quantities of the drug make them resistant". Fleming's prediction was correct. Penicilline-resistan staph energed in 1940s while the drug was in limited use. Following Dr. Fleming's warning, in due course resistance to several antibiotics began to appear in first hospital environments then in community. In England, concerns about antibiotics resistance, especially associated with antibiotics that were used both in human and food producing animal first as growth promoters later for prophylaxis and therapy purposes in pig and poultry farms led to the Swann Report in 1969. The report concluded that "the administration of antibiotics to farms animals, particularly at sub-therapeutic levels, poses certain hazards to human and animal health; in particular it had led to resistance in enteric bacteria of animal origin. Another health authority, Dr. Thomas Frieden, the director of U.S. Centers of Disease Control and Prevention, issued a disconcertingly frank warning in his speech at National Press Club luncheon on July 2014 "If we're not careful, we will soon be in a post-antibiotic era. And, in fact, for some patients and some pathogens, we are already there." A month before Dr. Frieden luncheon speech WHO Director-General Dr. Margaret Chan addressed Ministerial Conference on Antimicrobial Resistance on 25 June 2014 in The Hague, Netherland and warned." Antimicrobial resistance is not a future threat looming on the horizon. It is here, right now, and the consequences are devastating." She also indicated that "some analysts have compared the threat of antimicrobial resistance with the threat from climate change. Both are caused by human activities. And both are global threats that demand global solution. As a global concerted effort, the first "WHO Global Strategy for Containment of of Antimicrobial Resistance" was introduce in 2001 and finally, a global action plan on antimicrobial resistance was endorsed by the World Health Assembly in May 2015. In meantime WHO, through the coordination of its Regional Offices, has developed regional strategies on prevention and containment of antimicrobial resistance. During this period OIE and FAO and many national Health and Veterinary organization also participated global endeavors for the prevention and containment of antimicrobial resistance. On the margins of the US-EU summit in 2009 the President Obama and then-President of the EU, Prime Minister Fredrik Reinfeldt from Sweden, established a transatlantic taskforce on antimicrobial resistance (TATFAR). In November 2011 European Commission published "a strategy to tackle antibiotic resistance"., Finally US Administration released " The national Action Plan for Combating Antibiotic-Resistance bacteria" Today there is a common consensus that Resistance to antibiotics is a natural evolutionary response of bacteria to antibiotics. It is a inevitable biological phenomenon. As Prof. Jacques Acar indicate Antibiotic resistance is a complex issue" We cannot develop effective strategies and implement actions by blaming human health or agriculture communities "Three

important factors impact on the emergency and spread of antibiotic resistance: intrinsic (natural) mutations, transferable resistance genes and selective pressure created by anthropogenic irrational use of antibiotics in human health and in agriculture sectors. But, in Medical and Agricultural communities debate continues regarding whether or not resistant bacteria in humans can be linked to antimicrobial use in food animals. Some studies suggest a relationship between such use in food animals and human resistance trend, and other studies and risk analysis find no such relationship. New molecular tools and integrated surveillance programs' results will elucidate this dilemma. Another recent area of human health concern is the effect of antibiotic resistance bacteria, resistance genes in the environment. We, as a mankind, cannot fully stop it; but we can slow down its development and spread. Socially speaking resistance is a complex behavioral, economic, regulatory and educational problem and its control requires a comprehensive and holistic approach. Can we change present course?.Since we cannot stop development and spread of antimicrobial resistance development and spread, can we slow down progress? I THINK WE CAN… First, we all stakeholders dealing antibiotic resistance problem should take into consideration: first, challenger, bacteria has almost 4 billion years old adaptation capability to all adverse environmental conditions; second, as indicated by Dr. Brad Spellberg organisms us 1022, outweigh we should us by a factor 100 million-fould, replicate 500.00 time faster than we do, and have been doing this for replicate 500,000 time faster than we do. Therefore, as proposed by Dr. Stuart Levy and Dr. Dave Gilbert we must develop strategies to live in a peaceful coexistence with bacteria using new generations of vaccines, and new antimicrobial agents using them as rationally as possible in humans and animals for the sake of human and animal health and welfare. Antimicrobial stewardship programs should also be extended to animal breeding sector. It is hoped that financial aspects of the Global Antimicrobial Action Plan, recently developed by WHO is solved and the plan being implemented globally soon. Recent advances in the fields of bioinformatics, genomics, human and animal microbiota, metagenomics, tools for editing such as CRISP, and the development of a new device called an iChip by Slava Epstein, a microbiologist at Northestern University USA, made possible a new class of antibiotics called teixobactin. This discovery may pave the way for the discovery of many new class of antibiotics soon. As Winston Churchill once said," Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of beginning". I hope this will be true for the question I have tried to analyze.

The Veterinary Role and the Antibiotic Stewardship

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Antimicrobial resistance is a growing concern for everyone, including veterinarians as the medical professionals who protect our food supply. The use of antimicrobials in food animals is one piece of a very complex puzzle. Understanding how resistance develops and spreads is essential in ensuring that antimicrobials are used appropriately now and into the future. The Veterinarians' Oath ethically charges the profession with promoting public health and protecting animal health and welfare. With that also comes the responsibility to be cognizant of the human impacts that may occur as a result of any decision made. Veterinarians possess a unique skill set gained through our extensive education and training and thus, are best suited to make optimal medical decisions regarding the responsible use of antimicrobials in animals. In today's world, a veterinarian's decisions often affect more than just a single animal or person. When a veterinarian makes the decision to use a drug, any drug, he or she must consider the individual animal, other animals that may come into contact with that animal and the people who may come in contact with that animal. If the veterinarian is caring for food animals, the decisions made affect an entire herd or flock, and potentially hundreds or thousands of people who might consume the many foods that are produced from a single animal. As the only health professionals who routinely work at the interface between human and animal health, veterinarians are the most knowledgeable about the impact of animals and animal diseases on people. Therefore, any use of antimicrobials should involve the veterinarian in the decision-making process if we are to protect animal health and welfare, as well as public health and food safety. The responsible use of antimicrobials by veterinarians in food animals ensures healthy animals and in turn, those healthy animals provide safe food products. It is crucial that veterinarians be able to judiciously and effectively use antimicrobials now and into the future so that we can ensure the health and well-being of animals and keep our nation's food supply safe.

A European Perspective on Data Collection and Risk Assessment in order to Address the Spread of Antimicrobial Resistance Through the Food Chain

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Antimicrobial resistance (AMR) in bacteria is the cause for increased morbidity and mortality in human infections. Food is one of several possible transmission routes, although its relative importance compared to other sources (direct contact with animals, environment, human-to-human transmission) is currently very challenging to estimate in a quantitative way. Definitive studies with unquestionable proof for an animal-to-human transmission event are rather scarce. However there is circumstantial evidence and are clear indications of the same resistant clones, resistance genes, and related genetic "environments" (plasmids, integrons, transposons …) being present in bacteria from these 2 compartments, suggesting that an exchange takes place between them. A historical perspective with specific examples will be presented. Lack of data makes it difficult to determine the extent of human exposure to AMR bacteria/genes via food. Nevertheless, the high prevalence of resistance to some antimicrobials in enteric bacteria may pose a significant health threat. The emergence of resistance to critically or highly important antimicrobials such as fluoroquinolones, cephalosporins and carbapenems are particular concerns. Scientific opinions provided by EFSA have addressed the problem of AMR in food-producing animals and food, and have given advice for policy makers. Some relevant examples on "hot issues" such as MRSA, ESBLs, and carbapenemases will be discussed. In former opinions EFSA concluded that the main risks factors for resistance to cephalosporins in animals are: (i) the use of antimicrobials, (ii) the extensive trade of animals in EU, and (iii) the spread from the top of some production pyramids. EFSA has given advice on potential control options and risk managers are the best placed to decide on the most appropriate strategies to be applied. Amongst these, there is an urgent need to promote prudent use in animals and to educate veterinarians and farmers on strategies to minimise AMR. Since AMR is a complex multi-factorial issue, control of all the routes by which AMR bacteria can arise in the human patient requires a response from all stakeholders. It is extremely important to improve and harmonise the data collection systems both for occurrence of resistance, and consumption of antimicrobials in animals and humans. With this information, it would be easier to quantitatively assess the risk posed by AMR. EFSA has produced technical guidance to improve the harmonisation of monitoring and reporting of AMR in animals and food. AMR is commonly found among bacteria from food and animals in the EU. There are large differences in the levels of AMR in animals and foods in the EU, and in the temporal trends between the Member States (MSs). The AMR levels observed in most indicator bacteria tend to be higher in the Western and Southern MSs when compared to other parts of Europe as observed in the annual EU Summary Report on AMR. The integration of AMR occurrence data and antimicrobial consumption data in animals has been achieved for the first time in a joint EFSA/EMA/ECDC report, from which conclusions will be presented.

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ORAL PRESENTATIONS

Application of Multiplex Real-time PCR Panel for Detection of Microbiological Agents Causing Bovine Respiratory Disease Complex in Necropsy Cases

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Bovine respiratory disease complex (BRDC) is one of the leading causes of respiratory losses in cattle industry. Various bacterial and viral pathogens are known to be implicated in BRDC. Conventional diagnostic methods for etiological identification are time-consuming, laborious, and their results are often confounded by aggressive antimicrobial treatments. To investigate the presence of bacterial and viral agents causing BRDC, a reliable, fast, and simultaneous detection of microorganisms is needed. Nasal swabs and lung specimens from 39 cases of BRDC-associated mortalities and 6 cases of non-respiratory bovine mortalities were investigated for the presence of selected bacterial agents (Mycoplasma bovis, Mannheimia haemolytica, Pasteurella multocida, Histophilus somni), and selected viral agents (bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine corona virus (BCV), bovine herpes virus type-1 (BHV-1)) by using two separate one-step multiplex real-time PCR assay (hereafter BRDC PCR panel; one for bacterial targets, the other for viral targets). The lung specimens were also cultured for bacterial growth for comparison. By using BRDC PCR panel, prevalence of the agents in lung specimens from BRDC-associated mortalities were M. bovis (61.53%), M. haemolytica (56.4%), H. somni (33.3%), P. multocida (25.6%), BVDV (30.8%), BRSV (23.0%), and BCV (20.5%). BHV-1 was detected only in nasal swabs (7.7%), and not detected in the lung specimens. In 74.3% of the lung specimens, more than one agent was detected. Excepting BCV in one case, no other agents were detected in lung specimens from non-respiratory mortalities. Prevalence of bacterial agents in lung specimens by culture were lower for M. haemolytica (43.4%) and H. somni (7.6%) compared to BRDC PCR panel. Excepting BHV-1, all other agents were detected in significant numbers in lung specimens from BRDC-associated mortalities by using BRDC PCR panel. BHV-1 was detected only in nasal swabs by this method. Traditional culture methods detected fewer bacterial agents in lung specimens than BRDC PCR panel, possibly due to aggressive treatment of animals with antimicrobials which may ultimately suppress the growth of bacteria in culture media. The application of BRDC PCR panel can provide fast and accurate diagnostic tool and aid bovine industry in identifying specific agents of BRDC. As with all diagnostic tests, results should be interpreted in the context of clinical and pathological findings.

Keywords: Bovine respiratory disease complex, multiplex real-time PCR

Therapeutic Alternative for the Control of Rhipicephalus Microplus in Cattle, Based on the Acetone Extract of Gliricidia Sepium

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The cattle is an outstanding activity in the Colombian economic context, generating 27% of agricultural gross domestic product (GDP) and 64% of livestock GDP; ectoparasites, specifically Rhipicephalus microplus tick has been associated with large economic losses to the livestock production screed. In this vein, there is a need to implement within the integrated pest management systems sustainable beef production, therapeutic alternatives from an environmental perspective and effective process control of this ectoparasite. Gliricidia sepium is a promising plant species to be part of strategic management in the control of external parasites. In the present investigation the ethnopharmacological use information matarratón tree (Gliricidia sepium) and external parasite in cattle production systems of low tropics was validated. Preliminary phytochemical march of acetone extract of G. sepium determined the presence of groups of secondary metabolites: flavonoids, terpenoids, coumarins, cardiac glycosides, saponins and tannins; found using the technique of thin layer chromatography and colorimetry. The acaricidal activity of acetone extract of leaves of Gliricidia sepium was held in larvae and engorged Rhipicephalus microplus, using larval immersion test (LIT) and adult immersion test (AIT), respectively. The acetone extract showed Gliricidia sepium LC50 of 78 mg / mL (IC 71 to 83 mg / mL) and an LC90 of 146 mg / mL (IC 128-182 mg / mL) in the bioassay LIT On the other hand, the AIT test shows acaricidal effect of G. sepium on R. microplus engorged with an LC50 of 100 mg / mL (IC 82-118 mg / mL) and an LC90 of 143 mg / mL (IC 123 - 164 mg / mL). Additionally, this study established inhibiting oviposition in 56.7% of teleogines exposed to the concentration of 53 mg/mL of acetone extract of G. sepium, and a 100% control of reproduction and reproductive efficiency 0.0% for teleogines used in the study, exposed to the same concentration above. All data were organized and processed according to the logistic regression test given by the probit method for obtaining lethal concentrations 50 and 90; likewise, there were significant differences ($p < 0.05$) between treatment levels negative control (excipient 2.5% Tween 80 in distilled water) and positive control (Amitraz 0.025 mg/mL in distilled water); and in turn between the levels of treatment of different concentrations (2.5, 5, 10, 20, 40, 80, 160 mg/mL) of acetone extract of G. sepium cited and the negative control. According to these results the acetone extract of G. sepium generates a phenomenon of inhibition of the hatching process, the eggs ovopositados entirety by this fitopreparado teleogines exposed within the antiparasitic tests. In cattle herds parasite load of the population is set to 92% associated agroecosystem, only 8% or is less direct parasitic stage (in the host); so that this promising plant play an important role in the plans of parasitic integrated control by providing therapeutic resources in the control of parasitic free stages of the tick, meeting called international protection of the environment, powering plant resources adapted the edaphological and ecological zone generally low tropics; besides being a plant species recognized by producers and socio-cultural environment of the rural sector of the Orinoco region of Colombia.

Keywords: Cattle, parasitic integrated control, phytopharmacology, rhipicephalus microplus

Pathomorphological and Immunohistochemical Studies of Tumors of the Urinary Bladder in Water buffaloes in Marmara, Middle and Western Black Sea Regions of Turkey

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Enzootic haematuria syndrome occurres in cattles and water buffaloes after a long lasting eating period of the natural plant cover of the area which is often infested by bracken fern. In this syndrome, non-neoplastic and neoplastic findings are seen in urinary bladder. In our study, we aimed to determine non neoplastic and neoplastic lesions of the urinary bladders of water buffaloes in Marmara, central and western Black Sea regions and identificate the species of the bracken ferns eaten by water buffaloes while grazing in the same provinces. Urinary bladders of water buffaloes, which were 3 to 8 years old from both genders, were collected from slaughterhouses of İstanbul, Adapazarı, Bolu and Samsun in which chronic enzootic hematuria was previously detected. Totally 163 urinary bladders with lesions were evaluated. The bladders were fixed in 10 % neutral formalin and evaluated macroscopically. Tissues were processed routinly and embedded in parafin, sectioned at 4-6 µ thickness and stained with haematoxylin-eosin (H&E). For immunohistochemistry streptavidin- biotin- peroxidase complex method was used. All sections were taken to positive charged slides by microtome. All slides, deparaffinized, hydrated and put in citrate buffer pH 6.0 for antigen retrieval. Microwave pressure cooker was run 20 min under 800 watts. As primary antibodies; mouse monoclonal anti-Uroplakin III antibody (for urothelium) and rabbit polyclonal CD31 antibody (for endothelium) were used. Then biotinized secondary antibody and streptavidin-peroxidase were dropped on the tissue sections. After this process, sections were visualized with 3-amino-9-ethylcarbazole (AEC, C01-12, GBI) chromogen. Background was colorized with Gill's (I) Hematoxylin. Slides were covered with aqueous mounting medium. Macroscopically; diffuse and petechial hemorrhages were observed at some of the urinary bladders. Varying sizes of the white foci were seen from the surface of the bladder and some of them were in cauliflower appearances. In histopathology, chronic cystitis was observed at 67 urinary bladders. 74,6 % of these cystitis were diagnosed as follicular cystitis. In 39 of cases, neoplasia was observed. These neoplasms were diagnosed according to The World Health Organization Classification of Tumors in 2004. According to this classification; 43,8% of all tumors were papilloma; 17,9% were papillary urothelial neoplasm of low malignant potential (PUNLMP); 33,3% were low grade papillary carcinoma, 5,1% high grade papillary carcinoma and 2,5% were hemangiosarcoma. In one of the urinary bladder; low grade papillary carcinoma was observed as well as hemangiosarcoma. Immunohistochemically, positive staining was observed at the cytoplasms of umbrella and intermediate cells of epithelial tumors with UPIII. In one case; CD31 was positive at the cytoplasms of the tumor endothelial cells, diagnosed as hemangiosarcoma. *Athyrum filix-foemina (L.) Roth, Dryopteris dilatata (hoffm.) Gary, Dryopteris filix-mas (L.) Schott, Polystichum aculeatum (L.) Roth, Polystichum setiferum (Forsk.) Woynar, Polystichum woronowii Fomin, Polypodium vulgare L., Pteridium aquilinum (L.) Kuhn.* species were identificated. In conclusion, water buffaloes cannot be fed with intensive breeding like cattle because of the fact that, they are animals that are supposed to be bred in extensive conditions. So, they consume all kind of grasses including bracken ferns which will cause the syndrome called as chronic enzootic heamaturia. This study will be informative for all researcher and breeders.

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Keywords: Bracken fern, chronic enzootic haematuria, neoplasia (tumor), pteridum spp, water buffalo

Investigation of Epidemiology of Peste des Petits Ruminants Virus (PPRV) Infection Using N and F Gene Targeted Different PCR Methods

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The first aim of the study, investigation of the genetic relationship of F and N genes of PPR viruses isolated from different provinces of Turkey, with those from neighboring countries, via sequencing and phylogenetic analysis. The second aim was to evaluate the sensitivity and specifity of three different PCR methods for diagnosis of PPRV in field samples. In this study, RT-PCR was used to test blood, nasal swabs, lung, spleen, rectum, small intestine and lymph node samples taken from animals (n=574), sheep (n=473) and goats (n=101). The samples were submitted to the Pendik Veterinary Control Institute from 50 provinces located different geographical area of Turkey during 2010–2012. Each animal greater than 6 months of age was evaluated as an epidemiologically independent flock. In addition, they were never vaccinated against PPR. Viral RNA was extracted from tissue and blood samples by RNeasy Mini Kit (Qiagen, Germany) and by High Pure Viral Nucleic Acid Kit (Roche, Germany) according to the manufacturers instructions respectively. One-step RT-PCR methods were used for the detection of PPRV RNA (Ozkul et al., 2002; Kerur et al., 2008; Kwiatek et al., 2010). PCR products were purified with High Pure PCR Product Purification Kit (Roche, Germany) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) on an ABI 3130xl DNA Analyzer (Applied Biosystems, USA). Purified products were sequenced in both direction. Using N gene based real time RT-PCR, PPRV RNA was detected in 255 suspected samples (255/574, 44,4%). In addition, all samples were tested based on F and N gene by conventional RT-PCR. PPRV RNA was detected in 204 suspected samples (204/574, 35,5%) and 226 suspected samples (226/574, 39,3%), respectively. Conventional RT-PCR investigated 53 samples of F gene and 60 samples of N gene were selected for sequencing. They were deposited in GenBank under the accession numbers of JQ388615-JQ388664, JQ519907-JQ519965, JX117877-JX117880. Phylogenetic analysis identified that investigated PPR viruses were located in lineage IV and had three different subgroup according to the F and N gene regions. Analysis of F gene partial nucleotide sequences showed that homology was found to be 98,2-100% among themselves, 97,9-98,9% and 91,3-92,4% between Turkey2000 (the Turkish isolate was used as a reference virus) and Nigeria/75/1(PPR vaccine virus was also used as a reference virus) sequences, respectively. Homology of N gene partial nucleotide sequences was found to be 94,2-100% among themselves, 94,2-98,3% and 89,3-90,9% between Turkey2000 and Nigeria/75/1, respectively. Molecular detection of PPRV genome showed that PPRV is circulating in goats and sheep in Turkey. Compared three different PCR methods, real time RT-PCR targeted N gene was found to be more sensitive and specific than conventional RT-PCR targeted to F and N gene. Molecular characterization of PPRV partial nucleotides of the F and N genes show that the virus belongs to lineage IV. We conclude that PPRV infection is endemic in our country, considering animal transportation restriction difficulties the most convenient method of protection is vaccination of the sensitive population.

Keywords: Goat, phylogenetic analysis, PPRV, RT-PCR, sheep, Turkey

Ultrasonographic Findings in the Ovine Udder During Lactogenesis in Healthy Ewes or Ewes with Pregnancy Toxaemia

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Objective of the study was to record, by means of ultrasonographic examination, changes occurring during lactogenesis in the udder of healthy ewes and of ewes with pregnancy toxaemia.

The work was carried out in 28 ewes, 16 with pregnancy toxaemia (group A) and 12 healthy controls (group B). B-mode and Doppler ultrasonographic examination of the udder of ewes was performed. Appropriate data management and analysis were carried out. No abnormal findings were recorded in mammary glands of all ewes in the study. Mean quantity of milk collected from group A ewes was 82.3 mL and from group B ewes was 103.6 mL ($P < 0.02$). During the last month of pregnancy, grey-scale intensity values of mammary parenchyma in group A were significantly greater than in group B ($P = 0.007$), as was also the progressive increase in grey-scale intensity values in both groups ($P < 0.001$). After lambing, grey-scale values decreased sharply compared to those in pregnancy ($P < 0.01$); during lactation, changes over time were not significant ($P > 0.6$), but differences between the two groups were still prevalent ($P = 0.046$). There was a significant reverse correlation between grey-scale intensity values and milk quantities ($P < 0.035$). The progressive increase in the diametre of the external pudendal artery was significant ($P < 0.001$), but no significant differences were evident between the two groups ($P > 0.35$). Throughout the last month of pregnancy, but not during the first week of lactation, progressive changes in blood flow parametres were significant ($P < 0.02$, $P > 0.12$, respectively), although no significant differences were recorded between the two groups ($P > 0.06$). Results of B-mode ultrasonographic examination suggested that there were differences in remodelling of the mammary parenchyma between healthy ewes and ewes with pregnancy toxaemia, possibly as an effect of reduced milk production by the latter animals. Perhaps, ultrasonographic udder examination at late gestation may have a prognostic value for the milk yield during the subsequent lactation period. Results of Doppler ultrasonographic examination indicated that blood flow to the mammary gland progressively increased at the last stage of pregnancy.

Keywords: Doppler, lactogenesis, mastitis, pregnancy toxaemia, sheep, ultrasonography

Clinical, Bacteriological and Cytological Findings during Uterine Involution in Ewes that had Developed Pregnancy Toxaemia

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Objective was to describe the findings of uterine involution in ewes with pregnancy toxaemia during the last stage of the preceding gestation. We used 9 ewes, which, during the last stage of their gestation, had developed pregnancy toxaemia, as confirmed by increased blood concentration of β-hydroxybutyrate (>=1.2 mmol L-1 in two samples collected after the 129th day of pregnancy) (group PT), as well as 5 healthy control animals (group C). On the day of lambing and 1, 2, 4, 7, 10, 15 days after lambing and every five days thereafter, animals were examined clinically (general examination, vaginoscopy). Samples (swab for bacteriological examination, cyto-brush for cytological examination) were also collected, initially from the interior of the uterus (up to 4th day post-partum) and later from the posterior entrance of the cervix (7th day and 10th day post-partum) or the anterior part of the vagina (15th day post-partum and thereafter). Biopsy samples were collected from the uterus laparoscopically. From 4 ewes of group PT samples were collected on days 20 and 40 post-partum and from the remaining animals on days 10 and 30 post-partum. From group C, samples were collected from 2 and 3 ewes, respectively. On each occasion, samples were collected from one uterine horn only (first sample from left horn, second from right horn). Conventional bacteriological and cytological techniques were employed from processing of samples. Among group PT, 4 ewes developed post-partum metritis, one of which also had retention of foetal membranes. General (fever, anorexia, depression, refusal to suckle the lambs) and local (copious and malodorous, mucous or purulent vaginal secretion) signs were observed. Among group C ewes, one developed metritis and retention of foetal membranes. Bacteria were isolated from vaginal swabs on at least one occasion from all animals into the study. Median time of first bacterial isolation from the vaginal swabs was 1 day for PT group ewes and 1.5 days for C group ewes (P=0.16). Median duration of infection was 12.5 days for PT group and 7.5 days for C group (P=0.076). Organisms isolated were identified as Escherichia coli, Proteus spp., Streptococcus spp. or Trueperella pyogenes. Bacteria were isolated from uterine content samples collected 10 days after lambing from all group PT ewes; subsequent samples from these animals (30 days post-partum) did not yield bacteria. Also, bacteria were not isolated from any other Pt group ewes (sampled 20 and 40 days post-partum) and from C group ewes. There was a significantly higher frequency of intra-uterine infection in PT group ewes (P=0.007) immediately post-partum, as well as a significantly longer duration of intra-uterine infection (P=0.039). There was similarity in the results of bacteriological examination of genital tract and intrauterine samples (positive, negative) in 63% of samples; in the remaining cases, only the genital tract samples yield bacteria. Immediately post-partum, neutrophils predominated in genital tract samples collected from animals of both groups; in samples from PT group ewes, lymphocytes were also seen. Progressively, number of neutrophils decreased and epithelial cells were observed. In intra-uterine samples, a few neutrophils were seen in group C animals, whilst, in contrast, abundant neutrophils and lymphocytes, as well as erythrocytes were evident in group PT ewes. The results indicate that although there was no increased risk for infection of the genital tract between animals of the two groups, ewes in group PT developed metritis more often; duration of intra-uterine infection was also longer in these ewes. The result indicate that ewes with pregnancy toxaemia should receive increased veterinary care in the immediately post-partum period to prevent infections.

Keywords: Escherichia coli, metritis, pregnancy toxaemia, sheep, trueperella, uterus

Use of the Combination of Clinical Examination and California Mastitis Test for Selective Intramammary Administration of Antibiotics at the end of a Lactation Period in Ewes

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Intramammary administration of antibiotics at the end of a lactation period, i.e. at the start of the dry period, is an important preventive measure against mastitis in ewes. However, administration of antibiotics in dairy ewes has raised concerns in consumers of dairy products. Hence, selective administration of antibiotics, which takes place only to ewes with a confirmed mammary problem has been advocated. On-farm methods of evaluation of potential problems include clinical examination of the udder and application of the California Mastitis Test (CMT), which provides a good estimation of the cellular content of milk. Objective of this study was to evaluate the combined use of clinical examination and CMT for identifying ewes, which are in need of administration of antibiotics at the end of a lactation period, before the start of the dry-period. We carried out a trial in a commercial sheep dairy farm in Greece, in total involving 80 multiparous ewes (i.e., 160 mammary glands). Before start of the dry-period, detailed clinical examination of the udder was performed in all ewes into the study. Further, milk samples were collected for evaluation by means of CMT. Based on the results of the clinical examination and the CMT, mammary glands were then classified in: (a) mammary glands with lesions (L+), i.e., glands with pathological findings during the clinical examination and/or increased CMT score in milk or (b) mammary glands with no lesions (L-). In all L+ mammary glands, a combination of antibiotics (penicillin, streptomycin, nafcillin) was administered intramammarily (A+). Most L- mammary glands also received the same combination of antibiotics (A+), although some remained as untreated controls (A-) (Table 1). Finally, within 10 days after the subsequent lambing ewes were again clinically examined and milk samples were collected for CMT. There were significantly (P<0.01) fewer mammary glands with abnormal findings (clinical lesions or increased score in CMT) after lambing in ewes that received antibiotic treatment at the end of the previous lactation period. Further, after lambing, cure rate of glands with abnormal findings was >70%. Details are in Table 2. The results indicate that, before the start of the dry-period, clinical examination and CMT can be used to identify animals with mammary abnormalities, for intramammary administration of antibiotics.

Keywords: Dry-period, mastitis, sheep

Table 1

		Intramammary administration of antibiotics	Intramammary administration of antibiotics
	Mammary glands (n)	A+	A-
Clinical lesions and/or increased CMT score	L+	64	0
Clinical lesions and/or increased CMT score	L-	79	17

Results of clinical examination and California Mastitis Test in ewes at the end of a lactation period, before start of the dry-period (L+/L-: presence/absence of abnormal findings, A+/A-: administration/no administration of antibiotics intramammarily)

Table 2

At the end of the lactation period	Mammary glands (n)	After lambing	Mammary glands (n)	%	P
L+/A+	64	Abnormal findings+	17	27	<0.001
		Abnormal findings-	47	73	
L-/A+	79	Abnormal findings+	4	5	<0.001
		Abnormal findings-	75	95	
L-/A-	17	Abnormal findings+	4	24	0.002
		Abnormal findings-	13	76	

Results of abnormal findings in clinical examination and California Mastitis Test in ewes after lambing, in relation to their condition and the intramammary administration of antibiotics at the end of the previous lactation period (L+/L-: presence/absence of abnormal findings, A+/A-: administration/no administration of antibiotics intramammarily)

Immunophenotype Classification and Molecular Diagnosis of Canine Lymphomas

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The purpose of the study is to determine the accuracy of immunophenotypic information between three Methods: immunocytochemistry (IC), immunohistochemistry (IH) and heteroduplex polymerase chain reaction for antigen receptor rearrangement (heteroduplex PARR) in spontaneous canine lymphomas. EDTA blood, fine needle aspiration and tissue biopsy from enlarged peripheral lymph nodes prior treatment were collected from fifteen dogs. Cytopathology and histopathology were also examined and classified using updated Kiel. IC and IH were performed by appling anti-Pax5 and anti-CD3 antibody as for B- and T-cell marker respectively. Gene of neoplastic lymphocytes from cytology and blood were evaluated by heteroduplex PARR. Sensitivity and specificity from these three tests were noted. Two dogs were low grade (1 Macronucleolated medium-sized cell, 1 centroblastic/centrocytic) and thirteen of them were high grade lymphomas (2 centroblastic polymorphic, 5 immunoblastic, 3 pleomorphic mixed, 1 pleomorphic large cell, 2 lymphoblastic). Immunophenotyping results from IC and IH similarly showed eleven B-cell lymphomas (Pax5+/CD3-) and four T-cell lymphomas (CD3+/Pax5-). In addition, immunophenotyping by heterodulplex PARR illustrated IgH genes in five dogs and TCRγ genes in six dogs. Three samples showed dual genes and one was indeterminate. The sensitivity of heteroduplex PARR was 60% with no difference in specificity to IC and IH. Methods for immunophenotyping should not relied on only one method. IC or IH with heteroduplex PARR should be concurrently applied for immunophenotypic data in canine lymphomas.

Keywords: Canine lymphoma, immunocytochemistry, immunohistochemistry, immunophenotype, heteroduplex PARR

Infection Status of Oral Cavity, Stomach, Bile, Liver and Feces of Dogs by Helicobacter SPP

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In dogs, the gastric Helicobacter spp. have been well studied, but there is little information regarding the other parts of the alimentary system. also the mode of acquisition of gastric Helicobacter spp. infection in dogs has not been determined. It is suspected that oral-oral and faecal-oral transmission may be involved. We sought to determine the spatial distribution of Helicobacter spp. in the gastrointestinal tract and the hepatobiliary system of stray dogs using culture-independent methods. Samples of oral cavity, stomach, feces, liver, and bile from fourty – eight stray dogs were evaluated for Helicobacter spp. by genus and gastric Helicobacter spp. The presence of Helicobacter spp. was determined by single PCR evaluation of DNA extracted from saliva, dental plaque, fondus, body, bile, liver and feces. Genus-specific PCR positive samples were evaluated for H.felis and H.pylori using specific primer pairs. Additionally gastric samples were studied by quantitative rapid urease test and cytology. Helicobacter spp. DNA was detected in 39(81/3%) saliva, 38(79/2%) dental plaque, 39(81/3%) fondus, 39(81/3%) body and 41(85/4%) feces. But no helicobacter spp were present in bile or liver of these stray dogs. In generall 100% dogs screened by single PCR were found to harbour Helicobacter spp. DNA in the oral cavity (dental plaque and/or saliva) and stomach. This study demonstrates that in addition to the stomach, the oral cavity and feces of stray dogs can be colonized by helicobacter spp. These findings support the possibility of oral-oral and fecal – oral transmission between dogs.

Keywords: Dog, helicobacter

Prevalence of Household Dogs' Infection to *Dirofilaria. immitis* Using Some Laboratory Diagnostic Methods, in Rasht, Northern Iran

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Dirofilariasis which is also called canine heart worm disease (HWD) is a prevalent disease in Iran, particularly in north and northwest temperate zones. Because of some limitations due to religious beliefs of these areas' people, there are no enough cares about the dogs' health. Based on available literature, no survey had performed about household dogs dirofilariasis in north Iran up to the date of our study so this study aimed to evaluate the prevalence rate of the disease and compare the potency of some available laboratory diagnostic tests in household dogs referred to small animals veterinary clinics in Rasht, northern Iran. One hundred blood samples were collected from clinics which had obtained from dogs with no apparent signs of HWD from early fall 2010 up to the late spring 2011. Consequently the samples were examined using a sandwich immunochromatographic Kit (Speed Diro- BVT, France) as the gold standard and Modified knott test and direct smear test as the frequent diagnostic tests in vet clinics, for comparing the results. The results showed the rate of 5% (5 out of 100) infection using rapid test in the studied samples, whereas 3% and 2% of the dogs had diagnosed as infected using modified Knott and direct smear methods respectively. All of the infected dogs were in 4-7 year age group which was statistically significant comparing to" 1-4 " and " more than 7 " age groups ($P < 0.05$). Despite the fact that, 4 out of 5 infected dogs were male, no significant difference was observed considering the gender ($P > 0.05$). Also, all of the infected dogs were outdoor kept animals which 4 of them (80%) has a bad sanitary condition which in statistical analysis, both of them have a significant difference compared to indoor kept and appropriate sanitary condition groups respectively ($P < 0.05$). It can be concluded that HWD has moderate prevalence in Rasht, northern Iran and both of modified Knott and direct smear tests failed to diagnose appropriately comparing to rapid test. Except to gender impact, all of the studied parameters impact on the rate of disease was in accordance to the results of other studies, but considering that occult infection can complicate the clinical outcomes of the modified Knott and direct smear tests diagnostic results it is recommendable to use more sensitive tests such as immunochromatographic rapid kits.

Keywords: Dirofilariasis, dog, Iran, laboratory diagnosis, rasht

Dog Patient's Quality of Life in Idiopathic Epilepsy Treatment

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The main objective of the study is to present the significance of the good communication with the patient's owner. Additional aims are: client education in following the treatment regime and monitoring of disease–related quality of life. The authors also attempt to prepare the life quality assessment protocol. This protocol would have been useful in decision making of possible euthanasia when the side effects of the therapy outweigh its benefits. Selected five cases of idiopathic dog epilepsy were presented in this study. Due to the single drug resistance a whole group was treated with multiple – drug protocol. The average survival rate was 2.9 months. In all cases seizures responded to the treatment with phenobarbital with potassium bromide and gabapentin. Moreover, decision of euthanasia in these cases was made due to advanced cognitive dysfunctions. Idiopathic epilepsy is the most common canine chronic neurological disease, affecting approximately from 0,5% to 5 % of the population. The epilepsy is recognized as ”idiopathic” when there are no identifiable underlying causes. It usually requires higher and higher drug doses often balancing on the edge of the toxic references. It helps veterinarian in clinical management of the disease (seizures treatment), but in some cases it influences dramatically the patient's quality of life. The process is not rapid and results probably from the reduction of the drug tolerance. In this context both the owner and veterinarian face the difficulty of the decision of euthanasia. The good communication with the owner is very important during the treatment of idiopathic epilepsy. Veterinarians should educate the owners about the nature of the idiopathic epilepsy and how to take a good care of the animal. The owner should observe the behavioural patterns of the animal and assess the animal's quality of life especially when the treatment of seizures dramatically reduces the quality of life. Decision of euthanasia is very difficult because in this context the animal is not painful and its discomfort is subjective. The cognitive function disorders are the main problem in this disease. Cognitive function is altered and the interaction with the family is decreased. We observe loss of house-training, disorientation, decreased greeting behaviour and change in activity. The life quality assessment protocol would be helpful in making decisions of humane euthanasia.

Keywords: Euthanasia, idiopathic epilepsy dog, quality of life

Foot and Mouth Disease in Turkey: History Present Status and Future Challenges

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Foot and mouth disease (FMD) is a highly contagious, acute, viral disease of domestic and wild cloven-hoofed animals. There are seven serotypes known as A, O, C, Asia 1 SAT1, SAT2, and SAT3. Disease caused by one serotype does not confer immunity against the other serotypes. FMD is one of the most important health problems that endanger the safety of animal-derived protein production and supply. Even some authorities describe it as a food production problem other than an animal health problem. The combat against foot and mouth disease and the control instruments are required to be more strict than similar diseases because FMDV (foot-and-mouth disease virus) can spread very fast, has high adaptation capability and the economic losses due to the high mortality rate in young animals, meat and milk production in adult animals. The first records of foot and mouth disease in Turkey was published by Ottoman Administration for Statistics in 1914. Due to high economic losses caused by the wide spread FMD outbreak in 1957, modern control policies based on vaccination and quarantine measures were established. Control and intervention policies differ according to geographical location, development, community awareness and the extent of FMD. Turkey's FMD control policy primarily based on vaccination and has given highest priority to Thrace to stop FMD spreading European countries since 1960s. This policy represents a unique case in the animal health World as a country protecting neighbors more than its own farmers. The World Animal Health Organization (OIE) has been recognized Turkish Thrace free with vaccination since 2010, while rest of the country continues to struggle with endemic status, even severe in East Anatolia. Today, there are approximately 100 endemic countries in Africa and Asia where FMDV is circulating persistently. Recent global control strategies such as progressive control pathway to interrupt persistent virus circulation in endemic areas in the context of Turkey will be discussed.

Keywords: Epidemiology, FMD, Turkey

Loop-Mediated Isothermal Amplification (LAMP) Assay for Detection of Burkholderia Mallei

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Glanders is a contagious, acute or chronic, usually fatal disease of Equidae caused by Burkholderia mallei and characterized by serial development of ulcerating nodules that are most commonly found in the upper respiratory tract, lungs, and skin. The organism is infectious for people, with a 95% fatality rate in untreated septicemia cases, and is considered a potential bioterrorism agent. The principle of LAMP is based on auto-cycling strand displacement DNA synthesis facilitated by Bst (Bacillus stearothermophilus) DNA polymerase using a set of four to six primers, including forward inner primer (FIP) and backward inner primer (BIP), outer primers (F3 and B3) and loop primers (LF and LB). In the present study an isolate of B. mallei from one Siberian tiger passed away at the Tehran zoo in December 2010 with common clinical signs of glanders and the standard strain was used. Two other strains of B. pseudomalei and P. aeruginosa from microbial collection of Tuberculosis Department at Razi Vaccine and Serum Research Institute were also used to detect the specificity of the primers designed for the LAMP technique. A total of six primers were designed using the Lamp primer designing software: PrimerExplorer V4 (htpp://primerexplorer.jp/elamp3.0.0/index.html). To find the optimum time and temperature, the reactions were carried out at 63 and 65°C for 30 to 240 min. Finally each reaction was incubated at 63 °C for 60 min. Positive and negative controls were included in each run. LAMP products were directly detected with naked eye and also by adding 2µl of SYBR Green I to the reaction tube and observing the color of the solution under UV trans illuminator. In addition, LAMP products were electrophoresed on 2% agarose gel (Invitrogen agarose in lx TBE buffer) with ethidium bromide staining and evaluated in UV gel doc. The assay showed high sensitivity and specificity and it was concluded that our proposed LAMP assay is a rapid, sensitive and practical tool for the detection of B. mallei and would be useful in the early diagnosis of glanders disease caused by the organism.

Keywords: Assay, Burkholderia mallei, loop-mediated isothermal amplification

The Regional Distribution of Foot and Mouth Diseases Virus (FMDV) Types in Turkey

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The number of FMD outbreaks were seen in last 5 years in Turkey as 1.782 number in 2010 Year, 1.960 number in 2011 Year, 1.148 number in 2012 Year, 1.311 number in 2013 Year, 299 number in 2014 Year The dominant types according to years: O type in 2010 Year, A type in 2011 Year, Asia-1 type in 2012 Year, A type in 2013 Year, O type in 2014 Year. If the assesment make according to regions: The Black Sea Region in 2010 Year, The Black Sea Region in 2011 Year, The Aegean Region in 2012 Year, The Eastern Anatolia Region in 2013 Year, The Central Anatolia Region in 2014. Antigenic epitopes on the foot and mouth disease virus isolates were identified by using monoclonal antibodies. Mab panels were kindly provided by WRL, IZSLER, AFFSA and Turkey. The working dilutions of Mabs was determined. Total of 95 A and 31 O isolates were profiled. Percent reactivity of field strain was compared to parental FMD strains. Mab Profiling ELISA was applied in this study. Most of the field isolates showed epitope similarity with parental A22 Iraq vaccine strain. Available r1 values: [1096: 0,61], [570: 0,34], [552: 0,50], [584: 0,58]. 12 out of 95 isolates showed low profile with at least 3 of the 5 NA Mabs. Available r1 values: [552: 0,08], [560:0,20].PAN FMD A neutralizing 4F6 (IZSLER), which is reactive with all isolates, could be a good scientific tool for FMD type A works. 4 out of 12 Alfa-05 showed low profiles with at least 6 of the 10 NA Mabs. Alfa-05 could be a O1 Manisa vaccine escape variant. r1 value: O/ISR/1/2005*: 0.07 VNT.O/TUR/1/2005* (0,55) O/TUR/4/2005* (0,56) O/TUR/5/2005*(0,57), O/TUR/474/2005 (0,51) Non of the NPA isolates showed low profile with NA MAbs. r1 value: It is generally high with the first isolates of NPA-05s. Epitope on 2G5 (IZSLER) could be a crucial neutralizing epitope on O1 Manisa. There is no 2G5 epitope in alfa-05 strains, while almost conserved in NPA-05s.

Keywords: Epitope, foot and mouth disease virus, monoclonal antibodies, region

Duration of Antibody Response to 3PD50 and 6PD50 Oil Adjuvant FMD Vaccines in Cattle

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Foot and Mouth Disease is a highly contagiouse viral disease of clowen hoofed animals with milk and meat loss, and economic consequences because of international trade sanctions. The one of the most important method to combat with the disease is vaccination. Vaccination takes an important part of the control measures in Turkey for a long time. Implementation of vaccination has been brought success on control and eradication of the disease in many countries. For routine prophylactic use, generally minimume 3 PD50 dose of vaccines are recommended. However 6PD50 cattle dose is more commonly preferred by the countries that are in endemic condition. High potency vaccine not only confers protection for a prolonged time but also impedes persistency and emerging new variants indirectly. Besides it has the potential to cross-react different subtypes in the same serotype. In this study, determination of duration of immunity for low and high potency vaccines are targeted. For this purpose and to simulate of field conditions during vaccination campaigns, 2.5-4 months old calves that have maternal antibodies were used in a state farm. The animals were bled at the day 0 and vaccinated with two different vaccine contains 3PD50 and 6PD50 dose of antigen produced by FMD Institute Ankara/Turkey. At day 28 the animals were bled and divided into two more groups that were booster and non-booster groups. Booster groups were vaccinated with same vaccine according to the belongings to the high or low potency groups. Every month the farm was visited and blood samples were taken. VNT assay were performed according to OIE manuel. Results have showed that under the presence of maternal antibodies 3PD50 vaccines can not confer protection beyond 3rd month without booster. However, 6PD50 vaccine confers protection up to six month with or without booster dose.

Keywords: Duration of antibody response, foot-and-Mouth disease, foot and mouth disease virus, high potency vaccine, vaccine immunity

VNT antibody titers of the sera

Days	3 PD50	3 PD50 with Booster	6 PD50	6 PD50 with Booster
Day 0	1:47,73	1:48,04	1:45,15	1:42,86
Day 28	1:13,20	1:11,90	1:18,66	1:12,99
Day 60	1:40,58	1:80,10	1:69,68	1:199,27
Day 90	1:9,06	1:39,10	1:38,40	1:74,12
Day 120	1:5,66	1:12,75	1:27,19	1:32,65

VNT results are expressed as geometric means of groups

Implementing Effective Veterinary Biosecurity Programs in Aquaculture that Meet International Standards & National Regulations

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Facing progressively increasing risks and impacts of disease on aquaculture productions in all countries, over more than a decade at numerous conferences, symposia and workshops, a large number of individuals have discussed and debated what procedure that should be incorporated into biosecurity programs. A key feature has been determining which procedures will meet International Standards (i.e. processes and procedures in OIE Codes & Manuals) and National regulations. In balancing these requirements with practical approaches that aquaculture producers can implement, and are effective and useful for all stakeholders around the world (from producers to governmental regulators), the following were recognized as priorities for all biosecurity programs:

a) be practical and economic;
b) focus only on infectious and contagious diseases;
c) include procedures that address disease prevention, control and eradication in definable epidemiological units;
d) be based on well-established, sound scientific-justifiable veterinary procedures;
e) incorporate internationally accepted standards in the OIE Code and Manual; and,
f) involve public-private partnerships and collaboration between producers, aquatic veterinarians and paraveterinary professionals, and governmental regulators. In focusing on these principles, the International Aquatic Veterinary Biosecurity Consortium (IAVBC) has tested the procedures in Figure 1 with stakeholders at several conferences and workshops in Norway, South Africa, Chile, and elsewhere, that involve an integrated approach for developing, implementing, auditing and certifying effective aquaculture biosecurity program. At the core of a biosecurity program is defining an epidemiologic unit (EpiUnit), a well-defined geographical population of animals, on which all biosecurity steps or processes will be implemented. An EpiUnit might be an establishment (farm), a compartment (different locations that are all managed as an integrated operation, usually under one ownership), a zone (typically a region of a country), or a whole country. To some degree, each EpiUnit population is separated from other populations, allowing control over the spread of disease. However, within the EpiUnit, infectious and contagious diseases transmission between individuals is relatively easy. A second important principle is that all procedures implemented for a selected EpiUnit must be thought out ahead of time, and well documented. This requires both an *a priori* evaluation of the EpiUnit, and a written biosecurity plan that addresses all steps and processes to be implemented in the EpiUnit, and documentation of all procedures that are implemented over time (i.e. a biosecurity implementation record). Along with periodic on-site evaluation of operations and animals on the EpiUnit, the written plan and the documentation of implemented procedures become the focus for auditing and certification. Every biosecurity plan will be specific for an individual EpiUnit. To be effective and justifiable the processes and procedures need to involve several formal processes, including: hazard and risk analysis (hazard identification and prioritization, risk assessment/evaluation, risk management/mitigation and risk communication); analysis and remediation of critical control points (including evaluation and mitigation plans for correcting practices where disease could enter or leave the epidemiological unit); epidemiological principles (including necessary diagnostics, surveillance, monitoring and determining the status or freedom of diseases in the epidemiological unit); emergency preparedness (contingency protocols for disease control and eradication); and, auditing of procedures and records, and certification (providing assurance of disease freedom and useful as compliance incentives). This presentation will outline and provide an overview of the importance of each procedure, and how these can be implemented and integrated (Figure 1). This outline will be useful for other veterinarians or government officials to assist producers in developing effective and efficient biosecurity programs in aquaculture operations and larger EpiUnits.

Keywords: Aquaculture biosecurity, epidemiological units, national regulations, OIE standards

Making it Easy to Quantify and Display Disease Risks for Biosecurity Plan Clients in Data Poor Environments

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It is estimated that the human population will be nine billion by 2030 and according to the FAO the only agricultural industry with sufficient potential to meet the protein requirements of this population is aquaculture which currently provides around 50% of the fish and shellfish we eat. One of the primary constraints to achieving this objective is disease losses which were estimated by the World Bank in 1997 to be US$3 billion annually. It is currently estimated that 40% of insured losses are due to disease which causes problems in the operation and development of the aquaculture insurance market. Consequently the implementation of appropriate biosecurity measures (the prevention, control and possible eradication of disease) is seen as vital. However, compliance with biosecurity measures is often poor being related to level of education, training, responsibility and perceived economic benefits. Global estimates of disease losses may appear remote and inapplicable to farmers and producers who are often faced with having to make the rational choice from scarce data and often with even scarcer resources. This can and does lead to failing to recognise the actual risk being taken and can lead to disease incursion with catastrophic consequences for the business. This presentation looks at how a veterinarian can easily use commercial software such as Microsoft Excel or @Risk and free software such as PopTools to assist in designing a relevant biosecurity plan for an individual farm or local grouping. This can be achieved by using running spreadsheets with minimal input. Minimize sampling costs for determining the probability of disease presence and ongoing surveillance Improved accuracy in determining risk probabilities leading to smarter decisions. Better understanding of complex results for the client through use of visuals such as graphs. Overall this will achieve greater compliance through managing expectations, maximizing cost benefits and improved use of resources.

Keywords: Biosecurity, cost, risk

Advanced Fish Diagnostic Techniques

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What are the next steps in diagnosing fish diseases after having completed a physical examination, skin and gill biopsy examinations and bacterial cultures? How about hematology, radiology, endoscopy, sonography, or even computed tomography (CT) and magnetic resonance imaging (MRI)? These advanced diagnostic techniques can be used with fish as easily as with other pet patients. Diagnosing diseases in fish, especially internal problems, can be made easier with the use of radiology, ultrasound, and endoscopy equipment already present in the veterinary clinic. Techniques such as CT and MRI, where available through specialty practices or veterinary colleges, can also be of great assistance in diagnosing abnormalities of the cardiovascular system, intestinal tract, gas bladder, and abdominal organs in larger fish, such as a koi with abdominal distension. These techniques, along with microscopic analysis of fluid and tissue biopsies are very helpful in the diagnosis of fish diseases. Drawing blood samples from larger fish, especially koi, can be done from the caudal vein below the spine in the caudal peduncle. To collect a blood sample from an anesthetized fish, the needle is inserted at an angle pointing craniodorsally from the ventral midline of the caudal peduncle until it hits the vertebrae. Withdraw the needle slightly and it should be in the caudal vein. Light aspiration on the syringe plunger should be applied to collect the blood. Radiographs of fish can be taken successfully with standard veterinary radiology equipment. The radiographs can be taken without anesthesia by briefly restraining the fish in a sealed plastic bag with a small volume of water. Fish can also be anesthetized and then taken out of the water and positioned for radiographs. Ultrasound imaging can be performed on fish confined in a small container of water, as the water serves to couple the transducer to the fish's body, eliminating the need for ultrasound gel. The transducer can be held several centimeters away from the fish if it is in the water, and the transducer repositioned until the desired image is obtained. Endoscopic examination of the oral cavity, gill arches, and the pharynx can be performed by passing the endoscope into the mouth or gill operculum of an anesthetized fish. Flexible endoscopes can be passed through the esophagus into the stomach or intestines. A small surgical incision can be made through an anesthetized fish's body wall to insert an endoscope. The small incision can be closed with a simple interrupted absorbable suture, or sealed with methacrylate tissue adhesive. Computed tomography scans can be performed on fish while they are in a small container of oxygenated water. The water does not affect the image in a CT scan. This can provide a 3-dimensional radiographic image of skin, muscles, skeleton and other internal structures, or 2-dimensional views of sequential planes through the body. Magnetic resonance imaging, which uses nuclear magnetic resonance instead of x-rays to generate an image, can produce very detailed images of the soft tissue and internal anatomy, and is useful in diagnosing neoplasia. Positron emission tomography (PET) scans can be used to provide information about metabolic function and carcinogenesis. Using advanced imaging techniques and other laboratory tests can increase the diagnostic capabilities of a veterinarian treating fish, beyond the usual method of checking for parasites using biopsies and microscopic examination. The more diagnostic information the clinician has, the easier it is to successfully treat aquatic patients.

Keywords: Diagnostic, endoscopy, fish, hematology, radiology, sonography

Food for a Hungry World

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The objective of this paper is to demonstrate aspects of the impact of the increase in the world`s population in relation with food availability: " the world population can be expected to increase from the current 7.2 billion people to 9.6 billion in 2050 and 10.9 billion in 2100"*. Some countries are leaders in food production, like Brazil, the USA, New Zealand, Australia and some European countries. In this century Brazil has assumed an important position as a leader in food production. Not only for its own population (around 200 million) but also for export to several countries including China, Russia and the Near East. In less than half a century, Brazil was transformed from food importer to a large scale exporter. Presently it is the most important exporter of soy, sugar, bovine and chicken meat. It is also one of the major producers of important foods of animal and vegetable origin (see table below). The perspective and challenge of increasing the production is great. It is estimated that 40% of future needs for the increasing world population will come from the country. Some food production such as chicken and soy has been explosive. Moving from an agriculture previously based mostly on 'slash and burn' policies of forests, to advancement of applied scientific technology in many fields. For example, in animal production, food security, precision cultivation, direct seeding, computerized techniques for adubation, watering, harvest and use of areas previously deemed unproductive. Genetically modified organisms are currently used in soy and corn production. Cultivation of fresh water fish is expected to be the next food explosion with modern technology; in some places farmers are substituting soy for aquaculture of high-value Brazilian fish species. In the vast extension of Brazilian territory, there are several biomas with different areas for food production. Climatically diverse, drought and flooding may occur simultaneously in different parts of the country. The 'Cerrado' bioma (2 million sq km) in central Brazil is presently the most important area for food production, of mainly cattle and soy. The Amazonian bioma in the north represents more than half Brazilian territory within which could be located most of the European countries. Only a relatively "small" part of this huge area is being used for food production, mainly in it's southern and eastern borders. Food Security has the technical support of the Brazilian Veterinarians, presently more than 150,000 persons.

Keywords: Cattle, chicken, food, production

BRAZILIAN PRODUCTION OF SIX MAIN FOODS (2014)

1,000 tons	Meat		
	Bovine	Swine	Chicken
	9,160	3,462	12,875
Million tons	Grain		
	Soy	Corn	Coffee
	95,919	79,051	45,342

POPULATION(millions): Human 202,770 Bovine: 213,138 Swine: 36,438 Min. Agric., Brazil
** UN Population Projection. In: SCIENCE, 16 October 2014, Page 234*

Prevalence and Characterization of Salmonella Isolated from Chicken Meat in Turkey

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This study was conducted in a Turkish province (Samsun) to investigate the presence of Salmonella spp. in 150 chicken meat samples (75 carcasses and 75 pieces of meat) using two phenotyping techniques: classic culture technique and immunomagnetic separation. For the confirmation of the isolates at molecular levels, invA gene was detected in these isolates. The presence of invA, class 1 (Cls1) integrons and integrase (Int1) genes was demonstrated by PCR assay; and the resistance of the isolated Salmonella spp. strains to antibiotics was determined by disk diffusion test. All the cultural and PCR results were evaluated together; Salmonella spp. were detected in a total of 64 (42.66%) chicken meat samples. Contamination rate was higher in carcasses (53.33%, n=75) than in meat pieces (32%, n=75). When results of standard culture were compared with IMS technique, IMS (n=54) showed a clear superiority over the CCT (n=38). A very high resistance rate (>=89.28%) to vancomycin, tetracycline, streptomycin or nalidixic acid was found. Trimethoprim-sulfamethoxazole resistance was present in 32.14%. Relatively lower incidence of resistance (<=8.33%) to gentamicin, chloramphenicol, ampicillin and ceftriaxone was observed. Concurrent resistance to at least four antibiotics was detected in 92.85% of the isolates. Cls1 integrons and Int1 were positive in 80.95% and 95.23% of the isolates, respectively. However, Int1 alone was detected in 15.47% (n=13). In conclusion, the high prevalence of Salmonella spp. in chicken meat may pose a potential public health risk, and the presence of antibiotic resistant Salmonella spp. isolate together with Cls1 integron and/or integrase might play an important role in horizontal antibiotic gene transfer.

Keywords: Antibiotic resistance, chicken meat, integrase, integron, salmonella

Presence of *Escherichia coli* O157:H7 Bacteriophages in Slaughterhouse Wastewater and Biocontrol of *Escherichia coli* O157:H7 in Raw Meatball

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Escherichia coli (E.coli) O157:H7 is one of the major foodborne health concern pathogen, causing haemorrhagic colitis and hemolytic uremic syndrome in humans worldwide and considerable focus has been given to its biocontrol in foods with application of bacteriophages. In this study, we aimed to isolate virulent *E. coli* O157:H7 phages from slaughterhouse wastewater between July 2011 and June 2013, over a two-year period in Kirikkale, Turkey, characterize the phages according to their host range and efficiency of plating (EoP), identify them by electron microscopy and finally to choose the most virulent phage to use as a biocontol agent of *E. coli* O157:H7 on a highly risky ready-to-eat Turkish traditional delicacy food model called “raw meatball”, under refrigeration and room temperature storage conditions. A total of 31 *E. coli* O157:H7 phages were isolated and characterized according to their host range and efficiency of plating (EoP) characteristics on 5 reference strains and 28 wastewater or cattle originating wild type strains isolated throughout a parallel study. Phage M8AEC16, was selected out of 31 lytic phages as it showed a broad lytic activity towards many *E. coli* O157:H7 strains with O157 specificity and high efficiency of plating. According to the TEM analysis it was classified in *Myoviridae* family. *E. coli*O157:H7 ATCC 43895 (EC95) was used as the model bacterium in decontamination trials of raw meatballs. In general, efficacy of M8AEC16 was slightly higher in 22°C than at 4°C showing that storage conditions had minor effect on efficiency of phage biocontrol. On the other hand, viable *E. coli* O157:H7 reductions varied between 0.69 - 2.09 log cfu/g in the first 5 h of the replica trials. Major reductions of viable *E. coli* O157:H7 counts were observed in the beginning of the storage period, reaching up to 1.85 log cfu/g. This study is remarkable because of being the first one in Turkey that investigates applicability of phage biocontrol for a traditional food model such as raw meatball. Findings of this study were encouraging, as phages might be valuable in decontamination of other foodborne pathogens in raw meatball as well.

Acknowledge: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, project no 110R013) and Scientific Research Projects Unit of Kirikkale University (KU-BAP, project no 2013/75).

Keywords: Bacteriophage, biocontrol, E. coli O157:H7, raw meatball, wastewater

One Health in Action: An American Perspective

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At the center of America's agricultural heartland is Kansas City, a region home to a consortium of research institutions and assets, including the National Bio and Agro-defence Facility, the Animal Health Corridor, Google Fiber, Sprint Corporation, flourishing information technology and engineering sectors, four human medical schools, two colleges of veterinary medicine, diverse programs in environmental and plant science and many, many other assets. One Health3 (Cubed) is a coalition of stakeholders committed to leveraging the regions unique assets to integrate and implement interdisciplinary research and translate that research into clinical/practice applications. Human medicine, Veterinary medicine and Environmental Medicine (3) The practice of medicine involves the diagnosis and treatment of conditions that deviate from the state of health, as well as preventative interventions to preserve a healthy state. This applies to people, animals and the Earth. This is One Health. Integration, implementation, and interdisciplinarity (3) Recognizing that collaboration is about building relationships, developing trust, valuing diversity in skills, knowledge, experience and learning styles, stakeholders are investing in developing collaborative infrastructure using a systems thinking approach. Experiments involve the use of online asynchronous collaboration tools, network mapping, design thinking, systems modeling and simulations as a means of learning and scenario testing. Face-to-face workshops focus on the development of a shared vision, decision making protocols, and conflict resolution, all with underlying attention to strengthening interpersonal skills and appreciating differences in learning styles. A spirit of scientific entrepreneurship is fostered by meet-ups where industry, human medicine, veterinary medicine and environmental scientists "reverse pitch" their challenges to one another and to other stakeholders. The goal is to foster engagement around a common cause, collaboration, team formation and funding partnerships. It is expected that researchers equipped with collaborative skills and training in interdisciplinary scientific team best practices will achieve greater levels of innovation, work through expected conflict that comes with diversity, and achieve sustainable, equitable, measurable health outcomes. Government, Education, and Industry (3) The world is composed of inner connected systems. Dealing with massively complex problems such as climate change and the resulting downstream impacts requires a systemic problem solving approach engaging policy makers—government and non-government organizations, educators and industry leaders. No one institution has all the brain power, authority or resources to solve these problems. In fact, where there is failure to use a systemic approach and coordinate interventions across domains, there is potential for more problems than what present at the start. At the end of the presentation there will be a few examples of One Health research collaborations that reference the science as well as tell the story of how the collaboration was initiated.

Keywords: Collaboration, implementation, Integration, interdisciplinary, translational research, one health

Surveillance for Rotavirus Infections in Children and Dogs in Thessaly, Central Greece: Results of an Ongoing Study

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In recent years, there have been increasing reports of children with clinical diarrhea that were found to be caused by rotavirus strains. These were characterized and found to be phylogenetically close to strains of canine origin. There is therefore increased interest regarding the study of possible inter-species transmission of rotaviruses, e.g. from dogs to people and vice versa. In this ongoing study, cases of diarrhoea in children are studied in relevance to ownership of domestic dogs or close contact to stray dogs. In total, 111 children (2 months to 6 year old) with reported diarrhoea in the region of Thessaly, Greece, have been studied. All patients were attended at the main hospital of the University of Thessaly with clinical signs compatible to rotavirus infection. When a diagnosis of rotavirus infection was confirmed by using, in faecal samples, a species-specific rapid immunochromatographic test (ICT) commercial kit for detection of Rotavirus antigen and Polymerase Chain Reaction method, we undertook detailed investigation of dogs associated with the children. The investigation was carried out in dogs in the families of the sick children, as well as in stray dogs in the vicinity of the residential address of the sick children. The investigation included a detailed clinical examination of the dogs, as well as collection of faecal samples directly from the rectum. In total, 38 dogs were examined and sampled. Samples from dogs were processed as detailed above for samples from children. Of samples from children, rotavirus antigen was detected in 17 (15%) by using the ICT and in 20 (18%) by using the PCR. This indicates the increased sensitivity of the latter method when used as a screening tool for detection of rotavirus. One ICT positive sample was found negative in PCR and four of the ICT negative samples were found positive in PCR. All PCR positive results were confirmed by sequencing. Of samples from dogs, rotavirus antigen was not detected in any sample. No human rotavirus antigen was detected in any dog associated with the patients. This, possibly, indicates a limited potential of inter-species transmission of rotavirus from dogs to children. This is in agreement with the rarely reported human infections with rotaviruses of canine origin, despite the frequent close physical contacts between humans and these companion animals. Perhaps, other animal species can be of greater importance and more significant zoonotic potential in the transmission of rotavirus strains to humans.

Acknowledgments: This research has been co financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

Keywords: Children, dogs, Greece, rotavirus

Comparative Sequence Analysis and Zoonotic Potential of Group A Rotavirus Strains Isolated from Animals or Children in Thessaly, Greece

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Group A Rotaviruses (GARVs) are major enteric pathogens, affecting primarily children and young individuals of a wide variety of domestic or wild animals. In humans, Rotavirus infections are the leading cause of acute dehydrating diarrhoea, with significant mortality. In dairy herds, Rotavirus infections are ubiquitous in calves younger than three weeks and are considered to be the most prevalent cause of endemic enteritis. The salient, but not sole, clinical sign of disease is diarrhoea, although occasionally sudden death of infected neonates may also occur. In recent years, the increasing data of human and animal Rotavirus strains genotyping results has provided strong evidence that, under appropriate conditions, inter-species transmission of the virus may occurs in nature. Nowadays, typical bovine or bovine-like Rotavirus strains have been repeatedly isolated from children, underlying the role of cattle as a potential source of infection. Sofar, the available sequence data of Rotavirus strains circulating in children and calves in Greece is extremely limited. In the present study, the combined results of two separate ongoing surveillance programs are presented, in order to reveal genetic relationships between human and bovine Rotavirus strains and to evaluate the zoonotic potential. In total, 42 faecal samples were collected from calves in eight dairy farms in Thessaly, Greece. In all farms, there were clinical reports of repeated cases of diarrhoea in calves, which would not respond to antibiotic administration. All samples were tested for Rotavirus by using a rapid commercial test. Then, a Rotavirus specific one-step RT-PCR test was performed. Further, faecal samples from 11 children with diarrhoea, hospitalized in the Department of Paediatrics of the main hospital of the University of Thessaly, which had been tested by the rapid commercial test and found positive for Rotavirus, were tested again by using the one-step RT-PCR test. Finally, the VP6 genes of all Rotavirus isolates were partially sequenced. Rotavirus was detected in faecal samples from two calves in two different farms. Using PCR, two different rotavirus strains were detected. PCR test also confirmed Rotavirus in all 11 samples from children, leading to detection of 11 human strains. Phylogenetic analysis of bovine strains showed high similarity with previously reported, bovine strains isolated from children with gastroenteritis. In addition, one of the human strains clustered with bovine-like human strains and strains of animal origin. This is the first report of confirmed Rotavirus infections and the first virus detection and genotyping in calves in Greece. Moreover, this is the first in tempus broad study on Rotavirus strains between cattle and children. The high rate of Rotavirus infections in calves in our research is in accordance with high prevalence of Rotavirus infections in calves worldwide. The increased isolations of non-typical virus strains in both calves and children (50% and 9% respectively) suggest that in Rotavirus epidemiology there are no clear host range restrictions, enhancing the prevalent view.

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Keywords: Children, cow, diarrhoea, rotavirus, zoonosis

Brucellosis among Patients with Febrile Illness in Four Districts of East Wollega Zone, Western Ethiopia

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Brucellosis is a zoonosis of veterinary, public health and economic significance in most developing countries. Human brucellosis is a severely debilitating disease that requires prolonged treatment with a combination of antibiotics. The disease can result in permanent and disabling sequel, and results in considerable medical expenses in addition to loss of income due to loss of working hours. The level of the problem was not well studied in Western Ethiopia. Thus, The objective of this study was to determine the prevalence of Brucellosis among patients with febrile illness attending selected health institution in four districts of East Wollega Zone, Ethiopia. A cross-sectional study was conducted among 422 patients with fever illness attending Nekemte Referral Hospital, Nekemte Health Center, Getema Health Center, Arjio Gudatu Health Center and Sire health Center from August to September, 2014. The test was conducted using Rose Bengal plate test and those who are reactive on rose Bengal test was confirmed by complement fixation test. A pre tested and structured questionnaire was used to collect data on associated risk factors that are believed to influence the spread of Brucella infection. The overall prevalence of brucellosis ranges from 1.5 to 4.6 with average 3.3 by Rose Bengal plate test and 1.1 to 2.3 with average 1.2 by complement fixation test. Participants in slaughter of animal and absence of awareness about the brucellosis disease was statistically significantly associated with the diseases. The level of seroprevalence of brucellosis in the study area was not undermined. Thus, diagnostic test for the disease should be include in the health care system and proper health information should be given on how to handle animal during slaughtering and method prevention of the disease.

Keywords: Brucellosis, febrile illness, human, Western Ethiopia

Continuous Animal Activity Monitoring System for Early Detection of Health Problems in Cattle

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To investigate the potential for utilizing continuous, real-time, wirelessly streamed activity data for early detection of sick animals in a feedlot environment. Battery powered cattle eartags were outfitted with off-the-shelf triple-axis accelerometers (measuring activity in x, y, and z planes with a threshold value of 1.5g), a radio frequency transmitter (RF) operating at 902-928 MHz, and a helical antenna, packaged in a fabricated plastic housing. Packets of collected data were transmitted to a receiving server (up to a mile range) at 5-minute intervals, 24 hours/day. Activity data were collected from two groups of about 100 receiving steers, 24x7 for 30 days (8,640 packets collected from each animal/month). A mathematical algorithm was designed that computed hourly means of activity, conducted statistical analyses to assess significant differences (by hour) between groups of animals, and implemented the SaTScan temporal scan statistic to identify animals with low rates of activity which were thus potentially sick. The ability of the algorithm to identify sick animals was compared to a high quality, daily human assessment of animal health status (Gold Standard for this study). In one model, the combined results of the two groups of steers revealed that the algorithm correctly identified 46 of 58 morbid animals with an overall sensitivity of 79.3% and specificity of 47.6%. In its current state, this algorithm is identifying animals which are candidates for health evaluation and possible treatment with fairly high sensitivity (low false negatives) based on cattle activity data alone. In addition, there were several cases in which the algorithm triggered alerts on animals 1-7 days prior to identification by human assessment. Further investigation may reveal that an algorithm such as this may offer superior performance in detection of early disease onset than human observation alone. Larger field trials are scheduled to better assess the value of continuous animal activity monitoring in the feedlot and cow-calf production environments.

Keywords: Activity monitoring, early detection, health alerts

Physiological Constraints to Milk Production in High Yielding Dairy Cows

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Milk yields and their relationships with blood and milk metabolic parameters were assessed in 59 high-yielding Holstein cows over the course of their lactation to identify metabolic constraints to daily milk production. Over an 11 month lactation period the cows, which were milked thrice daily and fed a total mix ration (TMR), had a mean daily milk yield of 35.5 l. Blood parameters monitored were haematocrit (PCV), erythrocytes (RBC), leucocytes (WCC), haemoglobin, neutrophils, lymphocytes, monocytes, eosinophils, urea, protein, creatinine, triglycerides, cholesterol, magnesium (Mg), phosphorus (P), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), asparatate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and glutamic gammatransferase (GGT). Stepwise regression indicated that blood haemoglobin concentration was most closely and positively correlated with milk yield, indicating that oxygen-carrying capacity was potentially a limit to milk production. Secondly, milk Na was negatively correlated with milk yield, and milk protein yield was negatively correlated with milk Mg, Ca and Na, demonstrating lack of homeostatic control of these elements in milk. Principal component analysis identified a primary metabonomic axis of haemaglobin and RBC concentrations at one end and blood K, Na and milk lactose at the other, which appeared related to milk production. A second axis was apparent of milk divalent cations at one end and monovalent cations at the other. Constraints to milk production in high yielding cows may exist due to limited oxygen-carrying capacity of the blood, as well as monovalent cations.

Keywords: Blood, dairy cow, haematology, milk yield, minerals

Effects of Neonatal Diarrhea on Subsequent Productive and Reproductive Performance of Dairy Cattle

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Neonatal calf diarrhea is the most frequent disease in Iranian dairy herds. The aim of this study was to evaluate the effects of neonatal calf diarrhea on subsequent productive and reproductive performance. Records of 700 dairy cows (350 with and 350 without history of diarrhea during the first month of their life), were retrieved. For each cow, birth season, birth weight (under 35, 35-40, 40-45 and more than 45), birth type (normal, mild and severe dystocia), occurrence of diseases (diarrhea, pneumonia and other diseases) during the first month of their lives, disease morbidity from the first month to the first parturition, interval from birth to first service (days), interval from birth to conception (days), time from birth to first parturition (days), parturition status (normal, abortion, mild and severe dystocia) and 305-days milk yield in the first lactation period were recorded. Cox proportional hazard model showed that cows with history of diarrhea at the first month of their lives had longer interval of birth to conception [Hazard ratio: 0.85 (95% CI: 0.73 – 0.99)] and birth to first parturition [Hazard ratio: 0.84 (95% CI: 0.72 –0.98)] than those without ($P<0.05$). Also, cows which were born in summer and those born with dystocia had shorter interval of birth to conception and birth to first parturition than those born in spring and those born normally, respectively ($P<0.05$). Evaluation of factors influencing milk yield in the first lactation period using general linear model showed that cows which were born with a low birth weight (less than 35 kg) produced significantly lower 305-days milk yield in first lactation period than those born with birth weight of 40-45 (10415 vs. 10933 liters) and more than 45 kg (10415 vs. 10921 liters). These findings showed that occurrence of neonatal calf diarrhea and some individual characteristics of heifer calves can influence the subsequent reproductive performance and first lactation milk production of dairy cows.

Keywords: Calf, diarrhea, Iran, production, reproduction

Ascorbic Acid Inclusion in Semen Extender Improves the Post-Thawed Semen Quality of Sahiwal Cattle (Bos Indicus)

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The antioxidant effects of ascorbic acid at different inclusion rates were evaluated upon semen (n = 150) from mature Sahiwal cattle bulls (Bos indicus, n = 6). The semen was diluted at 37.0 oC in Tris-citrate egg yolk extender containing different levels (0.0, 1.0, 2.0, 3.0, and 4.0 mg/mL) of ascorbic acid. Semen was cryopreserved at -196 oC in 0.5 mL French straws. Semen straws were thawed at 37.0 oC to assess the spermatozoa indices in terms of motility, viability, plasma membrane and acrosomal integrity under phase-contrast microscope. Supravital staining, hypo-osmotic swelling test and normal acrosomal reaction analysis tests were performed for viability, plasma membrane and acrosomal integrity, respectively. The data were subjected to one way ANOVA. The results revealed significant improvement (P < 0.05) in post thaw sperm quality; motility, vitality, acrosomal and plasma membrane integrity by the increasing concentrations of ascorbic acid (1.0, 2.0, 3.0, 4.0 mg/mL) in semen extender. Quality parameters were higher (P < 0.05) when 3.0 mg/mL of ascorbic acid was added in semen extender followed by 2.0 and 4.0 mg/mL. In conclusion; the addition of ascorbic acid at the rate of 3.0 mg/mL in the semen extender may improve the semen quality of Sahiwal cattle bull.

Keywords: Ascorbic acid, cryopreservation, Sahiwal bulls, spermatozoa motility

Effect of ascorbic acid addition on post-thawed Sahiwal cattle bull semen quality cryopreserved in a Tris-egg yolk based extender

Treatment Groups	Treatment Groups	Semen quality indices	Semen quality indices	Semen quality indices	Semen quality indices
	Ascorbic acid concentrations (mg/mL)	Motility	Vitality	HOST	NAR
T0	0.0	50.63 ± 3.50d	61.27 ± 6.15cd	49.97 ± 3.62c	30.77 ± 3.15c
T1	1.0	53.67 ± 4.12c	60.43 ± 3.17d	51.37 ± 3.98c	36.20 ± 3.53b
T2	2.0	63.93 ± 3.87a	65.27 ± 2.97b	58.27 ± 4.48a	40.73 ± 3.33a
T3	3.0	64.97 ± 3.27a	69.10 ± 3.76a	60.10 ± 3.35a	41.57 ± 1.99a
T4	4.0	62.73 ± 2.80ab	62.73 ± 2.80c	54.20 ± 3.68b	37.60 ± 1.57b

a-dMeans ± SD within a column lacking a common superscript differ (P < 0.05). Hypo-osmotic swelling test: HOST, Normal acrosomal ridge: NAR

The Use of Contagious Ecthyma (CE) Vaccine Strain to Treatment of Lambs with Contagious Ecthyma Disease

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Contagious ecthyma (CE) is caused by the orf virus, a member of the family Poxviridae, genus parapoxvirus. Morbidity in affected sheep flocks is approximately 100%, while mortality varies between 1% and 10%. Live attenuated vaccines have been used for protection of sheep for a long time. The aim of this study was to reveal of treatment facilities with live attenuated CE vaccine of infected lambs with CE field virus. A total 294 lambs in 4 farms which infected with CE in Istanbul were investigated in this study. The rates of clinically CE positive animals in farms were 27.7%, 30.7% 40.9% and 19.3%. The detection of CE virus in tissue samples in oral lesions were used PCR. Two groups animals in each farm, one of both was negative control and other was experiment, were treated by mock and 1ml of attenuated CE vaccine ($E(P)CK_{22}$) titer with $TCID_{50}10^{7.0}$/ml, respectively. All of animals in all groups in farms were observed clinically during two weeks after inoculation. A total 50 lambs in experimental groups in 4 farms were showed a full recovery, however mock inoculated other animals were not showed any recovery in same time, clinically. In the vaccination of lambs against CE infection, the CE vaccine ($E(P)CK_{22}$) have been used by scarification route on scarificated skin of lambs at titles $DKID_{50}$ between 10^5 and $10^{5.5}$/50-100µl for per lambs in the field. Finally, we can speculate that if CE positive animals treated with higher dose CE vaccine by subcutanously, animals can recovery in short time.

Keywords: Contagious ecthyma (orf), lamb, treatment, vaccine

A Multiplex PCR Panel for Rapid Detection of Bacterial and Viral Agents Causing Abortion in Small Ruminants

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Reproductive failure causes significant economic losses and consternation to small ruminant producers. Abortion in sheep and goats can be caused by myriad etiologies, which makes conventional laboratory testing laborious and time-consuming. Many times etiological diagnosis cannot be established due to the suboptimal quality of samples received. The objective of this study was to develop and evaluate a multiplex PCR panel (hereafter referred to as "small ruminant abortion PCR panel") to detect various bacterial and viral agents commonly implicated in sheep and goat abortion. The small ruminant abortion PCR panel consisted of two one-step multiplex real-time RT-PCRs: one for bacteria (Campylobacter spp., Chlamydophila abortus and Coxiella burnetii) and the other for viruses [border disease virus (BDV), Cache Valley virus (CVV) and caprine herpesvirus type 1 (CpHV-1)]. Once primer and probe design for each of the target agents and running conditions of multiplex assay were optimized, the panel was applied to caprine and ovine cases submitted to Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) with clinical history of abortion. A total of 23 submitted abortion cases during 2012-2015 lambing season were selected and tested using the small ruminant abortion PCR panel. The diagnostic performance of the panel was compared to results of conventional and/or molecular assays which had been routinely performed at ISUVDL for the target bacterial and viral agents. For PCR testing, tissues (including placenta) or stomach contents from aborted or stillborn fetuses were homogenized in a balanced salt solution. Both DNA and RNA were simultaneously extracted from each sample using a commercial kit (MagMAX™ Total Nucleic Acid Isolation kit, Applied Biosystems, Carlsbad, CA, USA). PCR was done using standard protocol established in the laboratory. Of the tested cases, the prevalence of each target agent was as follows: Campylobacter spp. (47.8%), Chlamydophila abortus (4.3%), Coxiella burnetii (4.3%), CpHV-1 (4.3%). In comparison to conventional Campylobacter culture on the same cases, the PCR panel detected 4 more positive cases. Similarly, the small ruminant abortion PCR panel was able to detect Chlamydophila abortus in a case that was negative by immunohistochemistry. Neither BDV nor CVV was detected in any of the tested cases. Although the current study used samples from a small set of abortion cases, the preliminary findings to date suggest the small ruminant abortion PCR panel can be a useful tool for simultaneous and rapid detection of common bacterial and viral agents involved in sheep or goat abortion cases, improving the etiological diagnosis.

Keywords: Abortion, goat, sheep, multiplex PCR

Pregnancy Toxaemia as Risk Factor for Development of Mastitis in Sheep during the Immediately Post-Partum Period

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Objective of the work was to evaluate the potential predisposing role of pregnancy toxaemia in development of mastitis in the immediately post-partum period. Ewes were allocated in subgroups A1 or A2 (with pregnancy toxaemia) or B1 or B2 (with no pregnancy toxaemia). Ewes in A1 or B1 were challenged, on the 5th day post-partum, by deposition of Mannheimia haemolytica into the teat duct, whilst ewes in A2 and B2 were controls. Clinical, bacteriological and cytological examinations were performed, as well as pathological examination of the inoculated teat (after mammilectomy) and both mammary parenchymas (after biopsy). Mastitis developed in 8/8 ewes of subgroup A1, in 1/8 ewe of subgroup A2, in 4/8 ewes in subgroup B1 and in 0/4 ewes in subgroup B2. Comparisons between subgroups revealed that isolations from A1 or A2 were greater than respective isolations from B1 or B2 (for A1 versus B1, $P < 0.08$; for A2 versus B2, $P > 0.3$). Bacteria were recovered more frequently from tissue samples from A1 than from B1 ($P = 0.008$) and from A2 than from B2 ($P = 0.058$). The characteristic lymphoid follicles at the border between teat duct and teat cistern were observed in 3/8 ewes in A1 and in 7/8 ewes in B1 ($P = 0.019$). In A1, cumulative score for macroscopic and histological pathological findings in the teat was 18 and 23, respectively; cumulative score for histopathological findings in the mammary parenchyma ipsilateral to the inoculated teat was 24, whilst scores for A2 were 2, 9 and 5, for B1 were 5, 31 and 16 ($P <= 0.05$ compared to results in A1) and for B2 were always 0 ($P > 0.05$, compared to results in A2). The results confirm that pregnancy toxaemia can act as a potential risk factor for mastitis in the immediately post-partum period. Possibly, impairment of the lymphoid follicular structures present at the border between teat duct – teat cistern could have been the cause of reduced protection of the mammary gland.

Keywords: Mannheimia haemolytica, mastitis, pregnancy toxaemia, post-partum period, risk factor, β-hydroxybuturate

Efficacy of a Novel Vaccine Active against Biofilm Formation by Staphylococci in Protecting Ewes from Staphylococcal Mastitis

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Ovine mastitis is a significant welfare and financial issue in dairy sheep flocks. In milking ewes, the main causative agent of clinical mastitis is Staphylococcus aureus and of subclinical mastitis coagulase-negative staphylococci. Biofilm formation is a virulence factor of these bacteria, which contributes to their increased pathogenicity. Objective of the present study was to evaluate under field conditions the efficacy of a novel vaccine, which induces antibodies against the poly-N-acetyl β-1,6 glucosamine exopolysaccharide (PNAG), the main component of the extracellular biofilm matrix of Staphylococcus, and acts in preventing slime production and consequently biofilm formation by these organisms. Consequently, vaccination leads to avoidance of biofilm formation by these organisms. The trial was carried out in a dairy sheep farm in Greece. In total, 55 ewes were enrolled in the study, of which 30 were vaccinated (group V) and 25 were unvaccinated controls (group C). The product used is licenced in the European Union (Vimco®) and contains an inactivated slime-producing, biofilm forming S. aureus strain. Ewes were vaccinated initially approximately six to five weeks before the expected lambing date, which was followed by a repeat dose three weeks later. Ewes were examined clinically initially during the first 15 days after lambing and then at monthly intervals up to 75th day of the lactation period. At each examination, milk samples were also collected for bacteriological and cytological examination, which were performed by using established techniques. Staphylococcal mastitis was defined as the simultaneous isolation of staphylococci (S. aureus or coagulase-negative staphylococci) from a milk sample and concurrently increased cell content in the sample. Incidence risks were calculated for the total period up to the 75th day of the lactation period, as well as for the period 1st to 15th, 16th to 45th and 46th to 75th day after lambing. No cases of clinical mastitis were recorded in any ewe into the study. Further, 3 cases of subclinical staphylococcal mastitis (1 caused by S. aureus and 2 by coagulase-negative staphylococcal strains) were identified in group V and 9 cases of subclinical staphylococcal mastitis (1 caused by S. aureus and 8 by coagulase-negative staphylococcal strains) in group C. There was clear evidence that, for the period from lambing to the 15th day after lambing, incidence risk (IR) was smaller in group V ewes (IR=0.03) than in group C ewes (IR=0.27), which was statistically significant (P=0.007). For the periods 16th to 45th and 46th to 75th day after lambing, incidence risk (IR) was smaller in group V ewes (IR= 0.06 and 0.07, respectively) than in group C ewes (IR=0.15 and 0.22, respectively), although the differences were not significant (P>0.18). For the entire trial period, calculated incidence risk was 0.07 for group V ewes and 0.24 for group C ewes (P=0.057). There is little documentation regarding the field efficacy of this licenced vaccine against mastitis in dairy ewes. The results confirm the protective effect of the vaccine against staphylococcal mastitis. The effect was stronger during the initial stage of the lactation period, when incidence risk of mastitis is increased, due to the post-partum reduced immunity often observed in ewes.

Keywords: Biofilm, coagulase-negative staphylococci, mastitis, sheep, subclinical mastitis, staphylococcus aureus

Pathological and Molecular Investigations of Canine Herpesvirus-1 (CaHV-1) Infection Associated with Respiratory Disease and Acute Death in Dogs

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Canid herpes virus-1 (CaHV-1) has been defined as a member of canine infectious respiratory disease complex (CIRDC), causing respiratory distress such as nasal discharge, coughing and probably pneumonia in adult dogs. Outcome of CaHV-1 infection could be either self-limited or occasionally fatal. Based on our knowledge, no information of CaHV-1 circulation in Thailand has been reported then resulted in the lack of preventive strategies. This research aimed to determine the CaHV-1 prevalence in alive respiratory infected dogs and to describe the pathological and molecular investigations in naturally moribund dogs suffering from CaHV-1 infection. One hundred dogs suffering from respiratory distress were included. Nasal and oropharyngeal swabs were collected from each dog. Among them, 23 pleural effusions were aspirated in which were diagnosed by radiographs. All samples were kept at -80^{o}C until used for molecular assay. Moreover, six 3-month-old puppies, naturally moribund, were submitted to Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University for routine necropsy. Macroscopic and microscopic findings were systemically recorded. Various fresh tissues were separately collected for a panel of CIRDC-associated viral detection by PCR assay including CaHV, canine influenza virus (CIV), canine parainfluenza virus (CPIV), canine distemper virus (CDV), canine respiratory coronavirus (CRCoV), and canine adenovirus type 2 (CAV-2). Subsequently, selected CaHV-1 positive samples were further sequenced and phylogenetically analyzed. Bacterial culture and identification were performed from tracheal discharge of necropsied dogs. The CaHV was totally detected in 32 dogs, in which comprised of 17 dogs were positive from both sampling sites while 9 and 6 dogs were positive from nose and oropharynx, respectively. Additionally, the CaHV-1 was consistently detected in 6 plural samples. The co-infection with other CIRDC viruses was found in 22 dogs, accounted for 68.75%. All necropsied dogs were positive with CaHV only without bacterial infection. Prominently antemortem signs of necropsied puppies showed severe bronchopneumonia and inappetence. Postmortem examination revealed severe multifocal necrotizing nonsuppurative pneumonia with pulmonary congestion. Severe multifocal necrotizing hepatitis with sinusoidal congestion was markedly seen. Severe acute tubular necrosis with nonsuppurative interstitial nephritis and massive congestion at both cortex and serosa were described. The sequencing results from nasal and oropharyngeal samples were 100% homologous to canid herpervirus 1 glycoprotein B gene. Phylogenetic analysis revealed CaHV circulating in Thailand was CaHV-1, which genetically closed to phocid and bovine herpesviruses -1 (Figure 1.). Canine herpesvirus has been recognized as an important pathogen associated with respiratory illness dog worldwide. Importantly, dogs that were infected and recovered would be a reservoir for viral shedding. Abortion, still birth and neonatal death usually occurred in infected bitches. In this report, the CaHV-1 mostly co-infected with other CIRDC-viruses, meaning that it was either primary or secondary infections. Phylogenetic analysis revealed that CaHV circulating in Thailand was type 1, which is belonging to *Alphaherpesviridae* and genetically relating to Phocid and Bovine herpes viruses 1. All dead puppies were neither CIRDC-viruses nor bacteria were detected, confirming that CaHV-1 infection is a primary cause of the disease with consistently pathologic findings. Taken together, this is a first report of CaHV-1 incidence with molecular characterization and pathological investigation in dogs in Thailand. Based on the lack of prevention by vaccination, veterinarians should take action seriously when CaHV-1 infection is suspected. These findings also suggest that raising awareness of CaHV-1 infection in Thailand should be taken into account.

Keywords: Canid herpesvirus 1, CaHV-1, dog, pathology, phylogenetic analysis, respiratory

Microbiological and Histopathological Study of Canine Pyoderma in a Population of Iranian Domestic Dogs (2010-2014)

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We sought to investigate the clinical, microbiological and hispathological findings of canine pyoderma in a population of Iranian domestic dogs. 69 dogs (23.83% of all dermatological conditions presented to the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran from September 2010 to March 2014) were diagnosed to have pyoderma based on clinical, microbiological and histopathological features. Biopsies were obtained from 49 lesions and submitted for aerobic, anaerobic and fungal culture, and histopathological evaluation. Most frequently isolated bacterial species were Staphylococcous epidermidis (22 isolates), Staphylococcous aureus (7 isolates), Proteous (5 isolates), Staphylococcous saprophyticus, Streptococcous faecalis and Escherichia coli (each 2 isolates). Only one isolate of Streptococcous faecalis, Pseudomons and Streptococcous dysgalactiae was evidenced. Resistance was most commonly seen against Penicillin (82.35%), Amoxicillin (82.05%) and Ampicillin in Gram positive bacteria and Amoxicillin (80%), Oxytetracycline (80%) and Nalidixic Acid (80%) in Gram negative bacteria. Using ERIC-PCR genomic fingerprinting assay, 16 different patterns were recognized among 22 isolates of Staphylococcus epidermidis. Among the 7 isolates of Staphylococcus aureus, 3 different patterns were observed. Two strains of Staphylococcus saprophiticus had 2 different patterns. The 2 strains of Streptococcus faecalis did not produce any bands. As for the gram negative bacteria, one strain of E.coli did not produce any bands, while the other E.coli's pattern was completely compatible with that of Proteus. All dogs with detectable histopathologic lesions (n = 35) showed typical macroscopic changes. Histopathologic examination of all tissue samples identified acute superficial dermatitis, deep pyoderma, epithelial necrosis and formation of ulcer and crust. Hyperkeratosis, hydrophic degeneration of keratinocytes, spongiosis, scar tissue, sebaceous gland adenitis, hemorrhagic dermatitis and atrophy of hair follicles were also observed.

Keywords: Bacterial culture, canine pyoderma, ERIC-PCR, histopathology

Is the Heart Compensated in Anatolian Shepherd Dogs with Asymptomatic Degenerative Mitral Valvedisease, or not?

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Each of systolic function indices is unreliable in the setting of moderate-to-severe mitral regurgitation (MR) due to degenerative mitral valvedisease (DMVD). A multi-modality approach for assessment of systolic LV function in MR is suggested.Cardiologists often remark that dogs with moderate-to-severe MR have preserved LV function withhyperdynamic chambers, but it is difficult to quantify how high that value should achieve in a given case.On the other hands, wall stress is thought to contribute to progressive LV systolic failure. The goal of this study was to evaluate the systolic function and compansation of the heart in Anatolian shepherd dogs (ASH) with Asymptomatic DMVD. The severity of heart disease was classified using the CHIEF system based on radiographic heart and echogardiographic LA size. The control group consisted of 35 healthy ASH. In 38 ASH with DMVD (20 B1 and 18 B2), the clinical and cardiological examinations including systolic function (FS, EF, ESV-I, EPSS and CIandcardiac remodelling indices (LA/Ao, EDV-I, wall stress and NT-proBNP) were assessed. Increase in EF and FS were statistically higher in B2 group (73,8±1,08 and 46,8±0,39, respectively) compared with B1 Group (69,3±1,34 and 42,7±1,14, respectively) and normal control Group (66,2±0,70 and 35,8±0,54, respectively). Increase in ESV-I and EDV-I was statisticallydifferent in B2 group compared with B1 group and Control group. Decrease in CI was only statisticallydifferent in B2 group when it was compared with B1 group and Control group. There was no statistically difference in EPSS among the groups. The median LA/Ao was statisticallyhigherin B2 dogs compared with B1 dogs and control dogs (1,80, 1,44 and 1,20 respectively). The median plasma NT- proBNP concentration was statisticallyhigher in B2 dogs (2985±218) compared with B1 (1588±212) and control group of dogs (759±47). The wall stress values did not show any statisticallydifference among the groups (0,27±0,01 for B2 dogs, 0,28±0,008 for B1 dogs and 0,27±0,007 for control dogs. Myocardial systolic function assessed byESV-I was not correlated to LA/Ao, FS, EF, EPSS and CI. Cardiac remodelling assessed by LA/Ao was positively correlated to ECG findings, VHS, NT- proBNP. Cardiac overload assessed by NT- proBNP was positively correlated to LA/Ao, VHS, EDV-I. The significant higher FS, EF, ESV-I, EDV-I and LA/Ao, and increased plasma NT-proBNP concentration compared to control group were the indication of preserved systolic function,hyperdynamic phase of disease and cardiac remodeling. However, normal wall stress may indicate the compensation of the heart.Therefore,in ASH withasymptomatic DMVD, serial Echo-Doppler examinations, focusing on wall stress,can be recommended in order to identity and track these alterations over time and detect ongoing worsening of the disase.

Keywords: Anatolian shepherd dog, asymptomatic degenerative mitral valve disease, echocardiology

Determination of Renal Blood Flow with Doppler Ultrasound, Prevalence of Hypertension and Acid-Base Level in Dogs with Chronic Renal Failure

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Chronic renal failure (CRF) is an important cause of morbidity and mortality in dogs. To describe the renal doppler measurements, blood hypertension and acid-base levels in dogs with chronic renal failure. Twenty-six dogs with previously diagnosed chronic renal failure compared with twenty control dogs. A CBC, biochemical profile, urinalysis, blood gases, blood pressure determined and changes in renal blood flow were measured by renal doppler ultrasonography. Statistical comparisons (2-tailed t-test) were reported as mean-standard deviation. The CRF dogs had significantly higher serum blood urea nitrogen, creatinine and phosphorus concentration, significantly lower PCV and urine specific gravity than control dogs. It is determined that there is a high correlation between creatinine, renal RI and PI values. There were significant decreases in blood pH and HCO3 in our study. Indirect blood pressure measurements were slightly increased in CRF dogs. Renal doppler is a helpful tool in diagnosis of CRF and identification of acidosis and hypertension may help in developing treatments that slow the rate of progression of chronic renal failure.

Keywords: Blood gases, hypertension, renal RI, renal PI

Epidemiology of Brucellosis in Small and Large Ruminants in Relation Various Risk Factors in Punjab, Pakistan

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Brucellosis is a diseases of economic as well as zoonotic importance, which adversely affects almost all livestock species except cats. Aim of the present project was to carry out an epidemiological investigation of seroprevalence of brucellosis in small and large ruminants in relation to various risk factors like species, age, sex, bodyweight, and parity, and to find out whether these risk factors have positive correlation with the diseases prevalence or not. For this purpose, 2275 serum samples from ruminants (buffaloes, cattle, goats and sheep) were collected from December, 2010-12, from various livestock farms located in Punjab, Pakistan and stored at -20°C till analysis. Information regarding age, sex, body weight and parity was also collected. Initial screening was carried out with rose Bengal plate test (RBPT) and positive samples were subjected to indirect and competitive enzyme linked immunosorbant assay for further confirmation. Data thus collected were subjected to binary logistic regression analysis through a statistical software MINITAB 16.0 version. Seroprevalence was highest (8.01%) in buffaloes followed by cattle (6.94%), goat (6.73%) and sheep (1.91%) through c-ELISA. Similar trend was observed through i-ELISA also. The difference in prevalence among these species was statistically significant. In large ruminants (Buffaloes and cattle), in relation to various age groups highest prevalence was observed in sexually mature animals similar results were obtained in small ruminants indication that age has a positive correlation with the prevalence of the disease. Sex has not positive correlation with brucellosis prevalence in large ruminants But in small ruminants prevalence was significantly higher in males as compared to the females as observed in this study. Considering the body weight as a risk factor, seroprevalence was significantly ($P<0.05$) higher in animals having more than 600 Kg B.wt as compared to two other groups in cattle. But in all other species (Buffalo, goat and sheep) this factor did not affect the prevalence of disease in different groups. Similarly in relation to parity number, prevalence of brucellosis significantly differed in various groups of goat but in cattle, buffalo and sheep this risk factor have no correlation with the prevalence of the disease. It was concluded from this study that brucellosis is prevalent in small and large ruminants which are most common sources of milk and meat production in Pakistan. So chances of zoonosis are there for the human population, who are directly in contact with animals or consume the milk of these animals. But when we analyzed the percent prevalence in relation to various risk factors they were not consistent. The correlation of risk factor in one specie was positive but in others it was not. To control the diseases prevalence frequent movement of the animals without quarantine in and outside the herds must be controlled to prevent the economic loss as well as zoonotic threat.

Keywords: Brucellosis, ELISA, ruminants, serpravalence

Caprine Brucellosis: Prevalence and its Relationship with Various Risk Factors

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Goats are an important source of milk and meat in livestock raising areas of Pakistan. Moreover, their products like milk, meat and skin are important export commodities significantly contribute to the national economy. Various Infectious and contagious diseases adversely affect the production of milk and meat. Among these, brucellosis is a disease of major economic importance. Brucellosis in goats is caused by B. meletensis and is a worldwide trouble. B. melitensis is also a most common infection in human. The purpose of this study was to find out the prevalence of brucellosis at various farms and its relationship with various reproductive disorders. For this study a total 374 serum samples were collected from the animals maintained at the various Livestocks farms by following standard procedures and stored at -20ºC for till analysis. Initial screening was carried out by Rose Bengal Plate test (RBPT) as described by the Brown (1974) and then confirmation was carried out by Serum Agglutination test (SAT) following the procedure described by Hussain (2002). Data analysis was carried out by Chi-square test through MINITAB 16.0. Out of 474 serum samples of goats obtained from different government and private livestock farms of Punjab 18.98% were RBPT positive and the highest seroprevalence was recorded in Teddy goats (39.23%) followed by Beetal (15.38%) and crossbred animals (1.57%). When these samples were further confirmed by SAT, 9.89% animals showed sero-positivity with highest prevalence in Teddy (18.46%), followed by Beetal (9.40%) and crossbred (1.57%). Chi-square analysis revealed that the difference in seropositivity of brucellosis among different breeds of goats were significant by both the tests. On the basis of RBPT the prevalence of brucellosis in rams and does was 35 and 16.7%, respectively and it was statistically significant. Prevalence was also higher in males on the basis of SAT but it was not statistically significant. In various age groups a statistical significant difference was observed in both tests like RBPT and SAT. In goats with history of abortion, 16.12% were found seropositive and 12.75% were seropositive without history of abortion. Statistically, a non-significant difference was found associated with history of abortion or not. Brucellosis is prevalent in various goat farms in Punjab, Pakistan. Prevalence of caprine brucellosis is affected by breed and age, but not with sex and history of abortion.

Keywords: Brucellosis, goats, prevalence, risk factors

Seroprevalence of Bovine Viral Diarrhea Virus Infection in Cattle in Central State of Malaysia

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Bovine viral diarrhea virus (BVDV) infection which has been reported in many parts of the world with significant economic loss due to its effect on reproductive performance and deaths, has not been studied in Malaysia before. The aims of this study were to investigate seroprevalence of BVDV infection in cattle in the state of Selangor, Malaysia and its association with possible risk factors. A total of 407 blood samples were collected from five selected farms within Selangor. During sampling, animals were identified for their breed, age, lactation and pregnancy status. Serum or plasma samples, separated from the collected blood samples via centrifuge, were tested for presence of antibody against BVDV using a direct ELISA (PrioCHECK®BVDV antibody) following the protocol given by the manufacturer. Results demonstrated an overall 33.2% (135/407) prevalence of BVDV antibody; with all the farms tested were positive except one (Table 1). Individual farm level prevalence reached 75.9% (66/87) which was significantly higher than the other four farms which had a prevalence of 26.0% (66/254), 13.3% (2/15), 2.8% (1/36) and 0% (0/15). Animals grouped according to breed, age, lactation and pregnancy status showed significant variation in BVDV prevalence. Significantly higher number of adults with age > 9 months (36.7%) than young calves (15.2%), pregnant (42.9%) than non-pregnant (31.1%) and more lactating (51.1%) than non-lactating (25.8%) cows were affected. According to breed, dairy Friesian-Sahiwal and Jersey crosses were the most affected while beef cattle breeds such as the local Kedah-Kelantan cross were the least affected. In conclusion, the study revealed that BVDV infection is highly prevalent in cattle in Selangor that varies with individual farms, breed, age, lactation, and pregnancy status of animals. Further studies are required in order to determine BVDV prevalence at national level, virus genotype, impact of the disease and to identify possible future control strategies to be implemented.

Keywords: BVDV, cattle, direct ELISA, risk factors, seroprevalence

Table 1. Prevalence of BVDV infection in cattle in the State of Selangor

Farm	Number of animals tested	Number of animals tested positive	BVDV prevalence
A	87	66	75.9%
B	36	1	2.8%
C	15	2	13.3%
D	15	0	0%
E	254	66	26.0%
Total	407	135	33.2%

Aquatic Veterinary Educational Initiatives to Ensure a Well-Trained Workforce to Serve Client & Other Stakeholder Needs

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With the growth of aquaculture and the increasing impact of diseases there is a need to ensure an adequate aquatic veterinary workforce of well-trained individuals. A wide range of knowledge, skills and education/experience (KSAs) is required to provide a variety of veterinary or veterinary-related services. These include veterinarians needed in private practice, and those employed by governmental agencies, diagnostic laboratories and other aquatic animal health-related institutions and companies. Given the very diverse needs, roles, expertize and the responsibilities required of this workforce, a concerted and collaborative effort of academic institutions, government agencies, and veterinary and non-veterinary organizations is needed to ensure this workforce has the KSAs to provide these services. Fortunately, several programs are in place and are continually being refined to help ensure training and credentialing of sufficient numbers of experienced individuals, both at national and international levels. Although the utilization of private aquatic veterinary practitioners by aquatic animal owners and industries over the last several decades has been slow (primarily because of reluctance of producers to utilize and pay for veterinary services), the availability of education, training and credentialing programs for aquatic veterinarians has increased. These include courses in both veterinary and non-veterinary degree-earning curricula, and extracurricular continuing education and professional development (CEPD) programs offered by academic institutions, and veterinary and non-veterinary organizations. While it is difficult to determine the number of para-veterinary professionals with experience in aquatic veterinary-related fields, the number of veterinarians registered in an online Directory of Aquatic Veterinarians (at www.AquaVetMed.info) as increased progressively since 2006 to more than 3,000. However, given that there are now at least 19 aquatic veterinary or para-veterinary organizations which cater to aquatic veterinary medicine, the number of veterinarians actually engaged in this discipline is probably closer to 10-15,000. Preliminary results of an ongoing survey of all 30 veterinary schools currently accredited by the AVMA Council on Education in N. America and elsewhere, and a 2014 survey of European veterinary schools by the Federation of Veterinarians of Europe, indicate a large number of required and elective courses covering a variety of core aquatic-oriented subjects are currently in veterinary school curricula. Some courses have been offered for more than 20 years. While most of these courses provide sufficient training necessary to practice aquatic veterinary medicine after graduation, no veterinary school currently adequately covers all nine of the subject matters areas considered essential for aquatic veterinary practice. To address this, a new Certified Aquatic Veterinarian (CertAqV) Program has been initiated by the World Aquatic Veterinary Medical Association to recognize and credential KSAs acquired from a combination of academic, CEPD, or self-study sources at the level expected for a graduate of a University degree that allows a person to practice veterinary medicine (i.e. “Day-1” competency). Importantly, the subject matter required to earn CertAqV recognition covers requirements that compliment and support several initiatives such as those in N. America, Europe, Australia/New Zealand and elsewhere to ensure veterinary education meets contemporary society needs. Furthermore the program offers a template of what might be covered in veterinary curricula and addresses the needs of standard setting bodies such as the World Organization for Animal Health (OIE). In addition the program also compliments National Veterinary Accreditation programs designed to authorize and allow private practitioners to perform aquatic veterinary work on behalf of the government. At the same time it serves as an introduction for existing and developing aquatic veterinary Board Certification programs that recognizes advance KSAs above those of the average practicing veterinarian.

Keywords: Aquatic veterinary, certification, curriculum, day-1 competency, education, workforce

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AQUAVET® has been teaching aquatic animal medicine to veterinary students and graduate veterinarians from the USA and around the world since 1977. In the early 70s Dr. Don Abt at the University of Pennsylvania discussed the apparent need for veterinarians in aquatics including aquaculture and the care of aquatic mammals, reptiles, birds, amphibians and fish in practice and in the aquarium industry. The result was a new program to have experts in the field come share their knowledge with veterinary students and veterinarians. There are currently over 60 experts teaching the courses each year. Cornell was invited to join in the venture, which then became a joint program from both universities for about 40 years. It had been based at the University of Pennsylvania until this year. It is now based at Cornell University. AQUAVET®I is an intensive month long course on husbandry, conservation and medicine starting with water quality, then invertebrates, then fish (including fresh water, cold water, warm water, marine), common aquatic birds and reptiles, amphibians and finally marine mammals. It is meant to prepare students for further studies in fields such as aquaculture, private practice, aquarium medicine, research and conservation. AQUAVET®II is a 2 week course focusing on the diseases and pathology of economically important invertebrates and fish in aquaculture and in research. These first two courses are currently presented on the campus of Roger Williams University in Bristol, RI. AQUAVET®III is for a very small group of students to train skills necessary for the aquarium industry. This is a 5-week course including 2 weeks at the Georgia Aquarium, one of the largest in the world, which houses multiple species including whale sharks, manta rays, dolphins, beluga whales, penguins, etc. The course continues at the University of Georgia, where the students have hands-on experience learning to do anaesthesia, rigid endoscopy and surgery of fish and reptiles. The final 2 weeks focus on dolphin medicine and diagnostics. It takes place at Dolphinaris facilities in Cancun and Cozumel, Mexico.

Keywords: AQUAVET, aquatic animal medicine, continuing education, Cornell University, University of Pennsylvania, veterinary education

Koi Ulcer Disease – Lesion Assessment and Treatment Strategies

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Cutaneous ulcers in koi are one of the most common complaints for which the pet fish practitioner is called upon. Along with “mouth rot’ and “fin rot”, these form the complex described as koi ulcer disease. Most cases have been cultured out as being caused by Aeromonas hydrophila or sobria, although many other predisposing factors, such as trematode parasitism and poor water quality have been incriminated also. Treatment protocols will vary depending on whether the lesions are of the trunk, the mouth or the fins or in combination. It will also be dependent on the extent of the lesion(s), the stage of degeneration/regeneration and the treatment options available. It is important to determine the stage in the development and healing as local debridement will be useful in the degenerative phase, but counterproductive during healing. This would limit the use of topical treatment to only the first few days after ulceration has begun. Determination of stressors in the aquasystem leading up to the outbreak, such as parasitism and poor water quality, should be identified where present and corrected. Systemic antibiotics are often indicated, especially with multiple and/or extensive lesions. Additional considerations are maintenance of ideal water quality during the treatment period, especially if treating in a quarantine system and stabilized water temperature at 75 °F (24°C), the preferred optimum temperature for koi.

Keywords: Koi ulcer disease, lesion assessment, treatment strategies

Serotype Distribution of *Salmonella* Isolates from Turkey Ground Meat and Meat Parts

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The aim of the study was to find out the serotype distribution of 169 *Salmonella* isolates recovered from 112 *Salmonella* positive ground turkey (115 colonies) and 52 turkey meat parts (54 colonies). Conventional cultivation technique was used for the isolation of *Salmonella* spp. from turkey ground meat and meat parts. All colonies were confirmed with PCR by the detection of oriC gene. Serotyping of the *Salmonella* isolates was performed with lam agglutination and serum neutralization tests due to the scheme of Kaufmann-White. According to the results, 15 different serotypes were identified among 169 *Salmonella* isolates. Out of 15 *Salmonella*serotypes: *S*. Corvallis, *S*. Kentucky, *S*. Bredeney, *S*. Virchow, *S*. Saintpaul and *S*. Agona were identified as the predominant serovars at the rates of 27 %, 13 %, 12 %, 12 %, 11 %, and 10 %, respectively. Other serotypes were below 6 % of the total isolates. All *S*. Kentucky and *S*. Virchow; most of the *S*. Corvallis (39/46) and *S*. Heidelberg (9/9) serotypes were recovered from ground turkey. Likewise *S*. Heidelberg, *S*. Stanleyville, *S*. Montevideo, *S*. *subsp. I*, *S*. *group C*, and *S*. Newport were only recovered from ground turkey meat samples. As the predominant serotype, 39 of 46 isolates of *S*. Corvallis were recovered from ground turkey meat. However other two major serotypes, 90 % of *S*. Bredeney and 67 % of *S*. Saintpaul isolates were recovered from turkey meat parts. From the seasonal point of view, 39 (23,1 %), 26 (15,4 %), 53 (31,4 %), and 51 (30,2 %)*Salmonella* were isolated from turkey samples that were collected during the winter, spring, summer and autumn, respectively. Most of the isolates were determined in warm months. In the winter and spring 16 different serotypes, in the summer and autumn 19 different serotypes were recovered. As a predominant serotype *S*. Corvallis was detected in the spring, summer, autumn and second after *S*. Bredeney in the winter months. Also, 86 % of *S*. Kentucky, as the second major serotype of the study, was recovered in spring, summer and autumn. Ground turkey meat samples were collected from nine different companies and no correlation was observed between producing companies and serotype profiles. Unlikely, meat part samples were collected from three different supermarkets (supermarkets A, B and C) which were preparing the meat parts in their own butchery stores. Based on the results of this study, a relation is observed between supermarkets and serotype distribution of the samples. *S*. Bredeney and *S*. Saintpaul were the mostly recovered serotypes from turkey meat parts. In the study, 78 % of *S*. Bredeney and 58 % of *S*. Saintpaul serotypes were detected from meat parts of supermarket A. The results indicate that turkey ground meat and meat parts were contaminated with quite distinct*Salmonella* serotypes. This is the first study reporting *Salmonella* serotype distribution in turkey meat and *S*. Corvallis as predominant serotype in poultry meat in Turkey.

Keywords: Turkey meat, salmonella, serotype, salmonella corvallis, seasonal distribution

Investigation of Biotoxin Existence at Bivalve Molluscs

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The marine environment may be seriously affected by contamination due to the massive proliferation of toxic phytoplanktonic species for example toxic algae or 'algal blooms', which appear at certain times of the year under the influence of various environmental factors. This phenomenon is commonly known as a 'red tide'. These toxic algal blooms cause important socioeconomic damage to those regions that depend on aquaculture or fisheries industry, owing to their environmental and human health impacts. Seafood products are important both nutritionally and economically. Seafood poisoning from marine toxins is an underrecognized hazard for travelers, particularly in the tropics and subtropics. Furthermore, the risk is increasing because of factors such as climate change, coral reef damage, and spread of toxic algal blooms. These toxins cause important cause important human contamination and their impact has increased considerably over the last few years. For this reason, strict control of these toxins is necessary to prevent serious damage to health. These phytoplanktonic microorganisms are essential food for filter-feeding bivalve shellfish as well as other types of marine seafood. These accumulate in shellfish tissues and are harmful to man when contaminated bivalves are eaten. Other marine animals, such as gastropods, crustaceans and fish, can also accumulate the toxins through the food-chain and pose a threat to seafood consumers. Once contaminated, some shellfish depurate the toxins relatively quickly, whereas others, such as scallops, can retain the toxins for months and even years, particularly in the digestive gland and the gonad. The nutritional value and benefits to health attributed to seafood are well known. Apart from the high quality protein, essential ammo acids, vitamin and mineral content, epidemiological studies indicate lower risk for coronary heart disease, hypertension and cancer among populations eating seafood. Lipophilic marine biotoxins can be accumulated in different molluscan shellfish presenting a health risk to humans if contaminated shellfish are consumed. To protect public health, monitoring programmes for marine biotoxins have been estabilished in many countries for detecting the presence of these compounds in shellfish tissues. Vomiting and diarrhea are the typical gastrointestinal disorders in human symptoms. Chronic exposure may cause tumour promotion, especially in the digestive system. In this study, 975 bivalve mollusc samples sent by Province/District Food, Agriculture and Livestock Directorate, and 13 samples received through special request were used as material. The European Union Reference Laboratory for marine biotoxins method was used. The metod is based on the extraction of okadaic acid (OA-C), dinophysistoxin (DTX-1 and DTX-2), azaspiracid (AZA-1, AZA-2 and AZA-3), yessotoxin (YTX and hYTX) group toxins with %100 methanol from homogenized tissue. Extract are then filtered and directly analysed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) in order to investigate the presence OA-C, DTX-1 DTX-2, AZA-1, AZA-2, AZA-3, YTX, and hYTX. The limits of quantification for all toxin groups studied are expressed in µg/kg of whole tissue: OA-C (45.9), DTX-1 (57.5), DTX-2 (45.4), AZA-1 (3.0), AZA-2 (2.3), AZA-3 (3.1), YTX (102.9) and hYTX (94.7). In this study, from lipophilic biotoxins, OA-C, DTX-1 DTX-2, AZA-1, AZA-2, AZA-3, YTX, and hYTX analyses have been performed in 988 bivalve mollusc samples in 2014. As a result of the analyses no lipophilic biotoxin has been detected above limits specified at the Special Hygiene Regulation for Animal Foods. This situation reveals the consumption of bivalve molluscs does not threaten public health in terms of lipophilic biotoxins.

Keywords: Bivalve molluscs, LC-MS/MS technique, lipophilic biotoxins

Presence of Atrazine in the Biological Samples of Cattle and Its Consequence Adversity in Human Health

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-teriazine) is the most widely used S-triazine. Based on reports indicating increased mammary gland tumors in female laboratory animals triazines are considered as possible human carcinogens (group C). Because of the presence of different interferences in the samples, sometimes it is necessary to develop methods, having more selectivity, sensitivity and detectability to overcome co-extraction of interferences in the final solution. Cattle can accumulate herbicides in their body through ingestion plants infested with these compounds and one of the ways, by which, human beings are exposed to atrazine is through cattle meat and milk consumption. This study was aimed to monitor presence of atrazine in the cattle biological samples, using molecular imprinted solid phase extraction followed by high performance liquid chromatography. Sampling procedure: blood samples were taken from the jugular vein of 45 Holstein cows in 3 commercial dairy farms in Khuzestan province, Iran. Urine samples were also taken from the cows. All samples were transferred to the Clinical Laboratory of Veterinary Department, Islamic Azad University, Shooshtar Branch, Khuzestan, Iran. Serum samples were harvested and kept at -20˚c. Blood and urine samples were also taken from 5 normal cows with no corn silage in their ration. MISPE procedure: The SupelMIP Triazine 10 (Sigma-Aldrich Company, Germany) was used as a SPE media in this study. The mean ± SD concentrations of atrazine in serum and urine samples of the study group (0.739 ± 0.567 ppm and(1.389 ± 0.633 ppm, respectively) were higher ($P < 0.05$) than the concentrations in serum and urine samples of the control group (0.002 ± 0.005 ppm and 0.012 ± 0.026 ppm, respectively). Atrazine in the feed ingredients ingested by cattle could be transferred into the biological samples and consequently can be considered as a potential hazard for the public health.

Keywords: Atrazine, cattle, high performance liquid chromatography, molecular imprinted polymers

Using Multidisciplinary ThinkSpace-based Cases to Teach Food Safety Concepts in Non-Food Safety Courses

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Problems involving food safety and sustainable agriculture often require the perspectives of multiple disciplines to be solved effectively. Currently, graduates of programs in agriculture-related disciplines struggle to integrate information when attempting to address problems that span relevant disciplines or sub-areas of one discipline. We sought to address this problem by providing faculty members with a computer-based learning tool and relevant multidisciplinary cases to use for teaching food safety-related problem solving in their courses. Rather than seeking to design or implement stand-alone food safety courses, we sought to determine whether or not simply introducing several computer-based food safety cases into existing courses would improve students' knowledge of food safety. An on-line learning tool called ThinkSpace was used to make learning cases available to student participants in 4 courses at 3 veterinary colleges in the Eastern (College A) and Midwest (Colleges B and C) regions of the United States. The cases were introduced into 2 Parasitology courses (institutions A and C), a Regulatory Veterinary Medicine course (Institution B), and a Large Animal Medicine block (clinical teaching; Institution A). In the Parasitology course at Institution A, students (N=84) received a pretest regarding their knowledge of food safety, participated in the course utilizing 4 food safety cases, and then received a food safety-related posttest. In the other 3 courses, one or more groups of students (control) enrolled in the courses one or 2 years prior to implementation of the food safety cases and completed a posttest measuring their knowledge of food safety; this score provided a baseline regarding students' knowledge of food safety upon course completion for those courses. In one or more subsequent years (depending on the location), the food safety cases were used to supplement the regular course content, and the same posttest was again administered (intervention groups). In those 3 courses, a total of 729 students participated in the study, with 460 participating in control groups and 269 in intervention groups. The intervention consisted of 2-4 cases, depending on the course. In those 3 courses, scores for students in the baseline group(s) were compared with scores for students in the intervention group(s) using either an ANOVA or an independent samples t-test, depending on the number of groups being compared. Pretest scores were compared with posttest scores using a paired samples t-test. Effect sizes (Cohen's d for t-tests and η2 for ANOVA) were calculated for all comparisons using SPSS v. 23. Students and faculty were invited to respond to a survey regarding their impression of the ThinkSpace cases. Posttest scores for the Parasitology course at institution A were significantly higher than pretest scores ($p < .0005$; $d = 4.56$). Student posttest scores in the intervention groups were significantly higher than student scores in the baseline groups in the Regulatory Veterinary Medicine ($p < .0005$; $d = 0.59$) and Large Animal Medicine ($p < .0005$; $\eta 2 = 0.40$) courses. Scores were not significantly different between groups in the Parasitology course at Institution C ($p = .451$). Faculty and students somewhat agreed that the computer-based cases were beneficial for learning, but technical challenges limited overall satisfaction. Positive learning outcomes in 3 of 4 settings where the food safety cases were implemented suggest that implementing food safety-oriented cases in the context of a regularly offered course can improve students' understanding of food safety. Differences in implementation strategies likely produced the difference in learning outcomes between the Parasitology course at Institution C and the other courses.

Keywords: Case-based learning, computer-based learning, food safety, ThinkSpace

Water Injection: a New Technique for Brain Specimen Collection from Dogs as Definitive Diagnosis of Rabies

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In the definitive diagnosis for rabies of dogs, the opening of the skull has been usually used as a reliable technique for collection of brain specimen. However, it has risks of injury and infection, and takes much time and effort to the person concerning in the examination, especially by using a hand saw. This is a burden to them and even to the institution. Therefore, we developed a new technique that solves such problems. This new technique is usable for the dogs just after died or euthanized. It consists of only three steps as follows; Step 1: Using a dissecting knife and a scalpel, decapitate the dog at *atlanto-occipital* joint. Step 2: After cutting open the skin by a scalpel, using a hand drill of 4mm in diameter, perforate frontal bone to cranial cavity at the intersection point of centerline and horizontal line passing by *zygomatic process*. Step 3: Put the dog head on a cylindrical vessel, then insert the tip of 100ml plastic syringe to the hole made in Step 2, and after that inject 100ml of water into the cranial cavity with one shot. Collect brain specimens and water extruded from the *foramen magnum*. In the case of the dog having developed frontal sinus, attach a suitable nozzle for direct access to the cranial cavity on the tip of the syringe to get the specimens without effects by frontal sinus, which causes of water leakage from the nostrils. For biosafety, “Step 1” is desirable to be carried out in a necropsy room, and similarly “Step 2 and Step 3” should be done in a biosafety cabinet. In the situation without such safety devices, “Step 3” in particular must be carried out in sealed plastic bag. The brain specimens were collected without opening of the skull. They contained five parts (*medulla oblongata, pons, thalamus, cerebellum and hippocampus*), all of which are required for definitive diagnosis of rabies. Respective parts collected were not suffered serious damage in appearance, and completely distinguishable. The time consumed for the collection of specimens was a few minutes. In the trial stage, sufficient amount of brain specimens to inspect was obtained from twenty-seven of twenty-eight dogs of various breeds. The cause of one failure was insufficient water pressure owing to water leakage to nostril, because the dog had developed frontal sinus. It was settled by use of the nozzle in such case of dogs. This new technique “Water Injection” would satisfy everybody concerning in the collection of specimens, as regards its safety, simplicity, clarity, rapidity and reliability. These advantages free the person from hard and dangerous work using a hand saw. “Water Injection” would be used hereafter instead of the opening of the skull. We hope that this technique spreads all over the world, and also be applied to the other mammals, being improved by many investigators.

Keywords: Brain, dogs, injection, rabies, sampling, technique

Molecular Epidemiology of Rabies Virus Circulated in Turkey between 2013-2015 and Investigation of Rabies in Bats

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The aim of this study is to compare a 327 base pairs fragment of the rabies virus (RABV) nucleoprotein coding (N) gene of samples from different animal species and human across Turkey, the vast majority being obtained between 2013-2015. The N gene has been extensively used for genetic typing and evolutionary studies because of its relatively conserved variation among reservoir-associated variants and geographic lineages (Bourhy et al., 1993; Wiktor et al., 1980) and the nucleotide sequence data of the N gene is used extensively as a molecular marker to explain the patterns of the geographic distribution of RABV at the regional and global level (David et al., 2000). 134 bat swap samples from 10 cities of Turkey were tested against genotypes 1, 5 and 6 of rabies and rabies-like viruses. Total RNA was extracted directly from samples by Roche MagNA Pure Compact System (Instrument and Nucleic Acid Isolation Kit) and one-step RT-PCR was performed by Roche Transcriptor OneStep RT-PCR kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany) as described by Heaton et al., 1997. The products of positive PCR amplification were purified with QIAquick Gel Extraction Kit (Qiagen GmbH, Germany) and were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (ABI, Warrington, UK) according to the manufacturer's instructions. Automated fluorescence sequencing was performed with ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA, USA). Bat swap samples were tested using real time RT-PCR. For each virus, sequence reads in both orientations were assembled using SeqMan Pro (Lasergene 7.0; DNAStar Inc., WI, USA). Subsequent sequence alignment and comparison at the nucleotide and the amino acid levels were performed in BioEdit Sequence Alignment Editor v.7.2.5 (http://www.mbio.ncsu.edu/BioEdit/page2.html). Further sequences of RABV isolates from Turkey and neighbouring countries were obtained from GenBank. The phylogenetic tree was generated using MEGA6.06 (Tamura et al., 2013). Sampled bat populations were found negative from genotypes 1, 5 and 6. 327 base pairs partial sequences of the N gene was obtained from one hundred isolates since 2013 until March 2015 (more sequences will be completed until September 2015). Together with the further sequences of RABV isolates from Turkey and neighbouring countries which were obtained from GenBank, phylogenetic analysis confirmed that all RABV isolates were from genotype 1 and were distinct from the fixed Pasteur virus strain. Phylogenetic analysis also demonstrated the distinct geographical distribution of RABV variants within Turkey. In Turkey Middle East and Caucasus/Caspian clades co-circulate and sequenced RABV isolates in this study took place in these clades. Also, no species specific lineages were obtained. It is impossible to make a generalization about the results of bat swap samples because of the low sample capacity.

Keywords: Bat, molecular epidemiology, rabies, Turkey

Changes in Ruminal pH, VFA, and Microbial Composition of Cattle with Repeated SARA Challenges

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Subacute ruminal acidosis (SARA) is characterized by repeated bouts of low ruminal pH. Molecular techniques have shown that the ruminal bacterial community is very diverse, but factors influencing its composition remain unknown. The bacterial community in SARA cows has been reported, but the relationship between pH, volatile fatty acids (VFA), and microbial composition in the ruminal fluids of SARA cattle remains unclear. The ruminal pH, VFA, and microbial composition in cattle with repeatedly induced SARA were investigated to examine ruminal adaptation to acute and short-term changes in feeding. Eight rumen-cannulated Holstein steers (age, 8–10 months; weight, 180–200 kg) fed hay or a SARA-inducing diet (hay:concentrate, 2:8) for 7 days were used. Cattle were fed at 08:00 and 17:00, and the experiments were repeated four times. A wireless radio-transmission pH-sensor (YCOW-S; DKK-Toa Yamagata, Yamagata, Japan) was placed in the ventral sac of the rumen and the pH was measured every 10 min. Ruminal fluids were collected three times (08:00, 14:00, and 20:00) for the 7 days after each feeding. VFAs were separated and quantified using gas chromatography (Model 135, Hitachi). Microbial composition and bacterial number in the ruminal fluids were analyzed using 16S rRNA gene pyrosequencing and real-time PCR using bacterial DNA extracted from fluids collected at 20:00. We used one-way repeated-measures analyses of variance followed by Dunnett's multiple-comparison test to test for differences in pH and VFA from the first day of feeding (day 0) and the first sampling of the day (08:00). The 24-h mean ruminal pH values in SARA cattle were significantly lower than those in hay-fed cattle, and were slightly higher in the third and fourth experimental periods compared to the first experimental period. The 16S rRNA gene pyrosequencing analysis showed that the ruminal microbial composition was simpler in SARA cattle than in hay-fed cattle. Compared with hay-fed cattle at the genus level, the ratios of Prevotella and Eubacterium were lower, while those of Ruminococcus, Clostridium, and Butyrivibrio increased in SARA cattle. Real-time PCR analysis showed that the bacterial numbers of total methanogens, Fibrobacter succinogenes, and Selenomonas ruminantium were decreased significantly and those of Streptococcus bovis and Megasphaera elsdenii were increased significantly in SARA cattle compared to hay-fed cattle. In hay-fed cattle, the numbers of all bacteria, total methanogens, and Selenomonas ruminantium during the second experimental period were significantly lower than those in the first experimental period. Ruminal acetic acid levels in hay-fed cattle and butyric acid levels in SARA cattle were slightly higher than those in the respective other groups. In SARA cattle, the ruminal acetic acid, butyric acid, and NH3-N levels were significantly higher in the fourth experimental period than in the first experiment. Both ruminal pH and bacterial diversity were significantly lower and the microbial composition was simpler in the ruminal fluids of SARA cattle. Ruminal fermentation could guard against acute and short-term feeding changes, and changes in the ruminal microbial composition of SARA cattle may occur following changes in ruminal pH.

Keywords: Cattle, microbial composition, pH, rumen, SARA, VFA

Acaricidal Resistance in Tick Infested Cattle and Buffaloes of River Ravi Region, Lahore (Pakistan)

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Tick infestation and tick-borne hemoparasitic diseases (TBHDs) are still obstacles to livestock and dairy industries of developing countries like Pakistan. Wide range of health problems and economic losses in bovids are resulted to tick infestation, which is characterized by anemia, poor growth and performance, rough body coat, and act as a vector for many TBHDs. A chemotherapeutic trial of commonly used injectable acaricides was conducted in order to study comparative efficacy in tick infested cattle and buffalo population of district Lahore of Central Punjab. A total of 1258 bovids (n=726 cattle; n=532 buffalo) having tick infestation were included in the present study. Ticks were collected with the help of forceps, and morphological characters of ticks were identified under stereoscope according to key. For chemotherapy, A total of 28 bovids (n=14 cattle; n=14 buffalo) positive for ticks, was divided into two groups; A and B. Each group was comprised of 14 bovids (n=7 cattle; n=7 buffalo). The bovids of group A was treated with ivermectin at the rate of 0.2 mg per kg body weight (BW) subcutaneously (SC), whereas bovids of group B was treated with doramectin at the rate of 0.2 mg per kg BW SC. Efficacy of drugs was measured on the basis of disappearance of clinical signs and reversal of clinical signs of tick infestation at day 2, 4, 6 and 10 post-treatment. Data regarding the chemotherapeutic trial was analyzed by repeated measures logistic regression using Statistical Product and Service Solutions (SPSS) version 16.0. The results indicated that the study area is at a risk of tick infestation and TBHDs. Cattle and buffaloes were found infested with 2 genera of ticks i.e., *Hyalomma* and *Boophilus* in the study area during the course of study. Ivermectin cured 5 out of 7 (71.43%), while doramectin cured 7 out of 7 animals (100%) in the cattle population. Among buffaloes, ivermectin cured 6 out of 7 (85.71%), while doramectin cured 7 out of 7 animals (100%). Overall ivermectin cured 11 out of 14 animals (78.57%), while doramectin treated all 14 animals (100%). Chemotherapeutic trial of commonly used injectable acaricides against tick infestation revealed that doramectin was more efficacious drug as compared to ivermectin.

Keywords: Buffalo, cattle, doramectin, ivermectin, Lahore, ticks

Acute-Phase Proteins, Oxidative Stress, and Enzyme Activities of Blood Serum and Peritoneal Fluid in Cattle with Abomasal Displacement

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Blood serum and peritoneal fluid acute-phase proteins, oxidative stress indicators, and some enzymes could be used for evaluation of abomasal tissue damage because of displacement in displaced abomasum (DA) cases. The aim of this study was to investigate the concentrations of acute-phase proteins, oxidative stress indica- tors, and activities of enzymes in blood serum and peritoneal fluid in cattle with right displaced abomasum (RDA) and left displaced abomasum (LDA) and in healthy cows. A total of 60 Holstein Friesian cows at 2–4 weeks postpartum (14–30 DIM) were used in this study; 40 cows (2–6 years old) with displaced abomasum (31 LDA and 9 RDA without volvu- lus) and no other postpartum disease. As for control group, 20 healthy cows (2–4 years old) were selected from healthy cows in the same farm under the same environmental and management conditions. The control cows were also at 2–4 weeks postpartum (14–29 DIM) and diagnosed to be healthy by analyzing their clinical and hematologic status. DA diagnosis in dairy cows consisted of physical examination, laboratory, and specific DA tests. EDTA blood hemograma and gas parameters of heparinized bloodb were evaluated in DA cases and in control animals. Blood serum and peritoneal fluid (without anticoagulant, 5–10 mL) were analyzed by ELISA testsc for haptoglobin,d serum amyloid A,e malondialdehyde,f nitric oxide,g adenosine deaminaseh and myle- peroxidase.i Alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatinine kinase (CK), creatinine kinase-MB (CK-MB), c-glutamyl transferase (GGT), lactate dehydrogenase (LDH), amylase (AML), and total protein (TP) levels in blood serum and peritoneal fluid were measured by an autoanalyzer. In the RDA group, serum haptoglobin (HPG), serum amyloid A (SAA), malondialdehyde (MDA), adenosine deaminase (ADA), myeleperoxidase (MPO), aspartate aminotransferase (AST), creatine kinase (CK, creatine kinase–MB (CK-MB), and gamma-glutamyl transferase (GGT) activity increased significantly, and serum HPG, MDA, ADA, and AST concentrations increased significantly in the LDA group ($P < .05$). Peritoneal fluid HPG, MDA, ADA, MPO, ALP, GGT, and LDH concentrations increased significantly, whereas NO concentrations reduced significantly in the RDA group, and HPG, MDA, ADA, and TP concentrations increased significantly, whereas concentrations of NO reduced significantly in the LDA group ($P < .05$). It is concluded that there are acute-phase response, oxidative stress, and abomasal tissue damage because of displacement (obstruction and increased luminal pressure) in DA cases, and evaluation of serum con- centrations of HPG, SAA, MDA, NO, ADA, MPO, AST, CK, CK-MB, and GGT, and peritoneal fluid concentrations of HPG, MDA, NO, ADA, MPO, ALP, GGT, and LDH can provide information to help in understanding these changes. Although these results are promising, clinical and prognostic uses of these parameters warrant further research.

Keywords: Acute-phase response, LDA, malondialdehyde, myeloperoxidase RDA

Table 1. Blood serum biochemical values of healthy and displaced abomasum animals (mean ± SE).

Parameters	Control (n:20)	RDA (n:9)	LDA (n:31)
HPG ng/mL	39.5 ± 14.4c	1516 ± 105a	981 ± 90.5b
SAA ng/mL	23.3 ± 3.73b	66.7 ± 18.2a	41.8 ± 6.11ab
MDA mmol/L	4.44 ± 0.16b	7.51 ± 0.72a	6.49 ± 0.28a
NO µmol/L	23.4 ± 2.09a	18.6 ± 2.73ab	12.9 ± 0.91b
MPO Eu/mL	1.29 ± 0.48b	15.8 ± 5.43a	2.97 ± 1.31b
ADA U/L	38.8 ± 4.62b	202 ± 42.8a	229 ± 27.2a
ALP IU/L	46.6 ± 2.87ab	60.3 ± 10.9a	39.1 ± 3.77b
ALT IU/L	20.0 ± 1.23a	18.1 ± 1.85ab	14.5 ± 1.15b
AST IU/L	66.9 ± 1.86b	136 ± 27.0a	123 ± 16.5a
AMY IU/L	52.2 ± 8.21	44.0 ± 4.12	35.1 ± 1.77
CK IU/L	147 ± 16.7b	575 ± 162a	358 ± 52.5ab
CK-MB IU/L	244 ± 20.4b	782 ± 198a	502 ± 65.3b
GGT IU/L	21.5 ± 1.51b	82.1 ± 17.1a	34.9 ± 3.79b
LDH IU/L	1621 ± 52.7	1597 ± 157	1533 ± 73.1
TP g/dL	6.79 ± 0.09	6.51 ± 0.24	6.45 ± 0.08

Control, healthy animals; RDA, right displaced abomasum; LDA, left displaced abomasum; HPG, haptoglobin; SAA, serum amyloid A; MDA, malondialdehyde; NO, nitric oxide; MPO, myeleperoxidase; ADA, adenosine deaminase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase–MB; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; AMY, amylase; TP, total protein. a, b, c Different letters in the same line are statistically significant (Tukey test, P <.05).

Table 2. Peritoneal fluid biochemical values of healthy and displaced abomasum animals (mean ± SE).

Parameters	Control (n:20)	RDA (n:9)	LDA (n:31)
HPG ng/mL	15.2 ± 4.76c	1225 ± 147a	617 ± 72.8b
SAA ng/mL	61.1 ± 10.0	109 ± 19.0	107 ± 17.9
MDA mmol/L	3.85 ± 0.17c	6.89 ± 0.63a	5.85 ± 0.29b
NO µmol/L	60.2 ± 3.97a	27.1 ± 14.1b	13.9 ± 4.05b
MPO Eu/mL	1.48 ± 0.82b	15.8 ± 8.63a	1.68 ± 0.82b
ADA U/L	23.7 ± 3.76c	162 ± 24.9a	99.6 ± 11.0b
ALP IU/L	11.7 ± 2.12b	27.2 ± 6.86a	17.1 ± 3.59ab
ALT IU/L	2.65 ± 0.41	4.11 ± 1.43	3.96 ± 0.65
AST IU/L	33.4 ± 4.19	52.2 ± 11.2	55.4 ± 6.40
AMY IU/L	12.2 ± 1.88	18.6 ± 3.28	14.6 ± 1.38
CK IU/L	127 ± 38.8	132 ± 47.8	90.1 ± 19.5
CK-MB IU/L	146 ± 40.8	182 ± 57.8	118 ± 20.8
GGT IU/L	9.80 ± 1.54b	20.0 ± 3.12a	16.7 ± 2.22ab
LDH IU/L	348 ± 59.2b	736 ± 204a	482 ± 87.0ab
TP g/dL	1.97 ± 0.16b	2.66 ± 0.28ab	2.87 ± 0.20a

Control, healthy animals; RDA, right displaced abomasum; LDA, left displaced abomasum; HPG, haptoglobin; SAA, serum amyloid A; MDA, malondialdehyde; NO, nitric oxide; MPO, myeleperoxidase; ADA, adenosine deaminase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase–MB; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; AMY, amylase; TP, total protein. a, b, c Different letters in the same line are statistically significant (Tukey test, P <.05).

Investigation of Adaptability for a New Smart Herd Management and Decision Support System in Dairy Farms

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Aim of this study is to investigate new customizable “Smart Herd Management System-ASYST” by integrating various types of dairy farms’ milking systems and comparing herd management parameters within these farms via uniform and detailed data flow. During development of ASYST, “system engineering” for system related activities and “Software Engineering Paradigm” for software subsystems were used. “Iterative Incremental Process Model” and “Object Oriented Analysis Design Method” were used for work packages. “Open Unified Process (OpenUp)” and “Unified Modelling Language (UML)” were used. Integration of different milking systems was established either by software modules, report modules or existing databases. With this integration approach ASYST is capable to integrate with 10 different global milking systems. Integration to 2 top selling milking systems are fully completed. 2 different sizes of dairy farms (800 and 250 dairy cattles) which are using these milking systems were selected for this study. Upon completion of implementation and stuff trainings, ASYST is being used in these farms on regular basis. Data acquired from current milking systems of these farms include: Milking parameters, estrus tracking, insemination-calving records, general clinics records, body condition score, general and custom reports (milking process, inseminatin, general clinic records, etc.) It is proven in the field that after ASYST integration to these farms, detailed parameters and properties can be added to general parameters mentioned above and uniform data can be acquired from different farms via uniform and user friendly Graphical User Interface (GUI) either on site or via remote connection. Additional parameters and properties during this study were; Reproductive indexes for individuals and herds, individual reproductive and health interactive timelines, three dimensional (3D) linear type and body condition scoring, animal comparison, detailed herd management reports, individual and herd cost analysis, feed ratio calculations, inventory management and culling decision support. During this study, data acquired from current milking systems and data entered to ASYST are processed simultaneously with early warning and smart decision support algoritms embedded in ASYST. By doing so, these herd management parameters can be compared via uniform and various data acquired for all animals of these farms instead of limited sampling groups and limited variety. In the study it is proven; • Generation of early warning messages for mastitis and cost effectivity analysis are possible by processing milking data (milk amount, duration, conductivity) • Calculation of reproductive indexes (individual and herdwise) are possible by processing estrus tracking raw data and insemination records • Calculation of animal status (dry period, inseminated, etc.) and animal comparison according to relativity are possible by processing animal identification records • With advanced reporting module, it is possible to generate customized and high detailed reports (Eg: Follicular cists detected cows with 3 or more lactations during last 6 months) • After short training session, it is possible to perform 3D scorings (linear type and body condition scoring) and assign data to related animals in realtime under field conditions with mobile devices. After linear and body condition score assignments, users can correlate these values with various parameters (insemination index, insemination costs, distocia, etc.) • it is possible to correlate animal welfare scores with daily and lifetime milk yield, reproductive yield, cost effectivity, disease incidences, medications and prognosis by regular usage of animal wellfare scoring module. As a result of this study, various parameters like milk yield, reproductive yield, breeder quality, breeder matching, wellfare, condition score, clinical history, stuff performance and its effects can be compared and detailed statistical analysis can be performed with these uniform data sets which can not be accomplished due to software limitations and lack of GUI ergonomics.

Keywords: 3D scoring, herd, management, software, reproduction, welfare

Proteomic Analysis of Blood and Milk of Ewes with Experimental Mastitis Induced by *Mannheimia Haemolytica*

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Mastitis is a major welfare and production problem in sheep, caused by many microbial agents. In suckling ewes, Mannheimia haemolytica is the main causal agent. Objective of the present study was the evaluation of differentially expressed proteomes in ewes with mammary infection caused by M. haemolytica. Mastitis was induced by the deposition of a pathogenic M. haemolytica strain into the teat duct of ewes; the contralateral side of the udder was used as control. In order to confirm infection and to monitor progress of the disease, standard clinical, microbiological, cytological, ultrasonographic and histopathological methods were employed. Further, samples of blood (for plasma extraction) and milk (for whey extraction) were collected sequentially before and after challenge. In total, samples were collected at 6 or 7 time-points per ewe, from 2nd day before challenge up to 4th day post-challenge. For proteomics study, 1 mg of total protein was separated by two-dimensional gel electrophoresis (2-DE) for all samples. Differentially expressed proteins were detected semi-automatically in 2D-E gels. Spots were picked and the protein content was identified by using Matrix-assisted laser desorption/ionization Time-of-flight mass spectrometer (MALDI TOF MS). All animals developed clinical mastitis. After challenge, M. haemolytica was isolated from milk samples. Milk total somatic cell counts increased; in Giemsa-stained films, neutrophils predominated. Ultrasonographically, there was evidence of changes in mammary parenchyma grey-scale intensity values and of increased udder blood input into the udder. Histological changes observed in tissue samples included neutrophilic infiltration with mammary epithelial destruction and intra-alveolar haemorrhages; presence of some lymphocytes was also recorded. In blood plasma samples, we identified 24 differentially expressed proteins were identified; of these, after challenge, 20 proteins were upregulated (e.g., angiotensinogen, antithrombin-III, apolipoprotein A-I / A-IV, fibrinogen γ-B chain, gelsolin, haptoglobin, interleucin-4, serotransferin) and 4 proteins were downregulated (e.g., α-1 antiproteinase, fibrinogen β-chain, transthyretin). In milk whey samples from glands with mastitis, we identified 34 differentially expressed proteins; of these, after challenge, 26 proteins were upregulated (e.g., apolipoprotein A-I / A-IV, cathelicidin-1, chitinase-3-like protein 1, galactose-3, nectin, phakinin) and 8 proteins were downregulated (e.g., β-2-microglobulin, gelsolin, tuftelin-interactin). Moreover, we identified three proteins, namely gelsolin, phakinin and tuftelin-interactin protein, which were expressed differentially in plasma and whey samples after challenge. The results provide for the first time the proteomics analysis of ovine mastitis caused by M. haemolytica. The results indicate the protein changes in blood or milk after mammary challenge. The evidence can be used for elucidation of the pathogenesis of ovine mastitis, as well as in the identification of biomarkers for accurate diagnosis of the disease.

Keywords: Ewe, diagnosis, mastitis, pathogenesis, protein, proteomics

Trial of the Scandinavian Method of Ovine Artificial Insemination under British Sheep-Farming Conditions

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The aim of this study was to investigate whether the reported conception rates for the Scandinavian method of ovine artificial insemination (AI) by vaginal deposition of frozen-thawed semen were achievable in typical UK sheep-farming conditions. Semen was collected from a single adult Texel ram with a successful breeding history and was prepared and frozen in 0.25 ml straws by a commercial sheep breeding technology company (Innovis Ltd, Ormiston, East Lothian, UK). 40 Cheviot Mule ewes were synchronised by exposure to a vasectomised ram for 3 days (20-22nd October 2014). The vasectomised ram was reintroduced 14 days later. Oestrus was detected by marking of the ewes by raddle paint on the vasectomised ram. Ewes were checked twice daily, at 7.00 a.m. and 4 p.m. Oestrous ewes were inseminated, once, 8-21 hours after marking (3rd-7th November 2014). Two straws of frozen-thawed semen were used per insemination (maximum of 400 x 10^6 spermatozoa). The straws were thawed by submersion in water at 35'C for 12 seconds before being loaded into AI guns pre-warmed to 37'C. Ewes were kept in a separate paddock after AI. Once 20 ewes had been inseminated, the remainder were disseminated to the rest of the flock. A Suffolk ram with a different colour of raddle paint was introduced to the AI group 2 days after the last AI. The non-return to oestrus rate, as determined by ewes not re-marked by raddle paint, was recorded at 25 days after last AI. Foetal abdominal diameters were measured using trans-abdominal ultrasonography (6.5 MHz sector scanner, Mindray DP50, Mindray (UK) Ltd., Huntingdon, Cambs, UK) on 26th-28th January 2015 (80-86 days post-AI). At birth date, lamb number and lamb breed (Texel X or Suffolk X) was recorded. All statistical analyses were performed using Minitab 17 (Minitab Inc., State College PA, USA). A 25 day non-return to oestrus rate of 55% (11/20) was recorded. 10/20 ewes lambed in the period 31st March-2nd April and had Texel X offspring; a conception rate to AI of 50%. This is not statistically significantly different to that reported from Norway (67%, p=0.138) and Sweden (50%, p=1.000). The mean litter size was 1.7 lambs. All lambs were living. The foetal abdominal diameters measured ranged from 2.51 to 6.53 cm. There was a statistically significant difference in abdominal diameter as measured on 26th-28th January when ewes were divided by lamb paternity ($p<0.001$). Using known AI date and estimated conception date for non-AI pregnancies the relationship between foetal abdominal diameter and estimated foetal age was determined to be $y=7.552 x + 39.14$ (y=foetal age in days, x=foetal abdominal diameter). Vaginal deposition of frozen-thawed semen can achieve a conception rate of 50% under typical UK sheep-farming conditions. This is not statistically significantly different to that achieved in Scandinavia where the technique is widely used by sheep farmers themselves. This study indicates that this simple and non-invasive technique may have a role within the British sheep industry and is worthy of further investigation. The study also produced an initial estimate of the relationship between foetal abdominal diameter (as determined by trans-abdominal ultrasonography) and foetal age for Texel/Suffolk X Cheviot Mule lambs, which may be of use to veterinary surgeons investigating flock fertility problems as it provides a method of determining conception date during mid-gestation.

Keywords: Aartificial, insemination, sheep, ultrasonography

Comparison of Ovsynch and Flushing Based Synchronization Protocol in Beetal Goats during Breeding Season

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The objective of present study was to compare the effect of ovsynch protocol with conventional flushing based breeding during breeding season. Multiparous beetal goats were divided into two groups i) Ovsynch group (n=46) ii) and Control group (n=33). In Ovsynch group the first 4 µg GnRH injection analog was given im on day 0, followed 7 days later by an injection of 3.75 mg PGF2α intramuscularly. A second injection of 4 µg GnRH analog was then administered two days later, on day 9. Breeding occurred at a fixed time 16 hours later by introduction of bucks. After last GnRH shot estrus was evaluated twice a day (05h00) and 17h00 with intact bucks. In Control group Concentrate was used for flushing the goats. After flushing sudden introduction of bucks was done for natural mating. Estrus response was 88 % and 93 % in control and treatment group respectively. Pregnancy was diagnosed by transabdomonal ultrasonography. Pregnancy rate was numerically 13 points higher (80%) in ovsynch group as compared to flushing 67% but statistically non significant (P = 0.16, Chi square-test). Fecundity was also higher in Ovsynch (124%) in comparison with control group having 91%. Similarly litter size was 1.36 vs 1.54. Time frame for kidding was 69 days in flushing group but concentrated (within 26 days) in ovsynch group. Proportion of twins was statistically higher (P=0.012) in ovsynch group (71%) as compared to flushing based synchronization group (28%). On the basis of these results it has been concluded that flushing and Ovsynch treatments are comparable synchronization methods for beetal goats during the breeding season, but pregnancy, litter size and prolificacy rate was higher in Ovsynch group with marked reduction in kidding duration.This work was supported by Higher Education Commission(HEC) Islamabad under 5000 Indigenous PhD fellowship program.

Keywords: Beetal goat, breeding season, fecundity, flushing, Ovsynch, pregnancy rate

The Effect of Different Protocols and Vitamin-GnRH Applications on Oestrus Synchronization and Reproductive Performance in Anoestrus Dairy Goats

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The aim of the study was to evaluate the effects of different progestagens on oestrus synchronization and the effects of vitamin mix (A, D3 and E) and GnRH (buserelin acetate) singly or in combination on the fertility parameters in anoestrus dairy goats. The animals (Total n:186, Kilis breed n:93, Damascus breed n:93) were divided into three groups. Experiment I was treated with 20 mg fluorogeston acetate (FGA), Experiment II with 60 mg medroxyprogesterone acetate (MAP) andExperiment III with 3 mg norgestomet for 7 days. The goats which showed oestrus with these applications were mated naturally. Later, the goats were divided into four subgroups, randomly. The control goats received 2 ml NaCl immediately after mating, the vitamin group was treated with vitamins (1,000,000 IU A, 150,000 IU D3 and 100 mg E) immediately after mating, the GnRH group was treated with 4 µg buserelin acetate on the 12th day after mating and the vit+GnRH group received the combination of vitamins and GnRH at the same dose and day. Pregnancy diagnosis was performed by transrectal ultrasonography 35 days after mating. All fertility parameters were recorded. The oestrus rate was lower (P<0.05) and the interval to oestrus were shorter (P<0.05) in experiment I treated with fluorogeston acetate (Table 1). There were no statistically significant differences (P>0.05) between the subgroups in terms of the pregnancy rate, abortion rate, gestation length, twins rate and litter size (Table 2). In conclusion, the short term applications of FGA, MAP and Norgestomet successfully induced oestrus in anoestrus dairy goats. However, the vitamins and GnRH administrations post-mating did not improve the fertility parameters.

Keywords: Dairy goat, fertility, GnRH, oestrus, vitamin

Table 1:Estrus rate and interval to estrus in goats synchronized in the three experiment groups

Groups	n	Estrus Rate		Interval to Estrus	
		n	%	n	hour
Experiment I	62	55	b88.70	55	b26.01
Experiment II	62	62	a100.00	62	a32.35
Experiment III	62	59	a95.20	59	a32.32
Average	186	176	94.70	176	30.20

The values within the same column with different superscripts (a, b) are significantly different (P <0.05)

Table 2: Reproductive parameters in the control, vitamin, GnRH and Vit+GnRH subgroups

Groups	Pregnancy Rate		Abortion Rate		Gestation Length		TwinBirth Rate		Litter Size
	n	%	n	%	n	%	n	%	
Control (n:43)	16	37,21	6	37,50	10	148,20	5	50,00	1,50
Vitamin (n:45)	18	40,00	9	50,00	9	148,00	4	44,44	1,44
GnRH (n:45)	13	28,89	8	61,54	5	148,60	2	40,00	1,40
Vit+GnRH (n:43)	11	25,58	8	70,00	3	147,67	1	33,33	1,33
Average (n:176)	58	32,95	31	53,45	27	148,15	12	44,44	1,44

The values among the all groups are not significantly different (P>0.05)

Evaluation of Testosterone Effect on Femur Elongation in pre-Pubertal Term Rabbits

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Anabolic steroids are suspected to adversely affect longitudinal bone growth when use in young growing animals. Testosterone may accomplish this effect by its effect on chondrocytes of growth plate. This study was aimed to evaluate possible adverse effect of testosterone on longitudinal bone growth in growing rabbits. Sixteen female New zealand white rabbits of 42-45 days old were divided into two eight groups. The experiment group received weekly intra muscular testosterone. Survey radiographs were taken weekly and femur lengths were recorded for both groups. Mean Femur lengths (MF) increased significantly in control group and reached from 85.75±1.14 at the beginning of experiment to 105.29±2.88 at the end of forth week (P<0.001). In experiment group, MF was 82.90±2.78 before testosterone injection and reached to 88.67±2.36 that shows no significant increase (P=0.399). While the MF had no significant difference between two groups at the beginning (P=0.361), it was significantly more in control group as showed in the last survey radiographs (P<0.001). The results of this study show that testosterone may stop normal elongation of longitudinal bones in pre-pubertal term rabbits.

Keywords: Bone elongation, rabbit, survey radiograph, testosterone

Isolation and Identification of Viral Infections in Wild Birds in Poland

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Wild birds play significant role in spreading of infectious diseases. Mid- and long-distance migration of many wild bird species, as well as different habitats they reside, exposes birds to a wide range of pathogens. Viral infections are of special importance because of their morbidity and mortality rate resulting from many diseases of birds, mammals as well as humans. Only limited studies have been done for presence of viruses in wild birds. Relatively few reports are available about prevalence of particular viruses in different species of wild birds both raptors and waterfowl in Poland. Furthermore little information is provided about mortality rate caused by viruses in these animals. To our knowledge no reports on these aspects have been published in recent 30 years. Therefore such studies need to be conducted to better understand which viruses are involved in causing infections in wild birds. The aim of this study was to isolate and identify viruses from sick or dead wild birds using to standard virological and molecular biology methods. The samples were screened for herpesviruses, West Nile virus, adenoviruses, circoviruses, paramyxoviruses and influenza virus among wild birds in Poland. Wild birds were delivered to our laboratory from refuge for wildlife in Zlotowek where injured or dead birds from different parts of Poland were provided. Various organs of 12 species of wild birds were collected and analyzed. The examined birds included: raptors such as white-tailed eagles (*Haliaeetus albicilla*), saker falcon *(Falco cherrug*), common buzzards (*Buteo buteo*), goshawk (*Accipiter gentilis*) peregrine falcon (*Falco peregrinus*), kestrels (*Falco tinnunculus*), little owl (*Athene noctua*), brown owls (*Strix aluco*), bird hybrid between peregrine falcon and gyrfalcon (*Falco rusticolus*) and other species of wild birds such as: western capercaillies (*Tetrao urogallus*), mute swans (*Cygnys olor*) and wild duck (*Anas platyrhynchos*) Isolation of viruses was performed using cell culture and embryonated chicken eggs. Isolated material was subjected to slide haemagglutination test and transmission electron microscopy method (TEM). The polymerase chain reaction (PCR) nested-PCR and RT-PCR technique were performed on cultured virus and from DNA and RNA isolated directly from tissues. DNA of adenovirus and circovirus was not detected in any of investigated samples. One bird tested positive for herpesvirus DNA. The results of RNA viruses: WNV, influenza and paramyxovirus will be extensively presented at the congress. This study shows prevalence of many virus species among wild birds in Poland. Those results are important for the monitoring of health of wild bird population as well as for public health, especially in case of WNV and influenza, which might pose a threat for people.

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Keywords: Wild birds, viruses, PCR, cell culture, TEM

Hematology Values in Captive Spotted Paca (*Cuniculus paca*, Linnaeus 1766)

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Hematological evaluation in spotted paca (Cuniculus paca) a neotropical rodent is limited and the normal parameters have not been defined. For this reason, the aim of this study was establish reference values for complete blood count in this specie. Eight adults, female, clinically healthy, captive Paca weighting 9.2 ± 0.9 kg were subjected to hematologic exam every 15 days, in three periods. The ethics committee on animal use (CEUA-UNESP 027420/11) approved the whole procedure. Due to physiologic characteristics fasting was not applied and animals were chemically restrained with midazolam 0.5 mg/kg and ketamine 30 mg/kg IM, after physical restraint. A 20G under needle catheter was placed and fixed in dorsal metatarsal artery (dorsal face of metatarsal region) and blood sample (3 mL) was withdrawn in collection tubes containing ethylenediamine tetraacetic acid (EDTA). Immediately after collection, the blood was subjected to hematologic analysis, including count of red blood cells (RBC), leukocytes (WBC), platelet (PLT), fraction of the blood composed of red blood cells (Hematocrit - HCT) and determination of hemoglobin (Hgb), in electronic cell counter (ABC vet counter®). The differential count of leukocytes: basophils (BAS), eosinophils (EOS), rods, neutrophils (PMN), lymphocytes (LYM) and monocytes (MON) was performed by microscopical observation of blood smears stained with a mixture Methanol, May-Grunwald and Giemsa. ANOVA compared between periods and descriptive statistics were made for each parameter and built the confidence interval (CI) at a significance level of 95% through Student test. The blood collection technique proved to be effective and uncomplicated. The reference values determined by 95% CI for Paca were: RBC 3.99 – 4.43 (X106); WBC 7.36 – 10.4 (X103/µL); PLT 303 – 380 (X103/µL); HCT 34,18 – 38,84 %; Hgb 11.92 – 13.37 (g/dL); BAS 0 –39 /µL; EOS 0 –315 /µL; rods 0 – 6,2 /µL; PMN 3.5 – 5.53 (X103/µL); LYM 2.94 – 4.43 (X103/µL); MON 0.299 – 0.643 (X103/µL). No differences between periods for hematologic counts values. This study established the first reference values for complete blood count in Spotted Paca and these are similar to reported in capybara and agouti, species that are phylogenetically close. The Artery blood collection technique with chemical restraint was well tolerated, allowing quick and quality blood samples.

Keywords: Blood collection, complete blood count, neotropical rodent, South America

Effects of Retinol and Retinol Esters on Performance, Egg Quality Traits and Vitamin A Levels in Serum and Egg of Laying Quails

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This study was conducted to determine the effects of retinol and retinol esters supplementations into laying quail diet on performance and egg quality traits as well as on vitamin A levels in serum and egg. A total of 300 eight-week-old Japanese quails divided into one control group and four treatment groups containing 60 quails. The control group was fed with basal diet containing vitamin premix without retinol and carotene. The diets of the treatment groups were formulated to provide dietary supplementation of retinol and retinol esters [as retinyl acetate (R-acetate), retinyl palmitate (R-palmitate) and retinyl propionate (R-propionate), 3300 IU/kg]. Egg production and feed conversion ratio in all groups was higher than those in control group. Serum β-carotene and malondialdehyde levels were decreased in all groups, whereas serum retinol level was increased ($p<0,001$) in the same groups. Serum antioxidant activity level in R-palmitate and R-propionate groups was higher than those in control, retinol and R-acetate groups. Egg yolk color index in the 8th week decreased in the R-propionate group. Yolk retinol level in R-palmitate and R-acetate groups was higher than those in control and R-propionate groups. Egg yolk malondialdehyde level in experimental groups was lower than those in control group on days of storage. It may be stated that retinol and retinol esters supplementation into laying quail's diets has positive effects on some performance and quality traits, these supplements has a preventive effect on lipid oxidation in serum and egg.

Acknowledegements: This study was supported by Scientific Research Project Committee of Afyon Kocatepe University, Afyonkarahisar, Turkey (Project no: 13.SAG.BIL.08). This article is summarized from the MSc thesis of same title

Keywords: Egg quality, laying quail, performance, retinol, retinol esters

Immunologic Therapy with Monocyte-derived Dendritic Cells in Canine Liposarcoma

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The standard treatment of canine liposarcoma is typically restricted to a surgical reduction of the tumor mass, since a prolonged survival time after an additional postsurgical management with radiation or chemo therapy is not common. In this abstract, the postsurgical treatment using dendritic cell therapy in three dogs with liposarcoma is presented as an additional treatment option. Three male dogs were treated. A 9-year-old French Bulldog was pre-treated with surgery and radiation but had a recurrence of liposarcoma. A 3-year-old Irish Wolfhound mix and a 7-year-old Australian Shepherd had no prior treatment after pathological-histological diagnosis of liposarcoma. All dogs underwent tumor mass reducing surgery prior to the immunologic treatment, but no clean margin surgery was achieved. Immediately after surgery, the dogs were treated with primed dendritic cells derived from the patient's own monocytes. Autologous tumor lysate was used for priming. The cell suspension was applied intradermally. All dogs showed a positive immunologic reaction. After the second application in a cycle of three applications every four weeks, a very distinct demarcation and fistula-formation was observed in the area of the surgical site. Additionally, the quality of the patients' lives has been improved. The immunologic treatment with dendritic cells shows promising effects in canine liposarcoma after incomplete surgical resection.

Keywords: Ccancer, dogs, dendritic cells, immunotherapy, liposarcoma

Result of Endoscopically Assisted Gastropexy Technique in Dogs

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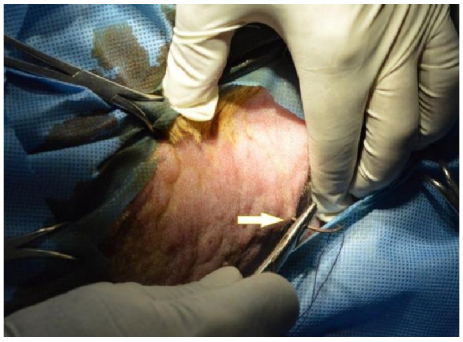
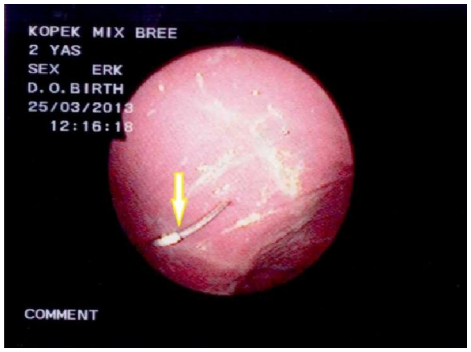
Gastric dilatation volvulus (GDV) syndrome is an acute condition with a mortality rate of 20% to 45% in treated animals. Medical treatment alone is inadequate and as many as 81% of affected dogs die within a year of initial treatment if surgery is not performed. Techniques commonly used to perform a gastropexy during emergency surgery for GDV can also be used for prophylactic gastropexy. The aim of the study to use of endoscopy in jointly with a gastropexy technique in dogs as a potential means to aid prevention and evaluation the long-term efficiency of this procedure of gastric dilatation-volvulus. 10 healthy adult medium- and large-breed dogs were used as materials. The dogs had no abnormal physical examination findings each underwent an endoscopically assisted gastropexy procedure. The dog was positioned in a left oblique recumbence. Endoscope was passed orally to them stomach, and the stomach was then insufflate with room air until rugal folds were minimally visible and adequate distension was achieved. External palpation across the body wall was then performed with a curved hemostatic forceps while the pyloric antrum was viewed to identify the chosen anatomic site. Once orientation was achieved, size-0 or size-2 polypropylene suture on a cutting needle (needle length, 80 and 90 mm, respectively) was passed through the right lateral aspect of the body wall (immediately caudal to the 13th rib) (Fig. 1); the needle and suture were viewed endoscopically as they entered and exited the stomach at the level of the pyloric antrum and then exited the body wall again through the skin (Fig. 2). An additional length of suture was then passed approximately 4 to 5 cm from the initial suture in the region of the pyloric antrum aborad to the first suture position. An incision was performed through the layers of the abdominal musculature between the 2 stay sutures until the stomach was visible. A longitudinal incision (approx 3- to 4-cm long) was then made through the serosal and muscular layers of the pyloric antrum. The seromuscular layer was sutured to the transversus abdominis muscle in 2 individual continuous patterns. The stay sutures previously placed were removed while the stomach was endoscopically evaluated and decompressed. After operation all the dogs has good condition. Operation procedure was followed by x-ray and ultrasonographic examinations. Data recorded included for anatomic location of the gastropexy, gastropexy length by ultrasonography, and duration of procedure and complications. Mean ± SD gastropexy length was 3.0 ± 0.25 cm by ultrasonography, and mean duration of surgery was 20 ± 5 minutes. There were no statistically differences for preoperative and post operative 7 days after gastropexy operation of (WBC), (RBC), (Hg), (Ht), pH, (PO_2), (PCO_2), (tCO_2), (HCO_3), (Na), (K), (Ca) and (Glu) levels. They were all references levels. Postoperative 7 days, there were no abnormality of direct radiographic examination. Left recumbent lateral view, the pyloric portion was well visualised. Gastric emptying was started within 15 minutes and stomach was empties within 4 hours. There were no seen any pyloric obstruction. Postoperative 7 days, the stomach was a similar layered appearance ultrasonographically. Mucosa and muscularis were corresponding by hypoechoic layers. The remaining layers were hyperechoic. Pyloric layers were shown wide hyperechoic area. Measurements of the stomach wall thickness ranges were 4 mm. Pyloric layers shown 6 mm thickness of the dogs. Gastric peristalsis was account for 6 contractions per minute. Pylorospasm were not seen. It appears that endoscopically assisted gastropexy is a simple, fast, safe, and reliable method of performing a prophylactic gastropexy in dogs. This procedure maximizes the benefits of decreased morbidity and shorter duration of anaesthesia associated with minimally invasive surgery.

Keywords: Dog, endoscopically gastropexy, ultrasound, X-ray



Once orientation was achieved, size-0 or size-2 polypropylene suture on a cutting needle was passed through the right lateral aspect of the body wall (immediately caudal to the 13th rib). The arrow indicates cutting needle.



The needle were viewed endoscopically as they entered and exited the stomach at the level of the pyloric antrum. The arrow indicates to view of the needle by endoscopically.

Sedative, Analgesic and Behavioral Effects of Nalbuphine-Xylazine and Nalbuphine-Midazolam Combinations in Dogs

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The objective of the present study was to evaluate the sedative, analgesic and behavioral effects of nalbuphine-xylazine (NX) and nalbuphine-midazolam (NM) combinations for neuroleptanalgesia in dogs. A prospective randomized cross-over study was done on six adult mongrel dogs (3 males and 3 females). Dogs were randomly allocated into NX and NM groups with a washout period 2 weeks. In both groups, nalbuphine was given at dose of 0.5 mg/kg i.v approximately 20 minutes before administration of the tranquilizer. Then dogs were randomly allocated into one of the two groups: NX group where dogs received xylazine at a dose of 1mg/kg i.v.; and NM group where dogs received midazolam at a dose of 0.5 mg/kg i.v. Dogs were evaluated for sedation, analgesia and behavioral parameters before administration of nalbuphine (baseline), prior to administration of xylazine or midazolam (0 time) and each 10 minutes till 60 minutes. Vital parameters including heart rate, respiratory rate and rectal temperature were also recorded. Nalbuphine in combination with xylazine or midazolam resulted in significant changes in sedative, analgesic and behavioral scoring resulting in a state of sedated consciousness with marked analgesia. Most of the sedation scores showed significant increase in NX 10 minutes and 20 minutes in NM group. Significant change in sedation was still recorded in both groups till 60 minutes. The degree of analgesia was at its peak 10-30 minutes in NX group. The analgesia score started to decrease at 40 and 50 minutes. At 60 minutes, animals had depressed reaction to painful stimuli. In NM group, the peak analgesic scoring was reported at 20 minutes and started to decline till 60 minutes. Most of the behavioral parameters were significantly decreased at 10 minutes in NX group and 20 minutes following injection in NM group. The combination of NX provided greater sedation and analgesia compared with NM combination. Nalbuphine-xylazine is a potent neuroleptanalgesic mixture which provides effective sedation and decreases anxiety-related behaviors. The combination of nalbuphine-xylazine provided greater sedation and analgesia compared with nalbuphine-midazolam combination. Nalbuphine is a promising drug that will be an important part in the field of neuroleptanalgesia due to its low cost, wide safety margin and non-scheduled classification.

Keywords: Dog, midazolam, nalbuphine, neuroleptanalgesia, xylazine

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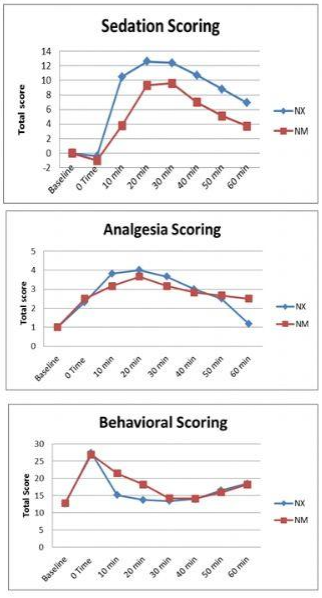
Sedation, analgesia and behavioral scoring in both nalbuphine-xylazine (NX) and nalbuphine-midazolam (NM) before administration of nalbuphine (baseline), prior to administration of xylazine or midazolam (0 time) and each 10 minutes till 60 minutes af



A Retrospective Study of Hepatoid (perianal) Gland Tumors

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Hepatoid glands are present in all dogs and especially in the perianal region. In this retrospective study, a number of canine biopsy specimens were observed in order to determine the incidence of hepatoid (perianal) gland tumors with pathomorphological findings. Between the years of 2010 and 2014, a total number of 685 canine biopsy specimens were received from the clinics of the Faculty of Veterinary Medicine, Ankara University and from some private veterinary clinics. For histopathologic examination, the tissue samples were fixed in 10% formalin and embedded in paraffin. Five µm sections were stained with hematoxylin and eosin (H&E). Twenty nine cases were diagnosed as hepatoid (perianal) gland tumors in the Pathology Department of the Faculty. These tumors were located on the anal and perianal region, tail, prepuce. Hepatoid gland tumors were obtained from dogs between the ages of 3 and 16. They were seen most commonly in males (79.31%) than females (20.69%). The risk of hepatoid gland tumors in Terriers and Mixed breeds was noticed to be higher than the other breeds such as Cocker spaniel, German shepherd, Husky, Golden retriever, Chiwawa. Among these twenty nine cases, 51.72% (15/29) were benign, 48.28% (14/29) were malignant. Hepatoid gland adenomas and carcinomas had similar gross morphology. On the cut section, they had white colour, elastic consistency and multilobular appearance. Microscopically; in the hepatoid gland adenomas, neoplastic cells resembling hepatocytes were forming solid areas. They were polygonal and had vesicular normochromatic nuclei, eosinophilic cytoplasm On the other hand, pleomorphic cells had generally hyperchromatic nuclei and little cytoplasm in the hepatoid gland carcinomas. The appearance of “bird’s eye” called cells were noticed. Besides, squamous metaplasia was observed in both tumors. In conclusion, this study will contribute to pathological evaluation of benign and malignant tumors of the hepatoid glands. They were more frequent in male, old dogs, especially Terrier breeds.

Keywords: Dog, hepatoid gland tumor, histopathology

Veterinary Leadership in Animal Welfare

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Appropriate and timely veterinary care is one of the fundamental welfare requirements for animals who are dependent on human guardianship. Veterinarians and nursing staff have the opportunity to represent the respect and empathy for animals that we wish to see reflected in our societies. We teach through our every action the consideration for animals that we strive to engender in communities and in the students who will rise to lead our profession. This begins with the manner in which we introduce ourselves to our patients, the skill and reassurance with which we restrain and handle them, and the care that they receive in our hands. Good welfare is not only the absence of suffering: we strive not only to correct disease or to relieve pain, but to make the lives of animals and the relationships that they share with people happy. By nature of requiring veterinary treatment, animals may already be suffering. They suffer metabolic and emotional stress as they heal and fight disease. They are often frightened and confused, whether from trauma or illness or when snatched from the street for sterilization. Our clinics smell of distressed animals and chemicals. There is a great deal of human activity and mechanical sensory assault. Being handled by strangers and confined against their wills add to the trauma of an already assaulted physiology. The stress that our patients experience under clinical care may therefore be profound. Within the provision of veterinary care, the mitigation of stress comprises a central role in the management of the patient's treatment. The inability of a patient to adapt to stressors constitutes distress, and compromises all the processes that we endeavor to support with veterinary care toward the physiological equilibrium of good health. Excellent veterinary caregivers are empathetically and cognitively aware of the distress that patients may be experiencing, pre-empt stressors, and de-escalate anxiety. Under conscientious care, even distressed and fearful patients may blossom into happy, confident animals in the course of hospitalization. Our demonstrated concern for the animal's emotional and physical well-being, whether during long hospital stays or only in a moment of vaccinating a patient, are paramount to professional veterinary practice and to the manner in which animals are valued in our societies. Veterinary caregivers have the opportunity to set a strong example in communicating respect for the inherent value of individual animal lives, regardless of the commercial, aesthetic or utilitarian worth that society may assign to an animal. We can promote veterinary professional and animal welfare standards no matter how sparse our resources may be when we work in the field, and most especially when we work in areas in which we seek to foster improved veterinary practices and attitudes of respect and compassion for animals. A moment of demonstrated affection and regard for a patient during an incident as brief as a vaccination procedure may change that animal's life through the way that her guardians see and value her thereafter. Our roles are not only to alleviate suffering, but to prevent it: and not only to prevent it, but to leave our patients with a happier future. This begins with our own hands and attitudes, and culminates in the hope that we engender in our fellow creatures toward a kinder planet for animals and for people.

Keywords: Animal welfare, charity work, veterinary practice, veterinary training

Bringing an Animal Welfare Focus to Clinical Veterinary Practice

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Animal welfare has received increasing attention in recent years within veterinary medicine. While there has been a significant focus on the veterinarian's role in enhancing animal welfare in food animal practice as well as the use of animals in research, less information is available regarding enhancing animal welfare in companion animal practice. A comprehensive assessment of animal welfare involves consideration of animal health and functioning, animal needs and wants, and consideration of aspects of natural living. It may be difficult to keep these considerations at the forefront when managing a busy practice. However, the role of the veterinarian as an animal professional gives veterinarians a monopoly to treat animals. Thus, the veterinarian must assess animal patients with animal welfare in mind and provide full disclosure of conditions and treatments to clients to fulfill public trust. There are several areas that veterinarians can focus on to improve companion animal welfare in practice settings and these include: optimizing the clinic environment, refining patient care by better management of pain and distress, use of quality of life assessments for end of life decision-making, and avoiding unnecessary procedures. In addition to direct actions in veterinary clinics, it is important for veterinarians to make their voices heard on larger societal issues concerning companion animal welfare, such as overpopulation, genetic abnormalities, and inappropriate uses. This talk will discuss ways in which veterinarians can become more actively engaged in promoting animal welfare of companion animals.

Keywords: Animal welfare, companion animals, ethics, social responsibility

Physiological and Behavioral Effects of Human Animal Bond to the Puppies

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Human Animal Bond (HAB) is a function of the interdependence between animals and their owners such that both are affected by the exchange. The HAB is complex but, overall, it is mutually beneficial. The link between animals and humans is symbiotic and complementary. The majority of research in this area has investigated the benefits of the human animal interaction to humans. On that point are limited researches aimed to investigate the benefits of animals. The aim of this study is measuring the effects of HAB to the dogs under 1 year old by using Pet Attachment Scale, Strange Situation Test and Jacketed External Telemetry System for the first time in Turkey. The study was supported by Uludag University Scientific Research Projects Unit (OUAP (V) 2014/5). After obtaining the Ethical Permissions from Clinical Research Ethics Committee of Uludag University School of Medicine (2014-3/1) and Animal Experimental Ethics Committee of Uludag University (2014-01/01), the informed consents of the owners were taken. The subjects were sixteen (8 male and 8 female) puppies whose ages ranged from 3 months to 1 year and their owners. The study consists of three parts: 1.Pet Attachment Scale: Dr. Hamano's (2002) Pet Attachment Scale with six divisions was translated and adapted into Turkish and applied to the owners to measure the strength of the bond formed with their dogs. 2. Strange Situation Test: Strange Situation procedure involves conducting controlled observations of a subject's response to being placed in an unfamiliar room, introduced to an unfamiliar adult stranger and subjected to short episodes of separation from the attachment figure. Therefore, it reproduces situations that dogs are likely to encounter in their everyday life, such as being in a new environment, meeting a stranger, and being separated from their owner for eight experimental episodes. The room equipped with two chairs ('stranger' and 'owner'), dog toys, a water bowl and two video cameras. The videotaped sessions were analyzed by two trained observers, and 12 mutually exclusive categories of behaviour were recorded. Test is adapted from the study of Palestrini et al., 2005. 3. Jacketed External Telemetry System: The behaviour of each dog during the eight experimental episodes was video recorded and heart rate (HR in beat/min), heart rate variability (HRV), ECG parameters (PR, PQ, QRS, QT interval duruatios (ms) and QT corrected for heart rate and HRV), respiratory rate (breath per minute, bpm), peak inspiratory and expiratory flows (PIF and PEF, mL/s), tidal and minute volumes (TV (mL) and MV (ml/min), body surface temperature, and locomotor activity was measured using a Jacketed External Telemetry system (Data Sciences International (DSI), Jacketed External Telemetry, St. Paul, MN, USA]) in order to allow a comparison between physiological paremeters and behaviour. Dogs were housed in their home for 2 days (30 min/ day) to begin acclimation to jacketing. The ECG skin electrodes were placed in lead II configuration. Continuous beat-to-beat Lead II ECG data, respiratory parameters and behavioral measurements using video cameras were simultaneously acquired from puppies using external telemetry system for 24 min (3 min for each short 8 periods). Results and Conclusion: The statistical results of the Pet Attachment Scale and Strange Situation Test will analyze with the results of Jacketed External Telemetry System. The new methodology for HAB in Turkey and the first results of the study will present in the Congress.

Keywords: Human animal bond, jacketed external telemetry system, pet attachment scale, physiological and behavioral effects, puppies, strange situation test

Risk Factors for the Development of Behavior Problems in a Population of Cats in Iran: Results of a Pilot Survey

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Prevalence and risk factors of behavior problems observed in cats presented to the veterinary clinics of Mashhad, Iran, were evaluated using a survey of feline behavior problems. Information was collected from 403 successfully completed questionnaires on various factors related to the demonstration of 12 common behavior problems: fearfulness (phobias), excessive activity, excessive vocalization, aggression towards people (owner, familiar and unfamiliar), aggression towards cats, aggression towards dogs, inappropriate elimination (urination and defecation), furniture scratching, compulsive disorders and sexual behavioral problems. The majority of respondents (297, 73.7%) stating that their cat exhibited at least one behavior problem. The main behavior problems reported by owners were furniture scratching (145, 36 %), fearfulness (134, 33.3 %), aggression towards cats (79, 19.6 %), inappropriate urine elimination (60, 14.9 %), excessive activity (59, 14.6) and excessive vocalization (47, 11.7 %). The cats acquired from breeders/pet stores were more likely to display fearfulness than those owned by the same person since birth (P=0.018) or acquired from friends/relatives (P=0.012). Persian cats showed significantly more unacceptable behaviors than other breeds, particularly, inappropriate urine elimination (P = 0.008) and scratching (P = 0.04). Male cats were more likely to exhibit sexual behavioral problems (P = 0.014) and inappropriate urine elimination (P < 0.0001) than their female counterparts. Puppies were less likely to exhibit aggression towards people (owner, familiar) (P = 0.047), and excessive vocalization (P = 0.029) than juveniles or adults. In addition, puppies showed significantly more compulsive disorders (P = 0.036) and excessive activity (P = 0.001) than juveniles or adults. Cats with outdoor access were more likely to display furniture scratching (P = 0.016) than those without outdoor access. Gray color cats were more likely to display inappropriate defecation (P = 0.034) and fearfulness (P = 0.043) than solid white color cats. In addition, these cats were less likely to exhibit aggression towards cats than solid white cats (P = 0.04). The evaluation of risk factors for feline behavior problems may be helpful to advise owners and improve animal welfare in Iran.

Keywords: Behavior problem, cat behavior, human–animal relationships

Anesthesiology in Pet Fish

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Learn which veterinary anesthetics can be used with fish, how are they administered and at what dosages, how fish recover from anesthesia, and what anesthetics are used for euthanasia in fish. Most fish anesthetics are added to clean, well-oxygenated water in a suitable glass or plastic container. The water is thoroughly mixed to ensure all the chemical is dissolved and dispersed evenly. The anesthetic solution should be the same temperature and pH as the aquarium or pond water. Material for anesthesia and tranquilization: Benzocaine (ethyl p-aminobenzoate) – Dose at 12.5 milligrams/Liter of water for a shipping sedative, 25–500 mg/L for anesthesia (may need to dissolve in ethanol first). Carbon Dioxide (CO2) – A dose of 100–400 mg/L bubbled through the water will cause unconsciousness, high exposure will cause death. Use with caution, under constant observation! Diazepam (Valium) – A sedative and muscle relaxant, used as a pre-anesthetic agent. Can be injected intramuscularly at 0.1-0.5 mg/kg, or given orally at 1-4 mg/kg. Ethanol (ethyl alcohol) – 1% added to the water will produce sedation, 3% or more will result in euthanasia. 20 ml of 100 Proof (50%) Grain Alcohol in 1 Liter of water will produce a 1% solution. Ether (dimethyl ether) – Dose at 10-15 ml/L water. Eugenol/Isoeugenol (clove oil) – Eugenol: 1 drop = 0.029 ml = 28.6 mg Use 30-60 mg/L (1-2 drops / Liter of water). Isofluorane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) – Dose at 0.5-1 ml/L water for anesthesia. Euthanasia dose 4 ml/L. Ketamine Hydrochloride – Dose at 1 gram/L water, or 66-100 mg/kg injected intramuscularly.Provides sedation and immobilization for handling or transportation. Propofol (2,6-diisopropylphenol) – Anesthesia induction dose is 1.5-2.5 mg/kg intravenously. Used as a sedative at 1 mg/L in the water. Quinaldine Sulfate (2-methylquinoline sulfate) – Dose at 5-10 mg/L for sedation, 25-200 mg/L for anesthesia. Acidifies low alkaline water, use sodium bicarbonate buffer in water as necessary. Tricaine Methane Sulfonate, MS-222 (3-Aminobenzoic acid ethyl ester) – Dose at 10-40 mg/L for sedation (handling/ shipping). Dose at 50-400 mg/L for anesthesia induction, 50-100 mg/L for maintenance. Acidifies water – buffer with equal volume of sodium bicarbonate or use hard water. When placed into the container with the anesthetic in the water, the fish will gradually begin to lie on its side and the respiratory rate will slow. In some cases, there may be an excitatory stage, so the anesthetic chamber may need to be covered to prevent fish from jumping out. After the fish is anesthetized in the anesthetic bath, it can be removed from the water for short-term examination or diagnostic procedures. If the fish is removed for longer procedures, anesthetic solution can be dripped across the gills. Have oxygenated fresh water on hand to syringe across the gills if the plane of anesthesia becomes too deep. Recuperation after anesthesia is accomplished by transferring the fish into a container of fresh, well-aerated water without any anesthetic. The longer the fish is under anesthesia, the longer it usually takes to recover. Monitor the fish until it has regained its equilibrium and is swimming normally and can be transferred back into the aquarium or pond. Euthanasia in pet fish is similar to anesthesia, but uses a higher dosage of anesthetic agent. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. Leaving the fish in the euthanasia solution for an hour after respiration ceases is a practical criterion for ensuring death.

Keywords: Anesthesia, euthanasia, fish, tranquilization

The Use of a Waterfall Bypass to Mitigate Water Temperature Fluctuation in Outdoor Koi Ponds

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Despite a relatively low utilization rate and high water concentration of oxygen during cold weather periods, difficulties maintaining ventilation of pond water during freezing periods can lead to hypoxic conditions. Furthermore, several mechanisms, such as pond deicers, heating systems, air bubblers and high circulation rates with waterfalls and spillways, which have been promoted to mitigate winter hypoxia, can themselves lead to medical problems. A thorough understanding of the inter-relationship between air and water temperature, water circulation and gas exchange is critical in evaluating cold season koi health problems. Gas exchange for ventilation in private koi ponds is most often accomplished by the use of waterfalls and spillways. As koi have adapted to overwintering at the bottom of lakes and ponds at a water temperature of 39°F (4°C), colder water can lead to medical problems such as koi winter “layover” syndrome, ichthyobodiasis and saprolegniasis. Water temperatures as low as 28°F (-2°C) have been recorded by this author where high water flow rates are combined with active waterfalls during periods with very low air temperature. A simple waterfall bypass has been used successfully to reduce pond water temperature fluctuation during periods of very low air temperature while maintaining adequate ventilation.

Keywords: Reduce pond water temperature fluctuation, waterfall bypass

The Important Terms of Marine Pollution

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The marine pollution is one of the most important parts of ecotoxicology. Contrary to organics, the inorganic contaminants do not undergo the process of disintegration that reduce their concentrations or toxicity and accumulate in the aquatic organisms. This accumulation is important both in terms of potential effects on the aquatic organisms and on the human health. For this reason, "biomonitoring" programs (biological monitoring) are required to determine the temporary and permanent effects of the contaminants on the coastal regions. The classic chemical analysis should be accompanied by the biological approach such uses "biomarker" that elucidates biological responses of pollution. Biomarkers have been considered as sensitive and suitable tools for detecting either exposure, or effects of, pollutants since they can provide more comprehensive and biologically more relevant information on the potential impact of pollutants on the health status of organism. "Bioindicators" such as fish, crustaceans, algae, protozoa, macrophytes, bacteria and plankton are the organisms which respond to the environmental pollution by changing the life functions or accumulating toxins in to their bodies. The evaluation of impacts of pollution on the marine ecosystem is based on two types of scientific Findings: a) Indicators of change of composition and/or stress of the ecosystem of the marines; b) Analysis of trend of levels of bioaccumulation of chemical contaminants in the ecosystem elements which are considered as indirect indicators of impact on the ecosystem. "Biomonitoring" is a scientific technique for assessing the environment including human exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's (bioindicators) tissues and fluids. The process of the increase in concentration of a toxic substance in an organism's tissues or organs which it exposes to the surrounding environment is called "Bioaccumulation". Bioaccumulation of substances taken in by the organism from water is only called "Bioconcentration". The increase in concentration of a substance in a food chain (not an organism) is called as "Biomagnification". Biomagnification can be regarded as a special case of bioaccumulation in which the chemical concentration in the organism exceeds that in the organism's diet due to dietary absorption. A biomagnification factor (BMF) can be defined as the ratio of the concentration of chemical in the organism CB to that in the organisms diet CA and can be expressed as: BMF=CB=CA. Two toxic mechanisms are available for contaminants: enzyme inhibition and replacement with essential elements. The bioaccumulation and bioconcentration process are initiated with species (benthic, demersal, pelajic), feed habitat, age of bioindicators, exposure time to contaminants, the concentration and chemical characteristic of other contaminants. Also there is significant differences between tissues and organs. Consequently chemical analysis provides baseline information on the occurrence of potentially toxic pollutants in the environment, but fails to predict synergistic, additive or antagonistic effects, that may give an important measure of potential biological effects. On the other hand bioindicators can detect direct and indirect effects of sublethal concentrations of toxic pollutants and offer additional biologically and ecologically relevant information - a valuable tool for the establishment of guide lines for effective environmental management. The terms details and differences must be well known for successful monitoring of marine pollution by biologically.

Keywords: Bioaccumulation, bioconcentration, biomagnifications, biomonitoring, marine pollution

Plasma Oxidative Stress and Antioxidant Parameters on Hypothyroidism and Hyperthyroidism in Rats

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Thyroid hormones regulate basal metabolic rate by affecting numerous activities of mitochondrial respiratory chain components. As a result of this process reactive oxygen and nitrogen species (ROS and RNS) may increased. Imbalance of ROS and RNS versus antioxidant defence mechanism can cause oxidative stress. Thus, we aimed to evaluate thiobarbituric acid reactive substances (TBARS), nitric oxide (NO), total glutathione (GSSG), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) levels during hypothyroidism and hyperthyroidism in rats. hirty-six male Wistar Rats (12 weekly age) were randomly and equally divided into 3 groups (n=6 each subgroups) as euthyroid (groups 1 and 2), hypothyroid (groups 3 and 4), hyperthyroid (groups 5 and 6). Hypothyroidism and hyperthyroidism were induced by administration of 6-n-propyl-2-thiouracil (PTU; 0,05%) and L-thyroxine (L-T4; 0,0012%) received into drinking water for 3 and 6 weeks period, respectively. Plasma TBARS, NO, GSSG, GPx, CAT and SOD levels were assessed by ELISA method. Statistical differences among the groups were analyzed one-way ANOVA followed by Duncan's multiple range test (SPSS statistical software, version 19). P<0,05 was considered statistically significant in the analyses. Our results showed that TBARS and SOD levels were increased (p<0,05) on 3th and 6th weeks both hypo- and hyperthyroid groups compared with control group. NO levels were decreased (p<0,05) in 3th week hypo- and hyperthyroid groups whereas it was increased (p<0,05) on 6th week in hypothyroid group compared with the other groups. GSSG level on 3th week and GPx level on 6th week hypothyroid groups were lower (p<0,05) than the other groups. CAT levels on 6th week hypothyroid and hyperthyroid groups were tended to increase compared to control but these data were not statistically significant. In conclusion, hypothyroidism and hyperthyroidism can cause of oxidative stress-induced damage however increasingly antioxidant levels may protect the tissues against oxidative stress and damage.

Keywords: Antioxidant, oxidative stress, plasma, rat, thyroid

Immunological Pathogenesis of Pulmonary Inflammation by Lipopolysaccharide (LPS) and Anti-Inflammation Effect of Honey in Animals

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Lipopolysaccharide (LPS) that is endotoxin of Gram-negative bacteria has a variety of immune activity and is contained within air and environmental smoke. LPS inhalation also induces neutrophils into the lung and causes lung inflammation in animals. Although neutrophils are important cells for bacterial killing by phagocytosis, these cells also induce inflammation. Honey is used as a traditional medicine for colds, skin inflammation but not edible. However, the induction mechanism of neutrophils by LPS and the anti-inflammation activity of honey on lung inflammation are not fully understood. Therefore, we investigated the mechanism of induced neutrophils and anti-inflammation activity of honey. 8-10weeks female C57BL/6 mice were used. C3H/HeJ mice were used as TLR4-deficient mice. Mice were inhaled 600μg of Japanese honey (Kyoto Sangyo University) and following 1 day later, mice were inhaled 60μg of LPS by intranasal administration. At the 24h after intranasal administration of LPS, BAL was performed by injection of phosphate-buffered saline (PBS) and 5 times for a lung (1ml/wash). Cytospin preparations were made from each BAL sample, then stained with Giemza. Differential cells were evaluated proportion for each leukocyte in cytospin preparation. The expressions of TLR4 and CD14 on alveolar macrophage (AM) and LPS-induced neutrophil were analyzed by flow cytometry using PE-labeled each monoclonal antibody. Neutrophils were incubated with 2',7-dichlorofluorescein diacetate and hydroethidine. After incubating at 37 ℃ for 15 min, reactive oxygen species (ROS) productions were measured by flow cytometry. After 24h from intranasal administration of LPS, neutrophils were isolated using Ficol-Plus from BAL cells and peripheral blood in untreated mice (control). IL-1β, TNF-α, CXCL1 and NF-κB mRNA expressions of isolated neutrophil from BAL cells were analyzed by RT-PCR. BAL cells counts were significantly ($p<=0.001$) increased with LPS inhalation compare with control. BAL cells counts were not increased in TLR4 deficiency mice. The number of neutrophils was significantly ($p<=0.01$) increased by LPS inhalation compare with control. Honey impacted the dot plots of BAL cells. Honey inhibited infiltration of neutrophils to the lung by LPS intranasal administration. Honey also changed cell size and intracellular structures in AM. IL-1β, TNF-α and CXCL1 mRNA expressions in AM were increased by LPS intranasal administration. NF-κB mRNA expression of AM was significantly increased by LPS intranasal administration. ROS productions were increased in lung neutrophils. We investigated the mechanism of induction of neutrophils and the effects of honey. BAL cells counts were significantly ($p<=0.001$) increased with LPS inhalation. However, BAL cells counts were not increased in TLR4 deficiency mice. The number of neutrophils was significantly ($p<=0.01$) increased by LPS. IL-1β, TNF-α and CXCL1 mRNA expressions of AM were increased by LPS. NF-κB mRNA expression of AM was significantly increased by LPS. ROS productions were increased in lung neutrophils. Honey inhibited infiltration of neutrophils to the lung. These results suggest that in the mechanism of neutrophils infiltration to lung by LPS intranasal administration, AM recognize LPS via TLR4 and then AM produce IL-1β and TNF-α, activated-AM by IL-1β and TNF-α produce CXCL-1 mediated with NF-κB, result in infiltration of neutrophils to the lung. And also honey indicated anti-inflammation activity via the suppression of induction of neutrophils in LPS intranasal administrated animals.Honey may be a candidate as anti-inflammatory drugs in the therapy of animals.

Keywords: Alveolar macrophage, honey, LPS, pulmonary inflammation

Evaluation of Bone Healing Using a Hydroxyapatite with Alendronate Compared to a Hydroxyapatite with Collagen

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The effects in bone healing of two types of hydroxyapatite on bone and osteogenic properties were evaluated. An osteoconductive resorbable hydroxyapatite with alendronate was compared with a mixture of hydroxyapatite with collagen; the hidroxyapatite is commonly used in several surgical procedures involving bone loss. Osteoconductive and osseointegration characteristics were measured according to the products ability to induce local cell differentiation into bone forming cells. These characteristics were evaluated in hydroxyapatite implants performed in 24 New Zealand breed rabbits, tested at 8, 15, 30, 60 and 90 days after the surgical procedure. Hydroxyapatite is an osteoconductive structure. This allows the material to be invaded by connective tissue from the surrounding bone, which will ossify later, keeping inside the characteristics of its origin. The material is an ideal platform on which new bone can grow, providing excellent osseointegration characteristics. Hydroxyapatite is an advance in the synthesis of bioceramic materials as an alternative to autologous grafting. A total of 24 adult male New Zealand white rabbits were used. The 24 animals were implanted in the right distal radius hidroxyapatite with alendronate, and the left distal radius hidroxyapatite with collagen. Animals were euthanized under a schedule as follows, group 1: at 8 days after implantation 6 animals, group 2: at 30 days of implantation 6 animals, group 3: at 60 days of implantation 6 animals, group 4: at 90 days of implantation 6 animals. The Cultures performed on all hydroxyapatites used for hidroxyapatite with alendronate implants and hidroxyapatite with collagen, showed no growth of any of these organisms. There were no differences between the hydroxyapatites with allendronate and hidroxyapatite with collagen ($p>0.05$) for the histological variables examined (macrophages, fibrin, lymphocytes, edema, congestion, fibroblasts, fibrosis, vascularity, osteoid, osteoblasts, trabeculae, osteocytes, osteoclasts, cartilage, lagoons, osteons and presence of implant). The Tukey found no difference among the treatments. Macrophages at 8 days there were high rates of absence of such cells. After 15 days, their presence was high in 25% of the cases, rising to 33.3% on day 30, and as time was progressing they began to disappear. At day 60, they were absent in 100% of the cases. Osteoconductive and osseointegration properties are important to evaluate an implant. Although there was a difference in shape for the hydroxyapatite with alendronate in the in comparison to Hydroxyapatite with collagen, the final response of the implants was similar as a result of the similarity in the particle size (300 µm in average for all implants). In this study, we found that bone formation rate was about 50% at four weeks of implantation. The above results ratify hydroxyapatite with alendronate quality in terms of clinical response, not producing cellular reactions different to the hydroxyapatite with collagen, confirming compatibility and good integration with the bone tissue.

Keywords: Bone regeneration, hydroxyapatite, osteoconduction, osseointegration, synthetic bone substitutes

Role of Oxidative Stress and Nitric Oxide in the Pathophysiology of Liver Injury in Streptozotocin-Induced Type 1 Diabetic Rats

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Type 1 diabetes mellitus (T1DM) is a severe chronic metabolic disorder characterized by hyperglycemia due to abnormalities in either insulin secretion or action. In this report it is aimed to investigate the cytotoxic effects of oxidative stress and to identify any correlation between effects T1DM related hepatopathology and hepatodegeneration. For that, male Wistar albino rats were randomly divided into two groups comprising ten animals in each group. Group I (normal control) received only vehicle 0.5% aqueous carboxymethyl cellulose. Group II diabetes was induced via a single intraperitonealy injection of streptozotocin (STZ) (65 mg/kg body weight). At the end of 20 days of experiment period, the rats were sacrificed, liver tissue samples were taken from rats for histopathologic and immunohistochemical analysis. Expression levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), for detection oxidative damage to DNA, superoxide dismutases (SOD), glutathione reductase (GR) and endothelial nitric oxide synthase (eNOS) were examined in liver tissues by immunohistochemistry. Results of the study revealed that the levels of 8-OHdG ($p<0.001$), GR ($p<0.001$), SOD ($p<0.001$) and eNOS ($p<0.05$) were remarkably higher in liver with T1DM than control. The most prominent finding of this study is the increased levels of 8-OHdG in only hepatocyte cytoplasm. These results suggest an involvement of oxidative DNA damage and oxidative stress might play a pivotal role and different mechanism of hepatodegeneration for understanding the cellular mechanisms of the process of STZ-induced type 1 diabetic rats liver. Furthermore, these results also suggested that STZ-induced hepatopathology might have multiple mechanisms of hepatodegeneration, by attenuating oxidative stress mediated high NO expression. Taken together, the results suggest a close link between oxidative stress, hepatic inflammation and degeneration and its closely implicated in pathophysiology of T1DM related hepatopathology. The results also clearly indicated that increased levels of NO might contribute to STZ-induced hepatopathology and oxidative stress biomarkers might indicate the progress of the T1DM.

Keywords: Hepatopathology, nitric oxide, oxidative stress, type 1 diabetes mellitus

Organized Veterinary Medical Education in a Global Society: AAVMC Celebrates 50 Years of Progress

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The Association of American Veterinary Medical Colleges (AAVMC) will celebrate 50 years of progress in 2016. Originally formed in 1966 with 18 colleges in the United States, the AAVMC now includes all of the veterinary medical colleges in the U.S. and Canada as well as 19 additional colleges throughout the world that are accredited by the AVMA Council on Education (COE). The AAVMC serves to analyze, catalyze and advocate for veterinary medical education and has initiatives in education, research, admissions, diversity, and One Health. This presentation will focus on the major program areas in global veterinary medical education and set the stage for the next 50 years of organized veterinary medical education.

Keywords: Admissions, advocacy, diversity, education, one health, research

Accreditation System for Veterinary Education in Korea

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Accreditation Board for Veterinary Education in Korea (ABOVE-K) was established in 2010. ABOVE-K was recognized as an accreditation agency by the Government that is in charge of the license of veterinarians. ABOVE-K has begun to evaluate and accredit veterinary schools in Korea from 2012. ABOVE-K enhances professional competencies by impartial evaluation and quality assurance of veterinary education. In Korea, there are 10 veterinary schools. One of them is private, the others are national schools. These schools produce about 500 veterinarians per year. Veterinary education is six year system. It is composed of pre-veterinary (2 yrs) and veterinary (4 yrs) course. The veterinary course consists of about 4,300 hours of pre-clinical (30%), para-clinical (32%) and clinical (38%) curriculum. Currently, there are 12,000 veterinarians in Korea. Sixty-three percent of them work in clinical practice and public health, and the others work in related fields such as quarantine, medical sciences, protection of infectious disease and zoonosis, food safety, meat inspection, milk plant, biomedical industries, zoo garden, feed industries and etc. The accreditation standards consist of five areas. Respectively, these five areas are composed of various factors as followings: organization and finances area (stretegy and planning, implementation, budget etc.), curriculum area (curriculum design, pre-clinical curriculum, para-clinical curriculum, clinical curriculum, professsional ethics, clinical clerkship etc.) students area (admission policy, mentoring and welfare system, welfare facilities, outcome etc.), faculty area (full-time faculty ratio, academic activity, community activity, self-development etc.), and educational resources area (educational facilities, research equipment and facilities, maintenance system etc.). The cycle of accreditation is 5 years in principle. Terms and conditions of accreditation are classified into 5 types: full accreditation (5 years), limited accreditation (2 years), provisional accreditation (within 2 years), unsatisfactory accreditation (no-pass) and revocation of accreditation (withdraw or rescission). Full accreditation is granted when the candidate school fully meets the criteria set by the accreditation standards. However, the accreditation period may be extended or shortened by the degree of compliance with the accreditation standards. The accreditation process begins from submitting of an application of a candidate school to ABOVE-K. And then the candidate school performs evaluation to determine in compliance with the evaluation criteria presented by ABOVE-K and submits a self-evaluation report (SER) to ABOVE-K. This SER is reviewed by a written evaluation and site-visit. Approval of accreditation shall be in accordance with the results of final evaluation results.

Keywords: Accreditation, competence, education, evaluation, outcome

Using the Diagnostic Pathfinder and Team Based Learning to Teach Clinical Pathology: The Effect on Long-Term Retention

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Current research on teaching and learning practices supports the benefits of engaging students in methods that emphasize carefully designed practice with helpful and timely feedback in addition to information presentation. Research in medical sciences education has demonstrated the instructional efficacy of this approach both in a carefully-designed computer-based context (using the Diagnostic Pathfinder[1]) and in a Team Based Learning (TBL)[2] context. Most existing studies, however, have measured the effects of such strategies over short time frames (e.g., at the end of a course), rather than long time frames. This study helped address that deficiency by measuring long term learning outcomes after using the Diagnostic Pathfinder/TBL to teach clinical pathology when compared with a traditional lecture-based approach. All students enrolled at a large College of Veterinary Medicine in the Eastern portion of the United States are required to take the introductory clinical pathology course taught during Spring semester of the second year. Two years following the required clinical pathology course, students could elect to participate in a clinical pathology rotation to learn more clinical pathology in an applied setting. All students participating in the required clinical pathology course in 2009 or 2010 who subsequently participated in the clinical pathology rotation were invited to participate in the study. Forty seven students (19 taking introductory clinical pathology in 2009 and 28 taking the course in 2010) participated. The nineteen students in the "traditional" clinical pathology group (spring semester 2009) learned clinical pathology in a format that included lectures, monthly quizzes, one multiple choice/short answer midterm exam and a similar final exam. They also had access to 10 optional homework cases that could be completed using the Diagnostic Pathfinder, a computer based tool designed to help students learn diagnostic problem solving, which has been described elsewhere[3]. Students in the DP/TBL group (Spring semester 2010) learned using Team Based Learning (TBL) as described by Michaelsen, Knight and Fink[4], with 60 required Diagnostic Pathfinder cases, and 15 TBL Group Exercises throughout the semester. Two years after taking the core clinical pathology course, all participants took a test on the first day of their optional clinical pathology rotation, requiring them to read descriptions of three different clinical scenarios and respond to multiple choice questions evaluating their ability to analyze the clinical laboratory data. Students in the DP/TBL group (M=17.3; 87%) scored significantly higher (p=.018; Cohen's d=.76) than those in the traditional group (M=16.1; 81%). The results of this study support the idea that engaging students in teaching and learning methods that emphasize carefully designed practice with feedback, in addition to information presentation, leads to better long term retention than a more traditional lecture-based method, when measured by applied case-based problem solving test items. Short term gains that have been shown to result from such teaching approaches are likely to reflect longer term gains as well.

Keywords: Clinical problem solving, diagnostic pathfinder, deliberate practice, feedback, team based learning

International Veterinary Congresses in Ottoman Era Archives

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Infectious animal diseases, which led to the foundation of first school of veterinary medicine in scientific terms, also paved the way for the organization of a series of international meetings and transnational cooperation efforts to fight these diseases. The first international veterinary congress, arranged upon the advice of Prof. John Gamgee from Edinburgh Veterinary College in the United Kingdom in an effort to protect Europe from Rinderpest (Cattle Plague), was named the "International Veterinary Congress", and was held on 14–18 July, 1863 in Hamburg, Germany. At the congress, 99 delegates from different countries heard John Gamgee emphasize the role of the transportation and movement of animals in the spread of animal diseases, leading to the adoption of the "Rules for the Fight against Animal Diseases", and it was decided to hold a second congress in Vienna, the capital of Austria, in 1865. The aim of the study was to make an evaluation of the international veterinary congresses held during the Ottoman era based on data garnered from the Ottoman archives. The research materials consisted of documents held in the Ottoman Archives of the Prime Minister's Office. An archive scan unearthed six documents related with three of the 10 international congresses held between 1863 and 1923, which were then translated and analyzed. The documents, which underlined the purpose of the second congress held in Vienna, Austria in 1865, contained information about the Ottoman delegation and their expenses, the letter of the Swiss President regarding the third meeting held in 1867 in Zurich, Switzerland and the cancellation of attendance to the 10th congress held in London, UK, in 1914. During the international congresses held in Europe in 1863, 1865 and in the following years, the Ottoman Empire was highlighted as the point of entry to Europe of Rinderpest, the most common infectious animal disease during the Ottoman Era, and as a precaution the borders were sealed against the import of animals and products of animal origin. It has been reported that the decisions taken in the international congresses were turned into law or regulations. In this study as well, a data was identified regarding this and it can be stated that the decisions adopted during the second congress held in Vienna were delivered to the provinces of the Ottoman Empire as a forward step in the fight against infectious animal diseases. Furthermore, it can be suggested from the archives and literature that the students returning to their home countries after receiving education abroad, have made contributions to the scientific treatment of infectious animal diseases and other topics (training, treatment methods, etc.). In conclusion, based on the data garnered from the limited number of documents in the Ottoman Archives related to the international veterinary congresses held during the Ottoman Era, it is apparent that the presence of important infectious animal diseases in the Ottoman Empire, as the point of entry of these diseases into Europe, was the leading reason for participation in international congresses and other cooperative efforts, although the attendance and the attention attributed to the issue were not sufficient, despite the measures taken and arrangements made.

Keywords: Congress, infectious, Ottoman, outbreak, veterinary medicine

Towards Effective Regulation of Veterinary Medicines in Africa

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The Veterinary Pharmaceutical Industry in Africa is presided over by various Government Ministries and Agencies involved in the sector however, Ministries responsible for Animal Resources are not providing the lead. Africa requires an effective animal medicine regulatory regime that includes systematic, evidence based means of documenting the safety and effectiveness of products before they are produced, marketed or used in a particular country or region. The value chain actors who include veterinarians, farmers, animal medicine users and the public require assurance that veterinary drugs and biologicals will be safe and effective in preventing and mitigating disease. Most African Countries lack focused National Veterinary Regulatory Authorities and instead rely on human medicines based Institutions that have limited regulatory and technical capacity to implement the essential regulatory functions. Most of the National Regulatory Authorities have inappropriate organizational structures to implement veterinary medicinal products regulatory functions since in most countries the entities responsible for regulation are units in the Ministries of Health. Although these entities are expected to be autonomous, fully fledged departments with statutory authority which comprise boards or commissions to ensure their independence, transparency and accountability in decision making, the reality is different in most cases creating the need for veterinary medicines regulatory reform. The World Health Organization (WHO) and partners such as New Partnership for Africa's Development (NEPAD) have been active in strengthening human medicines national and sub regional regulatory systems and promoting harmonization while the capacity of countries to regulate veterinary medicinal products has remained inadequate in Africa. Monitoring product quality and enforcing standards have been made more difficult by the increasing number of companies involved in the provision of veterinary drugs, mainly as a result of trade liberalization and the expiry of patents, both of which have allowed new manufacturers to enter the market. In many areas veterinarians, veterinary paraprofessionals and livestock owners have encountered problems with ineffective drugs with inadequate support from regulatory authorities. Adverse reactions or product failures are generally reported directly to the supplier by the distributor, although most suppliers feel that such events are rarely product related and often blame misuse or adulteration by either the retailer or end user. There is a great need worldwide for veterinary medicines that provide needed therapies for vast numbers of animals and animal species and, in the case of food producing animals, for medicinal products that enhance the productivity and efficiency of food production and ensure food safety when they are used in accordance with their approval specifications. Focused independent regulatory institutions help to prevent the presence of violative drug residues in animals for food safety while ensuring efficacy of medicines for guaranteed animal health and welfare. The veterinary professionals can competently ensure safe distribution and use of veterinary medicines due to their training and practice on farms and rural areas where these medicines are used in animals with specified input from pharmacists contributing to reduced antimicrobial resistance. There’s need for African governments to spearhead the creation of focused institutions to regulate the production, quality assurance, import and export, the marketing, sale and judicious use of veterinary pharmaceuticals, poisons, pesticides and ethno-veterinary medicines.

Keywords: Pesticides, pharmaceuticals, poisons

Assesment of Lumpy Skin Disease outbreaks in Turkey between 2013-2014

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LSD outbreaks and epidemics were mainly associated with climate changes. These changes caused serious outbreaks and recently outbreaks highly affected in countries (Isreal, Egypt) that have been existing of disease. The first entry of LSD into Turkey was at 2013. The aim of this study is to evaluate LSD outbreaks in Turkey between 2013-2014. Outbreaks were followed on TURKVET system which is governmental veterinary information system. The system includes all animal movement, animal bio data and disease information. The study was conducted from August 2013(after first disease report) to end of December 2014. Outbreaks also evaluated according to season, area, animal movement, festival of sacrifice and vaccination. While, 18 LSD outbreaks occurred in 2013, 784 outbreaks occurred in 2014. Most of the outbreaks was in August 2014. Some outbreaks proceeded in winter, December and January in 2013 and 2014. After animal movement began at September because of festival of sacrifice, new province was infected with LSDV even there was no outbreaks in neighbour province. Currently, it's widely agreed that LSDV is transmitted mechanically via arthropod vector. Recently, new evidence has been published reporting a possible role for hard ticks in transmission of LSDV also transstadial and transovarial transmission. Generally, outbreaks were associated with wet and warm weather condition with an abundance of blood feeding arthropods population. But, in winter 2013 also 2014, outbreaks was continued slightly. And LSD outbreaks also occurred at all heights, from 50m to1300m. This could be evidence that LSDV can be transmitted by other biological vector, which can survive in the environment for long time. Control measures was implemented effected area such as restriction animal movement, vaccination, slaughter of affected animal, protection and quarantine zone around the infected premises, vector control. But transmission of the LSDV is continuing. Subclinical infected animal movement has the most important role of transmission of LSDV to new province that have naïve animal population. Vaccination rate and vaccination time are very important for controlling of the disease.

Keywords: Animal movement, lumpy skin disease, outbreak, vaccine, vector

Anaemia, an Unusual Clinical Signs of Bluetongue in Greece

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Bluetongue, caused by an Orbivirus with worldwide distribution, is characterised by a variety of clinical signs. Anaemia had not been considered a clinical sign of the disease and, in the past, it had been reported as a sign to exclude bluetongue during the differential diagnosis. In May 2014, a new outbreak caused by a serotype 4 strain of the virus emerged in Greece. In this paper, we report investigations in anaemia that has been observed in sheep in Greece during this outbreak. Additionally to clinical signs reported in the literature as usually occurring in cases of bluetongue, we also observed anaemia in the affected animals. We performed detailed investigation of 75 clinical cases and assigned scores on a '1' ('red, non-anaemic mucous membrane') to '5' ('white, severely anaemic mucous membrane') scale. Mean anaemia clinical score at initial examination was 3.9±0.1 and at re-examination, one month later, 3.2±0.1. Mean values of haematological parametres were as follows: erythrocyte count 7.91±0.30 cells μL-1, haematocrit 30.5%±1.7%, haemoglobin concentration 8.75±0.38 g dL-1, mean corpuscular volume 38.31±1.29 fL, mean corpuscular haemoglobin concentration 29.51±0.70 g dL-1, leucocyte count 9,411±499 cells mL-1, thrombocyte count 340,737±40,512 cells mL-1. Virological examination was performed in whole blood samples from animals with or without clinical anaemia, after total RNA extraction and elution. A one-step conventional PCR assay targeting the NS1 gene of the virus was used. Reverse transcription and amplification was performed using commercial reagents. Product of expected size of 274 bp was detected in all samples from animals with signs of anaemia. In contrast, there was no evidence of severe Haemonchus contortus or of Babesia infection in any of these animals. Anaemia had not been previously considered to be a clinical sign of bluetongue in sheep. In this paper, we report this unusual finding and we indicate that anaemia was classified as macrocytic, hypochromic, regenerative and non-haemolytic. The findings confirm the multiple clinical manifestations that the disease may cause to affected animals.

Keywords: Anaemia, bluetongue, Greece, sheep, unusual clinical sign

Geographical Distribution of Cases of Leishmaniosis in Humans and Leishmania Infections of Dogs and European Brown Hares in Thessaly, Central Greece, and Phylogenetic Analysis of Isolated Strains of the Protozoon

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Leishmaniosis, a caused by a protozoan flagellate of the genus Leishmania, transmitted by phlebotomine sandfly species, is endemic around Greece. Our study reports the geographical distribution and the environmental parameters associated with Leishmania infection in hares and dogs in relation to human cases of the disease in the region of Thessaly, Greece. The study also infers the phylogenetic position of human and animal Leishmania strains isolated from Greece. Whole blood and bone marrow samples from children, which were attended at the main hospital of the University of Thessaly with clinical signs compatible to Leishmania infection, as well as canine lymph node aspirates and lapine spleen samples have been collected. PCR and sequencing was performed using primers which amplify a part of rRNA gene (ITS1 region). Four samples from children, three samples from dogs and seven samples from hares were sequenced. Molecular evolutionary analyses were conducted on nucleotide sequences of Leishmania isolates detected from childrens, dogs and hares in Greece and on Leishmania sequences that were retrieved from the EMBL database, using the program MEGA 6. Phylogenetic distances were calculated by the Kimura-2-parameter method and unrooted trees generated based on the neighbor-joining method. A bootstrap analysis with 1000 replicates was included. The regions where the human Leishmaniasis cases and the Leishmania infected dogs and hares were recorded, were used as background information. The data were statistically analyzed and Geographical Information System (GIS) analysis was also performed using the software ArcGIS Desktop 10. The phylogenetic analysis performed on 60 Leishmania ITS1 sequences, including the fourteen Greek isolates, revealed that the homology of the nucleotide sequences between the Greek isolates was 99.1%. The homology of the nucleotide sequences between the Leishmania isolates from Greece and the strains belonging to L. donovani complex, which were retrieved from GenBank, was 98.9%. The majority of the human cases was found in discontinuous urban fabric and permanently irrigated land΄mean altitude was 171 m. Human leishmaniosis cases have been recorded in 19 of the 26 local authorities of Thessaly region, with a range of 1-19 cases. Most cases (43% of all) were recorded in the county of Larissa. GIS analysis revealed that most Leishmania-infected dogs lived in urban and rural areas (irrigated and non-irrigated land) at low altitude. The Leishmania positive hares were found in shrubland with pasture land and in agroforestry formations with a mean altitude of 350 m above sea level; mean distance of these locations from villages and towns was 500 m. The similarities detected between isolates from people, dogs and hares indicates a possible overlapping of transmission cycles of Leishmania spp. Further investigation and analysis of additional Leishmania isolates from dogs and wild animals, will provide insight of their possible role in the transmission cycle of Leishmania spp in Greece. The study also indicates the relevance of the investigation of other possible reservoirs of Leishmania spp in endemic regions in order to reach conclusions regarding the epidemiology of the disease and to implement preventive measures for the protection of public health.

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Keywords: Dog, European brown hare, GIS, human, leishmania, phylogenetic analysis

Emergence of Multi-Drug Resistant Salmonella in Broiler Chicken Farms and Slaughterhouses in the Province of Skikda (North-eastern Algeria)

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The objective of this study was to elucidate the prevalence of Salmonella serotypes in broiler chicken farms and slaughterhouses in the province of Skikda (North-eastern Algeria) and to determine their antimicrobial resistance profiles. The present study was conducted from December 2011 to May 2013 on thirty-two broiler chicken farms and five slaughterhouses in the province of Skikda (North-eastern Algeria) in order to assess their hygienic and salmonellic infection status. For this purpose, 1194 different samples were collected during two sampling campaigns at two different age periods (15-30 days and 45-60 days). The sampling involved drinking water, food cloacal swabs and droppings. Surface wipes were sampled at 30 cm height from the floor on 400 cm² area of the floor walls. The poultry slaughterhouses were subjected to a one single visit and 90 samples were taken from the following matrices: caecum, liver, neck skin, carcass rinsing water, sticking knives and floor wipes. All samples were transported on pelleted ice to the laboratory for bacteriological analysis. The latter was performed according to the ISO 6579 standard (2002) and the NFU 47-101 standard (2005). The Salmonella strains were serotyped according to the Kauffmann- Le Minor's scheme and their antimicrobial susceptibility profiles were determined. The antimicrobial susceptibility testing and ESBL detection were performed according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2009). After 18-24 h incubation period, the results were read using an automate reader OSIRIS (Bio-Rad) and interpreted according to the CLSI criteria (CLSI, 2010). ESBL producing Salmonella strains were confirmed using the double disc test (SAEN, 2011). 34,37% of the broiler chicken farms were contaminated by salmonella. The isolation rates varied according to the sample origin: 6,25% for wipes, 4,24% for swabs, 3,12% for droppings, 2,18% for water samples. However, all food samples were negative. The results showed that samples taken at three weeks of age were more contaminated than those collected at the end of the raising period. The slaughterhouses were contaminated by salmonella. However, the rate of contamination varied according to the matrice sample: 12% for the caecum, 8% for the neck skin, 4% for the liver, 40% for the wipes, 20% for the sticking knives and 20% for the carcass rinsing water. Most of the isolated salmonella strains were identified as S. kentucky (n=22) S. heidelberg (n=14) S. enteritidis (n=4) and S.virginia (n=5). Distribution and antimicrobial resistance of Salmonella serotypes: A high resistance level to quinolones was noted in Salmonella isolates from both broiler chicken farms and slaughterhouses. 100% to nalidixic acid, 88,88% to ceprofloxaxin and 51,11% to levoflaxaxin. These strains were also resistant but to a less extent to cefalosporins, cephalotin (44,44%), cefazolin (44,44%) and cefotaxime (26,66%). A reduced susceptibility profile was revealed to other antibiotics: tatracyclines (48,88%) ampicilin (46,66%), ticarcillin (46,66%), sulfonamides (46,66%), nitrofurantoin (24,44%) and gentamicin (22,22%). ESBL- production was identified in 12 S. heidelberg strains. The results of the present study showed that salmonella infection is highly prevalent in broiler chicken farms and slaughterhouses of Skikda region with an increasing resistance to medically and veterinary important antibiotics. Human contaminations by these multi-drug resistant strains would have dangerous and costly public health consequences. Monitoring plans and strict biosecurity measures have to be implemented in order to identify the contamination sources and to limit the spread of these zoonotic bacteria in the local and national poultry industry.

Keywords: Algeria, infection, salmonella, farms, slaughterhouses

Avian Paramyxovirus Infections in Free-Ranging Birds in Turkey

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Avian paramyxoviruses are placed in nine groups (APMV-1 to APMV-9) on the basis of antigenic relatedness in serological tests. Newcastle diseases virus (NDV), the prototype of the paramyxoviruses (APMV-1) causes a highly contagious disease in domestic and wild bird populations and results in significant economic losses in the poultry industry around the globe. In this study, 105 strains of APMV-1 found in pigeons, exotic birds (pet birds) and wild birds in Turkey between 1993 and 2015 were investigated. Specimens were collected from affected pigeon lofts, dead feral pigeons, cage birds, free flying birds in wetlands and zoos in and around Antalya, Aydın, Bursa, Çanakkale, Denizli, Istanbul, Izmir, Manisa and Samsun provinces. Out of 105 isolates, 84 were obtained from the family Columbidae, 4 from the family Falconidae, 4 from the family Anatinae, 3 from the family Psittacidae, 2 from the family Corvidae, 2 from the family Phasianidae, 2 from the family Fringillidae and one from each of the families of the Laridae, Spheniscidae, Vulturidae, Sylviidae. The organ and feces samples were cultured in 9 to 11 days old specific pathogen-free embryonated chicken eggs for virus isolation. Real-time reverse transcriptase polymerase chain reaction (RRT-PCR) for detection of Matrix (M) protein was performed to indicate the presence of APMV-1. RRT-PCR for discrimination based on Fusion (F) protein between lentogenic and mesogenic/velogenic strains was also performed to show the presence of virulent NDV. A portion of the F gene was amplified and sequenced for determination of virulence and phylogenetic characterization. All suspected materials induced severe gross lesions in the embriyos and showed hemagglutination activity. In standard hemagglutination inhibition (HI) test with polyclonal NDV antiserum showed inhibition of heamagglutination of the isolated viruses. Out of 105 samples, 46 isolates with monoclonal antibodies 161/617 specific for pigeon isolates of NDV variants (pigeon paramyxovirus - PPMV) showed higher inhibition of heamagglutination titer than the polyclonal NDV antiserum. At the same time, all isolates were tested for Avian Influenza virus and they were found to be negative.The samples were tested by RRT-PCR for avian paramyxovirus M protein and 105 samples were found to be positive. Analyzed NDV isolates virulent F protein by RRT-PCR resulted in 53 positive and were typed as mesogenic / velogenic APMV-1. According to the virulence and phylogenetic tree analysis, out of 53 isolates, 48 were clustered into genotype (4b) VIb that is typical for pigeon paramyxovirus-1 (PPMV-1). This study showed that APMVs are prevalent in the free flying and wild birds, and that PPMV-1 found in pigeons and doves present a constant threat for domestic poultry. Therefore, for future studies, continuous surveillance may help better understanding the epidemiology of NDVs in free flying and wild bird populations and their relationship to NDVs in domestic poultry.

Keywords: Avian paramyxovirus (APMV), free flying birds, PCR, PPMV, virus isolation

Molecular Typing of Common *Salmonella* Serovars in Turkey

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In this study, extra laboratory work load due to phase 1 and phase 2 flagellar antigens not being expressed synchronously, time consuming work load and high cost due to probable cross-reactions requiring extra antiserum usage are envisaged to have been overcomed. Also, we decide to cope with some Salmonellae such as those which loss their O-antigen and turned into rough strain or which do not express flagellar antigens, or loss motility because of unsuitable environmental conditions in the field. In addition to conventional *Salmonella* typing with antisera according to Kauffman-White (K-W) scheme, It is aimed to optimize an alternative, cheaper, more rapid, sensitive, specific multiplex-PCRs and overcome time consuming and laborious laboratory process of conventional serotyping. And also, the isolates which are not retyped by multiplex-PCRs, will be anlayzed by ribotyping. For this purpose, 550 *Salmonella* isolates formerly identified by the slide agglutination method with a complete set of sera were retyped by multiplex-PCRs using specific primers designed for common*Salmonella* serovars circulating in the field. For serogroup typing; the primers amplifying O:B (O:4), O:C1(O:7), O:C2-C3 (O:8),O:D (O:9, O:9,46, O:9,46,27), O:E (O:3,10, O:3,19), O:D1(O:9) ve O:E1(3,10) somatic antigens were used (Herrera-Leon et al, 2007). In addition to sdf1 primers for *S*. Enteritidis, the primers amplifying H:i, H:z10, H:b, H:e,h, H:l,v, H:r, H:G complex, H:d flagellar antigens were used for determining phase-1 flagellar antigens (Echeita et al, 2002). For typing of phase-2 flagellar antigens; the specific primers amplifying H:1,5, H:1,6, H:1,7, H:1,2, H:l,w, H:e,n,x, H:e,n,z15 flagellar antigens were used (Herrera-Leon et al, 2004). A serotype of *Salmonella* is determined on the basis of antigenic variability at lipopolysaccharide moieties (O anti-gen), the flagellar protein (H antigen), and the capsular poly-saccharide (Vi antigen). Of the examined 550 *Salmonella* isolates, 218 isolates were fully identified. Briefly, *S*. Infantis, *S*. Enteritidis, *S*. Mbandaka, *S*. Braenderup, *S*. Typhimurium, *S*. Abony, *S*. Bredeney, *S*. Israel, *S*. Otmarshen, *S*. Potsdam, *S*. Kapemba,*S*. Anatum were the identified serovars. At least two antigens were determined in 254 *Salmonella* isolates. Altough the primers were enable to determine somatic and both flagellar antigens, among 254 *Salmonella* isolates, in 70 *S*. Infantis, 10 *S*. Mbandaka, 38 *S*. Virchow, 7 *S*. Hadar, 5 *S*. Livingstone, 4 *S*. Richmond serovars, 1 of 3 antigens could not be determined. In 78 *Salmonella* isolates, solely one antigen was detected. The primers for H:d phase-1 and H:e,n,x phase-2 antigens were found to be not amplified. Serovars, expected to be entirely typed yet, only two antigenic structure determined will be decided to be ribotyped. The primers amplfying H:d phase-1 and H:e,n,x phase-2 antigens were removed from the PCR reaction mixtures. The multiplex-PCRs were found to be reliable, fast and cheap to determine almost half of the total isolates accurately.

Keywords: Molecular typing, ribotyping, salmonella serovars

Monitoring Salmonella Contamination from Poultry House to the Packaging

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The objective of the current study was to monitor and model the prevalence of Salmonella spp. in broilers and in the environment where the animals were kept from the first week in the broiler house until the animals were slaughtered and packaged. For this purpose, in 3 different commercial broiler houses, cloacal swab samples, litter samples (by drag swab method), feed, and water samples were collected on weekly basis starting from the first day of the chicks. When the birds become ready for harvest, arrangements were made to ensure the slaughter of the birds first in the morning at the poultry slaughterhouse. Samples were taken from the animals during hanging, scalding and washing liquids, and from the carcasses at regular intervals. All samples were analyzed for detection of Salmonella spp. Change in the contamination of Salmonella in the chain between the farm and slaughter was modelled. A total of 345 cloacalswabsamples, 42 drags wabs, 26 water samples, 45 feed samples from 3 different poultry houses, and 9 scalding water samples, 10 inner-outer bird wash liquid samples, and 60 carcasses were analyzed for the presence of Salmonella spp. Results indicated that although presence of the pathogen differed between the 3 poultry houses, 39 cloacal samples and 6 drag swab samples were found positive. However, 31 of the 60 carcass samples were positive for Salmonella at the slaughter house. Serotypes of the isolates also differed from farm to farm, although Salmonella Infantis was the most common serotype. Theresults of the current study indicate that slaughter house plays the major role in contamination of the broiler carcasses.

Keywords: Broiler, modelling, prevalance, salmonella

STUDY OF P53 Domain Mutation in Ovine Pulmonary Adenomatosis

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P53 is a protein which plays a vital role in complex organisms, and regulates cell cycle. P53 mutations are the most frequent individual genetic changes in cancers. Among the best points to study mutations of this gene are the lung malignancies. In this study, 5300 lungs were studied in slaughterhouses of Kurdistan province-Iran, of which some had white, white-turquoise or nodule-like lesions like those seen in pulmonary adenomatosis (300 samples), which accounted for about 5.6% of all lungs. Fifteen cases were positive for ovine pulmonary adenomatosis. Following, positive blocks were colored to study mutation of P53 gene by immunohistochemistry method, of which 6 cases showed mutation. Next, positive samples were referred to DNA extraction stage, and then, were sent to GATC Company, Germany, for sequence finding by Sanger's method using sweep initiator in PCR (two-way reading) method. Based on the results, exon No. 5 was without mutation, intron No. 5 had 27 mutations, exon No. 6 had 6 mutations, and intron No. 6 had 17 mutations, and total number of mutations was 50. In all 6 samples, the highest number of mutations related to replacement of thymine for adenosine in base No. 201, and In 4 samples,by replacement of cytosine for adenosine in base No. 223. This study showed that in ovine pulmonary adenomatosis, like human adenocarcinoma, instances of mutation of P53 gene occur, and these exonic and intronic mutations can cause cancer to occur.

Keywords: P53, gene, mutation, ovine, pulmonary adenomatosis

Molecular Characterization of Cysticercus Tenuicolis of Slaughtered Livestock in Upper-Egypt Governorates

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The aim of this study is to present the molecular characterization of cysticercus tenuicolis of Taenia hydatigena from livestock isolates in Egypt, using the amplification of sequencing of the mt-CO1 gene. We introduce a detailed image of the Cysticercus tenuicolis infection in ruminant animals in Upper Egypt. Cysticercus tenuicolis inhabits such organs in ruminants as the omentum, viscera and liver. In the present study the infection rate of Cysticercus tenuicolis was found to be 16% and 19% in sheep and goat sample respectively. Firstly we report one larval stage of Taenia hydatigena detected in the camel liver in Egypt. Cysticercus tenuicolis infection manifested a higher prevalence in females than in males. Those above 2 years of age manifested a higher infection rate than younger animals. The preferred site for the infection was the omentum: a 70% preference in sheep and a 68% preference in goat samples. The molecular characterization using the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene of isolates from sheep, goats and camels corresponded to T. hydatigena. For this study, molecular characterizations of T. hydatigena were done for the first time in Egypt. Molecular tools are of great assistance in characterizing the Cysticercus tenuicolis parasite especially when the morphological character cannot be detected, because the metacestodes are frequently confused with infection by the Hydatid cyst, especially when these occur in the visceral organs. In the present study, Cysticercus tenuicolis manifested high identity in the goat and sheep samples, while differences were found more frequently in the camel samples (10 pairbase). Clearly molecular diagnosis for Cysticercus tenuicolis infection significantly helps to differentiate it from such other metacestodes.

Keywords: Cysticercus tenuicolis, ITS2, genetic, molecular and taenia hydatigena, qena

Practices in Administration of Anthelmintic Drugs to Small Ruminants by Veterinarians in Clinical Practice in Greece

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Objective was to investigate the use of anthelmintic drugs in clinical practice in Greece, by drawing information through questionnaires sent to veterinarians throughout the country. Veterinarians were selected at random from those active in farm animal practice. In total, questionnaires were sent to 143 veterinarians (58.5% of all veterinarians active, at the time, in that field in Greece) were selected. Questionnaires were sent by e-mail. Reminders were sent after four weeks and after another four weeks. Return rate for completed questionnaires was 35%, with a mean time to returning them 13.6 days. Reported mean numbers of administrations of anthelmintic drugs annually were 2.6 to sheep and 2.5 to goats (P=0.139). Most veterinarians reported administration of anthelmintic drugs in autumn (>95% of responders) or spring (>79%) (P<0.001 between seasons), as well as during the last part of pregnancy (>93%) or around mating period (including first part of pregnancy) (>62%) (P<0.004 for sheep, P<0.025 for goats between the various reproductive periods). No significant differences were reported between sheep or goats (P>0.55). Benzimidazoles (albendazole, fenbendazole, oxfendazole) or pro-benzimidazoles (febantel, netobimin) were the anthelmintic drug family reported more commonly administered Macrocyclic lactones (ivermectin, moxidectin), levamisole and various other drugs (closantel, oxyclozanide, rafoxanide) were also reported to be administered. Of the responders, 91% indicated administration of benzimidazoles or pro-benzimidazoles and 56.5% indicated administration of macrocyclic lactones (P<0.001), with no differences recorded between sheep or goats (P>0.11). Administration of formulations with two anthelmintics was reported by 44% of responders. Most frequently, responders reported use of 'bolus' or 'tablet' (>60% of responders) and of 'oral drench' or 'oral suspension' (>41% of responders). Smaller proportion (>41%) reported use of 'injectable solution'. Differences between use of the three forms were not significant (P>0.065), nor were differences in use to sheep or goats (P>0.29). Evaluation of potential association between use of the various pharmaceutical forms and administration of the various anthelmintics revealed that for use of 'injectable solution' and administration of macrocyclic lactones P<0.001, for use of 'bolus' or 'tablet' and administration of benzimidazoles P=0.082, whilst for all other potential associations P>0.16. Of the responders, 85% reported concurrent administration of anthelmintics with the anti-enterotoxaemia vaccine. The results provide for the first time information regarding use of veterinary drugs in small ruminant veterinary practice in Greece. Despite possible limitations, they are particularly useful for understanding administration of anthelmintics to sheep or goats. Administration during autumn often coincides with the last part of pregnancy, further supported by the extensive concurrent administration with the anti-enterotoxaemia vaccine. Administration in the spring often coincides with the start of the mating season in small ruminants. Extensive administration of benzimidazoles is associated with the infrequent detected resistance to these drugs in Greece. Widespread use of bolus or tablet pharmaceutical form reflects traditional approaches, which are still practiced. In general, it becomes evident that, to a large extent, veterinarians active in farm animal practice in Greece perform some targeted selective administrations of anthelmintics to sheep or goats.

Keywords: Anthelmintic resistance, benzimidazole, macrocyclic lactone, small ruminant

Evaluation of Oxidative Stress in Toxoplasma Gondii Infected Dogs by Measurement of Nitric Oxide Metabolites

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Toxoplasma gondii is a protozoan pathogen that infects wide range of animals including dogs. Clinical toxoplasmosis in dogs is often associated with immunosuppression induced by canine distemper virus infection. Clinical signs are usually most apparent in the respiratory and hepatic systems, and probably result from reactivation of a latent infection. Clinical signs can vary considerably and include neuromuscular, respiratory or gastrointestinal disorders. NO plays a complex and incompletely understood role in infectious diseases and has complex effects on immune system and metabolism. The aim of this study was to investigate possible change of this metabolite in dogs naturally infect with Toxoplasma gondii. Serum samples were collected from 83 dogs with known status of T. gondii, N. caninum and L. infantum infections. Measurement of nitrite and nitrate were based on the reduction of nitrate to nitrite by cadmium. Analysis of 83 serum samples showed that 30 dogs were infected with T. gondii. All analyzed serum samples were negative for infection with N. caninum and L. infantum parasites. Nitric oxide metabolites were significantly higher in dogs with T. gondii infection in compare to T. gondii negatives. Mean nitric oxide concentration (±SE) was 19.26±1.19 and 28.65±2.31 in T. gondii infected and T. gondii negative dogs. Possible role of nitric oxide in canine population is involving in a not completely known role in protection against this protozoan disease. As a cytotoxic/cytostatic effect or molecule, nitric oxide has been shown to inhibit the growth and function of a diverse array of infectious disease agents. Nitric oxide may stimulate immune system by activating cell mediated immune system.

Keywords: Dogs, nitric oxide, oxidative stress, toxoplasma gondii

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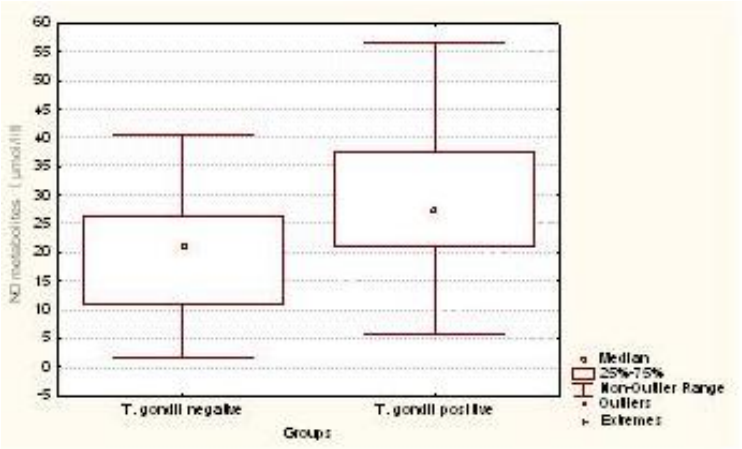
Figure-1
Nitric Oxide metabolites' concentration in two different groups



Antiproliferative Effect of Alpha-Tocopherol on Canine Mammary Gland Cancer Cells Culture

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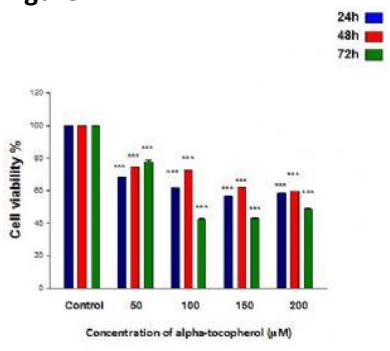
Breast cancer is the most common cause of cancer in female dogs. Studies that have been performed using cancer cell lines, animal models, and clinical trials have shown that vitamin E has anticancer activities due to its ability to induce apoptosis. The aim of this study was to evaluate invitro, the antiproliferative effect of alpha-tocopherol against canine mammary gland carcinoma cell line (CF41.Mg). CF41.Mg cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% heat inactived fetal bovine serum (FBS) and 100 mg/ml peniciline-streptomycin. The cells were maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37°C. Cell growth was estimated by direct counting of 0.4% trypan blue dye-excluding cells. The cells were seeded in 96-well plates at a density of 12000 cells/well. After one day of incubation for attaching the cells, the phenol red-free media with 10% FBS and different concentration (50, 100, 150 and 200 µM) of alpha-tocopherol that was purchased from Sigma Aldrich were added to the cells. After the cells were treated with different concentration of alpha-tocopherol, the 96-well plates were incubated for 24, 48 and 72 h at 37°C/5% CO2. After treatment, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2tetrazolium bromide (MTT) assay was used to detect cell viability. Briefly, 10 µM of MTT (at 5 mg/ml) was added to each well, at a final concentration of 200 µg/ml and incubated for 4 hours. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The results indicated that alpha-tocopherol inhibited proliferation of canine mammary gland carcinoma cells so that treatment with 100 microM alpha-tocopherol for 72 h resulted in a significant decrease (P<0.005) in cell viability. Since in this study we demonstrated that alpha-tocopherol (50-200 µM) induced cancer cell death, we suggest the alpha-tocopherol may be used a candidate drug for the inhibition of proliferation of canine breast cancer cells.

Keywords: Alpha-tocopherol, canine mammary gland cancer, cell culture, MTT assay

Figure-1



*The antiproliferative effect of different concentrations of alpha-tocopherol on CF41.Mg cells after 24, 48 and 72 h compared with the control group using MTT assay. Cell viability was calculated according to the following equation. Cell viability % = mean absorbance of sample/ mean absorbance of control * 100*

Diagnosis for Cells-Based Extragenital Canine Transmissible Venereal Tumor (CTVT) Cases by Polymerase Chain Reaction

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Canine transmissible venereal tumor (CTVT) is an only naturally contagious tumor in dogs which is transmitted during coitus or other social behavior, and occurring at both genital (GTVT) and extragenital (ETVT) areas such as eyes, skin, and oronasal cavity. GTVT is easy to diagnose from the anatomical site with cauliflower-like mass feature. On the other hand, ETVT diagnosis is more confusing. Fortunately, CTVT cell contains a specific genetic element, Long Interspersed Nuclear Elements (LINE), which inserted upstream to the *myc* gene. Taking this advantage, many researchers had established a molecular assay to identify and diagnose CTVT cases. Therefore, this study aimed to improve diagnostic accuracy by applying polymerase chain reaction (PCR) assay for FNA derived samples for imprecise ETVT cases. Twenty four tumor bearing dogs, regardless of sex, breed, and age were included in this study. All patients had subcutaneous mass at extragenital areas. Genomic DNA was extracted from FNA derived cells and then specific olionucleotide primers were used in PCR assay. Finally, specific amplicons, size 550 bp, were visualized on 1.5% agarose gel electrophoresis. Various sites of subcutaenous masses were prepuce, nasal cavity, conjunctiva and rectum. All 24 cases were cytologically classified as ETVT (19/24), histiocytic sarcomas (2/24), basal cell tumor (1/24), and melanoma (1/24). As expected, all ETVT cases gave the specific bands after PCR reactions. Furthermore, they also showed a 98-100% homology compared with deposited sequences from previous studies. Conversely, other samples which were histiocytic sarcoma, basal cell tumor and melanoma were negative by PCR. All ETVT cases showed the expected bands of PCR products accordingly to the hypothesis. So far, this demonstrated that ETVT in all parts of body had an association between tumor's location and specific molecular element (LINE), which is remained the same clonal origin as genital cases. Based on this finding, we proved that CTVT cells by FNA method can be used as the simple and practical specimen for diagnosis by PCR detection.

Keywords: CTVT, diagnosis, dog, extragenital

Histopathological Analysis of Domestic Cat (Felis Catus) Ovarian Tissue After Prolonged Cooling

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The study of ovarian tissue cryo-resistance is a crucial step to understand how long we can keep this tissue in good condition to use with Artificial Reproduction Techniques. Ovarian tissue bank has been showed to be a crucial component for conservation breeding of endangered species. The objective of the present study was to describe the morphological changes during cooling ovarian tissue for long periods through histopathological evaluations. Thus, ten queens were ovariectomized by elective chirurgical procedure and the obtained ovaries were placed in PBS plus amikacin (5mg/mL) and maintained at 5°C in a programmable refrigerator (Minitub®, Porto Alegre, Brazil) where they remained for periods corresponding to each of the 5 groups (0h, 12h, 24h, 96h and 120h). After the cooling period, they were transferred to a petri dish and divided in half, perpendicular to longitudinal axis, counting 8 hemi-ovaries per group. Each portion was fixed in buffered formalin for 24h and then put in ethanol 70% until the inclusion in paraffin. After this, 4µm sections were obtained, extended in slides and stained with Hematoxylin-Eosin to evaluate the histological quality. According to each refrigeration period there was obtained the following descriptions: 0h- Typical tissue morphology with no relevant changes; 12h- Discrete retrain of primordial follicles, eosinophilic nuclei and discreetly shapeless, larger follicles had no uniform spaces in granulose region discrete release of basal membrane and cell retraction; 24h- Cell retraction evident particularly in primary follicles, further eosinophilic cells, granulosa cells showed light separation and presence of pyknotic nuclei, and loss of demarcation between layers of follicle cells present in the superficial areas were more affected, but still showed some regions adequate preservation; 48h- cell retraction has become even more prevalent, lots of anucleate cells and vacuolated, the largest follicles showed moderate degeneration with cell detachment and the zona pellucida is presented separately, with moderately disorganized cells; 96h- loss of follicles architecture with little distinct of cytoplasmic limit and increased cytoplasmic eosinophilia, the antral content found itself and basophilic amorphic without delimitation of medullar and cortical portion; 120h- loss of cortical and medullar definition of the organ with detachment of cells and loss of the follicle structure, loss of architecture was close to 100%. Thus, according to the histology evaluation it became apparent a progression in ovarian tissue degeneration, however, up to 48h despite evident changes, these stretched only in the most superficial parts of the body with certain areas still had their typical morphology, which may also have available viable oocytes for extraction and maturation. By 96h, the signs of degeneration become evident in more than 80% of cells analyzed and at 120h all tissue had lost its architecture. Therefore, it was possible to conclude that the maintenance of ovarian tissue under-cooling is possible for a period till 48 hours.

Keywords: Cooling, feline, gonad, morphology, transport

Body Temperature Monitoring System in Sheep during Transport

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Road transport is one of the main causes of stress that impair homeostasis and metabolism of animals, implying a risk of animal welfare impairment. Changes in core body temperature have been used to assess the stressfulness of transport in a number of livestock species, including sheep. However, the manipulations associated with rectal temperature measurement are a source of stress themselves. New technologies allowing to assess the thermal response in the short and long term using non-invasive techniques need to be tested to evaluate their appropriateness to assess animal welfare during transport. The aim of this study was to assess changes on core body temperature in adult sheep during long transport by means of non-invasive data logger (i-Button®) as a tool to evaluate transport effect on sheep thermoregulation. Forty-eight ewes were transported for 29 hours, while twelve were not subject to transportation and classified as control group. Environment (farm and truck) and animal's body temperatures were assessed before transport, at loading, during transport and at unloading with a continuous monitoring by means of the i-Buttons®, both during the day before transport and the day of transport. Mean body temperatures statistical analysis for different groups and time intervals was evaluated using a mixed model by the PROC GLIMMIX procedure of SAS. The application of statistical analysis showed significant differences between the control group and the transported ewes ($P < 0.05$), and the influence of time intervals ($P < 0.05$) on body temperature. In particular, an increase in body temperature was found in transported animals in correspondence of the first hours of loading, transport and unloading. After 8 hours of travelling, animals still had a significant difference in body temperature compared with the pre-transport values. However, this difference was not significant in the last part of transport (from 25th to 29th hours). The monitoring of body temperature at the day before transport, assessing a regular circadian processes of ewes, and the monitoring of environmental temperature on truck, included in the thermoneutral zone for adult sheep (-12°C to 32°C) for the whole trial, suggest that the changes in body temperature of transported ewes should be imputable only to the transport practice. The pre-trial data collection for assessing baseline measures of temperatures makes the present evaluation of variations in body temperatures consistent and robust. Data were analysed considering the time intervals that may have more adverse effects on animals' welfare. In conclusion long transport affect the core body temperature of sheep and the technology of intra-vaginal data logger is a feasible tool to evaluate the changes in body temperature due to transport practice.

Keywords: Animal welfare, body temperature, data logger, sheep, transport

Quality of Life of Cancer Patients Treated with Dendritic Cell Therapy

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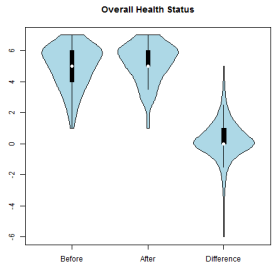
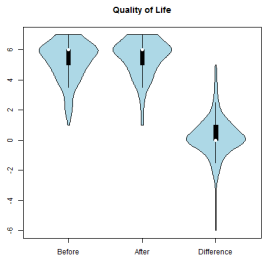
For cancer patients, often the quality of life is more important than a complete remission of the tumor. Traditional treatment approaches such as chemotherapy or radiation therapy are often immunosuppressive and induce unwanted side effects leading to a deterioration of the quality of life of the patient. Especially in situations where a curative treatment may not be possible, using these treatments means that the patients' quality of life is reduced for the limited time the patient still has. Furthermore, the owner often asks for a treatment which maintains or improves the quality of life for the patient instead of focusing on life expectancy. This analysis should show that immunotherapy can successfully be used to improve the quality of life of the patient. Using a standardized questionnaire, patients receiving dendritic cell therapy were evaluated by their owners. This main goal of this investigation was to find side effects of the treatment and give the owners a way to communicate if any undesired effects occurred during the treatment. With each treatment, the pet owner received a survey asking to evaluate the quality of life and possible side effects of the treatment. This survey should give the pet owner the possibility to give us (and the veterinarian) feedback about possible side effects, deterioration or improvements of the patient's health during the treatment. The pet owners were asked to fill out the questionnaire in the week after the application, therefore evaluating the situation right before the treatment and after the treatment. At no cost, the pet owner could send back the questionnaire in a provided envelope. For this analysis, responses from 87 patients with 129 observations have been evaluated. Since pet owners receive a survey with each treatment, multiple observations per patient are possible. Table 1 shows the frequency distribution of the observations. Most of the patients only have one observation, some have 2 and very few have more than 2. Since most of the patients received at least 3 treatments at the beginning of the therapy, this clearly shows that the willingness to participate in the survey decreases over time (with each subsequent treatment). This may be due to no changes in the observation (same as before) or because nothing "drastic" has to be reported. "The patient is fine". *Overall Health Status;* Question asked: How would you evaluate the overall health status of your patient? The data shows that the overall health status of the patients is increasing in the week after the treatment. The mean over all patients increases by 4.66% from 4.799 to 5.209 (p=0.164) (from 7 possible points). This improvement is especially present in dogs where the overall health status increases by 8.63% from 5.051 to 5.487 points (p=0.03). The small p-value indicates, that this improvement is statistically significant. The black bar in the figure in the graph for "differences" underlines these Findings: The tendency of differences (before and after) is clearly positive (above zero), indicating that there are almost no patients where the overall health status is decreasing. *Quality of Life;* The week after the treatment (injection of dendritic cell therapy), the patients' quality of life improves by 4.29% from 5.248 to 5.473 points (p=0.166) (7 being the highest possible quality of life). Especially, dogs show an improvement of quality of life of 6.44% from 5.372 to 5.718 points (p=0.056). Again, the tendency of differences (before and after) is clearly positive (above zero), indicating that there are almost no patients where the quality of life is decreasing.

Keywords: Cancer, dendritic cells, immunotherapy, quality of life

Overall Health Status



Quality of Life



The figure shows the distribution for the overall health status of the patients before and after the treatment as well as the distribution of the differences

The figure shows the distribution for the quality of life of the patients before and after the treatment as well as the distribution of the differences

Table 1 Frequency of Observations

Observations	1	2	3	4
Frequency	57	20	8	2

The table presents the number of observations for individual patients.

Table 2 Quality of Life

Overall (Dog, Cat, Horse)	
Mean before	5.248
Mean after treatment	5.473
Relative difference	4.29 %
P-Value	0.166
Only Dogs	
Mean before	5.372
Mean after treatment	5.718
Relative difference	6.44 %
P-Value	0.056

Results of the question "How would you evaluate the quality of life of your patient?" (1 lowest, 7 best)

Welfare of Rabbits in Animal Assisted Therapy Programs

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Animal-Assisted Therapy (AAT) is a method of complementary treatment in the rehabilitation of many human illnesses and conditions. Although the dog is the most widely-used therapy animal that is used an AAT program, the rabbit can be also used as an alternate animal species and complementary therapy for many situations in AAT programs. The rabbit has very good communication through its body language, and from the health point of view, rabbits are very clean animals. A special bond also exists between children and rabbits, and in the animal world of children, the rabbit is a very popular animal with children mainly through children's literature. As a result, rabbits elicit positive feelings in children and excite their imaginations. The companion animals used in two intervention programs in Greece, in a comprehensive-type kindergarten and in a Greek children hospital were two male rabbits, which participated in the events from the age of 3 months. Before and during the interventions they lived as domestic animals in a family-controlled environment, coming into contact with children and getting familiarized with cuddling, intense noises and high voices. No behavior problems were observed during this study, as well as changes in the normal vital parameters of the rabbits. Both, were sterilized at the age of 6 months in order to control their social behavior more efficiently Special care was taken both for children as well as for animals to ensure that diseases were not transmitted. The animals of the interventions were free from zoonotic diseases. Finally, particular attention was given so the children wouldn't get injured by the rabbit. One of the goals of this interventions with the rabbit, was to maintain children's contact with nature, even in a painful environment such as hospital, and as a new no familiar new environment of kindergarten during the early pre-school age. Based on our experience from these two AAT programs the rabbit was easily accepted by children with emotional or physical problems. In order for an AAT program with a rabbit to be a success, it is very important to guarantee the good health and the normal behavior of the rabbit, as well as its proper welfare. The contribution and the participation of a veterinarian during the design and the implementation of the two programs are also very important for assuring the success of an AAT program with rabbits. The intervention concluded similar results with corresponding which used other animals, such as dogs. The rabbit, however, has advantages that are crucial in situations of low cost, inadequate infrastructure and little experience or when intervention with other animals is difficult or impossible.

Keywords: Animal assisted therapy, children hospital, kindergaten, rabbit, welfare

Inability to Secure Appropriate Living Conditions for African Elephants in Polish Zoological Gardens

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The main objective of this study is to present the differences in behaviour between wild African elephants (*Loxodonta africana*) living in the Amboseli National Park in Kenya and elephants kept in captivity in Polish zoological gardens. We wanted to prove high social and environmental needs of this species and its extremely high requirements in enclosure. We believe that it will help to realize that Polish zoos could not meet these criteria. Observations and conclusions from this study should help the scientific environment, veterinarians and elephant keepers to visualise how important it is to keep high standards of breeding and welfare of these large mammals. The main base of the observation and analysis were elephant individuals in their natural habitat in Amboseli NP in Kenya and those kept in Polish zoological gardens. Subsequently their environment was analysed as well as enclosures designed for keeping them in vivarium conditions. Additionally, film material and photographs from zoo cameras from selected elephant rooms were analysed. It was the base material serving for preparation of the investigated issues. As a result of detailed observation of both the elephants, environment and relations between the individuals in their natural habitat and vivarium conditions, a detailed picture of the environmental needs, typical behaviours and pathological ones in consequence of environmental maladjustment has been made. Elephants have extremely high demands of enclosure maintenance confirmed by the studies on the representatives of this species in the wild. Amboseli elephants are one of the most analysed and least traumatised population in the world. In the contrary, PTSD symptoms and stereotypies were found in elephants in captivity. Furthermore, different defects, errors, omissions in maintenance characterising elephant pavilions in Polish Zoological Gardens were additionally presented. The research and discerning observations proved how important is the influence of the individual environmental elements on proper health maintenance, herd structure and individual behaviour. Their presence is an integral part of the basic psychological and behavioural parameters implementation and body functioning. A number of errors and shortcomings is faced by zoos, which vast majority is not able to provide the environment guaranteeing preservation of above-mentioned in the natural habitat. It consequently leads do metabolic, dermatological, reproductive and mental disorders and inadequate herd structure and its functioning, what makes the reproduction of this species in captivity highly difficult.

Keywords: African elephant, behaviour, PTSD, welfare, zoo

Efficacy of PRID®DELTA to Synchronize Estrus in Dromedary Camels (Camelus Dromedarius) During Breeding Season

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The present study aimed to evaluate the efficacy of PRID®DELTA to synchronize estrus in dromedary camels (Camelus dromedarius) during breeding season. Sixteen camels were received a new PRID®DELTA containing 1.55 g of P4 for 14 days. Ultrasound ovarian monitoring was done at day of insertion and every three days until day of PRID®DELTA withdrawal. Ultrasound examinations were continued day after day after PRID®DELTA withdrawal for ten days. According to the results of ultrasound examination, the percentage of camels belonged to breeding phase (follicles: 12-18 mm) or non-breeding phase (follicles: <=11 and >=19 mm) were calculated. Blood samples were collected day after day during the experimental period (24 days) started from the day of PRID®DELTA insertion. Serum was analyzed for P4 and E2 concentrations using ELISA kits. The sexual receptivity of camels was tested daily during the course of experiment. The results revealed that PRID®DELTA could not suppress follicular wave during the treatment. However, after 2 or 4 days of PRID®DELTA withdrawal, the percentage of camel in breeding phase was significantly higher (75 and 68.75, respectively) than those in non-breeding phase. The percentages of camels showed abstinence sexual receptivity during PRID®DELTA treatment were significantly higher than those showed incompletely or completely receptive. P4 level was increased significantly after PRID®DELTA insertion and reach its maximum level (5.55 ng/mL) on day 6. While, P4 level was significantly decreased and reached its basal level after 2 -4 days of PRID®DELTA withdrawal (0.67 and 0.65 ng/mL, respectively). Additionally, E2 level and sexual receptivity were significantly higher after 2 days of PRID®DELTA withdrawal in dromedary camels (22.95 pg/ml). In conclusion, treatment of dromedary camels with PRID®DELTA produced a uniform increase in serum concentration of progesterone but it could not suppress follicular wave. In spite of this, most of camels were in breeding (ovulatory) phase 2-4 days after PRID®DELTA withdrawal.

Keywords: Dromedary camel, estradiol, PRID®DELTA, progesterone, synchronization

Identification and in Vitro Evaluation of Potential Probiotic Activity of *Lactobacillus* Isolates from South American Camelids (*Vicugna Pacos*)

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The aim of this study was to isolate and identify *Lactobacillus* species obtained from young and healthy alpacas and to screen *in vitro* their probiotic activity. Fecal samples were obtained from rectal mucosa of 43 baby alpacas (*Vicugna pacos*) of 2-8 months of age, located in extensive or intensive farming, without registers of diarrhea and previous antimicrobial therapy. Pre-enrichment broth and culture in selective medium MRS for acid lactic bacteria were assess for each sample. Microbiological and molecular methods as colony morphology, Gram staining, catalase test and 16S-23S rDNA amplification and 16S rDNA sequencing were respectively performed for *Lactobacillus* species identification. Evaluation of in vitro probiotic properties included firstly the antimicrobial activity against pathogenic isolates of *Clostridium perfringens* type A and Enteropathogenic *Escherichia coli* (EPEC) by agar diffusion test, growth and survival at alpaca gastric conditions (pH, temperature, ruminal content) by optical density measured after growth in broth medium, adhesion to intestinal mucosa by crystal violet staining test and the screening of antimicrobial resistance profile by disc diffusion in agar. Three hundred and one (301) acid lactic bacteria were isolated and 187 colonies were presumptive of *Lactobacillus*. Eighty four (84) strains were definitely identify as *Lactobacillus* spp by molecular methods and the 46.43% belonged to *L. reuteri*, 23.81% to *L. helveticus*, 10.71% to *L. mucosae* and *L. ingluviei*, 2.38% to *L. vaginalis* and 1.19% to *L. crispatus*, *L. animalis*, *L. murinus*, *L. salivarius* and *L. gallinarum*. Thirty (30) *Lactobacillus* strains showed inhibitory activity against both *C. perfringens* and EPEC and were finally selected for following probiotic tests. None of these isolates grew at pH 3.0, but the 26.6% at pH 5.0 and 36.7% at pH: 5.0 showed the highest growth index. The 63.3% survived at 39ºC and ruminal content in anaerobic conditions. The 33.3% of selected *Lactobacillus* had a moderate adhesion to intestinal mucosa. The 10% showed sensitivity for five antibiotics, 60% to three antibiotics and 30% were to less than three antibiotics. Healthy alpacas are an abundant and diverse source of potential probiotic acid lactic bacteria. One strain of *L. mucosae* showed the best antimicrobial activity against pathogenic *C. perfringens* and EPEC, while different strains of *L. reuteri* showed highest index of intestinal adhesion and sensitivity for common use antimicrobials. Further studies are necessary to demonstrate *in vivo* the potential use of probiotic *Lactobacillus* in order to reduce and prevent cases of intestinal clostridiosis and colibacillosis in neonate alpacas.

Keywords: Alpaca, antimicrobial activity, enteric diseases, Lactobacillus, probiotic

A Study of the Structural Properties of the Digital Cushion on Cadaver Equine Feet View of Gas-Chromatography, Histological Analysis and Magnetic Resonance Imaging (MRI)

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In this study, we aim to investigate the structural properties of digital cushion, which plays an important role in the biomechanics of equine foot, using gas chromatograpy (GK) and magnetic resonance image(MRI) scans and histological dates. The study was planned to be conducted on three groups which consist of an equal number of stallions, mares and foals. To this end, a total number of 72 equine feet cadavers were examined in detail. Each foot was scanned individually using 1.5T MRI scanner in transversal, sagittal and axial positions.After the scanning, the digital cushion tissues were dissected first as a whole, and then further into cuneal and toric parts. Tissue samples were extracted from each dissected part in order to be analyzed both histologically and gas-chromatographically. The data aquired from histological analysis revealed that digital cushion segments are composed of loose connective tissue. It was observed that univacuolar white adipose cell groups were surrounded by fibrous connective tissue. Significant deviations between the three groups in terms of the amount of adipose tissue that these are made out were detected.It was discovered that the total adipose content of digital cushion was considerably higher in foals than in stallions and mares. It was also discovered that, in all groups, the ratio od adipose tissue in the toric parts of digital cushion was higher than that of the cuneal parts. Fatty acids analysis results obtained via tha GK procedure revealed a statistically significant ($P < 0.01$) difference in adipose tissue rations between toric and cuneal parts. The GK analysis also indicated that the adipose tissue rate of mare were significantly lower than that of stallions and foals. Evidently, the histological and the GK analysis results validate each other. Additionally, we discovered that, in all the three groups, the adipose tissue ration in toric parts were significantly higher than those of cuneal parts, in both front and hind feet. Although there is no statistically significant difference in SFA between the three groups, the SFA content in cuneal parts were invariably higher than that of toric parts. As a side note, we found out that MUFA is the highest in the foals and that PUFA is the highest in mare. The MRI scan results of all groups proved to be quite valuable in examining digital cushion. However, the MRI scans did not exhibit any readly recognizable differences between toric and cuneal parts. The histological and GK analysis results support each other regarding the structural properties of the digital cushion, and the MRI scans do verify these conclusions, yet they are not of much use when it comes to determine the structural differences between cuneal and toric parts of digital cushion.

Keywords: Equine digital cushion, gas-chromatograpy, histology, MRI

Feasability of Assessment of Equine Microcirculatory Parameters During Induced Hypotension and Pharmacological Intervention Using a Point of Care Sidestream Dark Field Microscopy Unit (MicroScan®)

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To describe the usage of a handheld, point of care side stream dark field microscopy unit (Microscan[1]) to assess changes in equine microcirculatory parameters in response to induced hypotension and subsequent pharmacological intervention. Seven healthy adult (average age 17, range 10-29 years) horses (4 mares and 3 geldings) were included in the study (average weight 468 kg, range 418-575 kg). Each horse underwent a physical examination before inclusion in the study. Each horse was prepared for anesthesia according to standard protocols for our hospital. Horses were pre-medicated with intravenous xylazine hydrochloride (1.1 mg/kg), and induced into anesthesia with intravenous diazepam (0.05 mg/kg) and ketamine hydrochloride (2.2 mg/kg). Once anesthetized, the horses were orotracheally intubated and placed into dorsal recumbency. The ventral abdomen was clipped and aseptically prepared in standard surgical fashion. Isoflurane was delivered in 100% oxygen, and mechanical ventilation was used to maintain end-tidal CO2 between 35-45 mmHg. Intravenous polyionic fluids were delivered initially at 10 ml/kg/hr and adjusted at the discretion of the anesthesiologist. Direct cannulation of the facial artery for invasive blood pressure monitoring was performed and maintained throughout the study period. Measurements were taken at nine time points and included microcirculation (microscopy), vital parameters, cardiac output via lithium dilution, electrocardiography, end tidal CO2, oxygen flow rate, inspired oxygen, tidal volume, exhaled isoflurane, peak inspiratory pressure, electrolyte concentrations and blood gas analysis. Microcirculation videos (Figure 1) were obtained at each time point from the oral mucosa, rectal mucosa, and the serosal mucosa of the pelvic flexure region of the large colon using the Microscan system (Figure 2). The pelvic flexure was accessed by exteriorizing the large colon following a ventral midline celiotomy performed prior to the first time point. The first time point was obtained when the patients during a normotensive (70-80 mmHg) state. Hypotension (<60 mmHg) was then induced by increasing the administered isoflurane and the measurements were then repeated. Pharmacological intervention (dobutamine, vasopressin, phenylephrine or norepinephrine) was administered until normotension was achieved. Measurements were repeated once normotension was achieved, followed by discontinuation of the agent and recurrence of hypotension. This process was then repeated for the remaining pharmacological agents, with the stated measurements repeated at each time point. The order of administration of the agents was randomized and the investigators were blinded to the treatment order. Following completion of the study, the subjects were humanely euthanized while under general anesthesia. The study was approved by the University of Georgia Institutional Animal Care and Use Committee. Interpretable video images and subsequently microcirculation parameters were successfully obtained from the mucosa of the oral cavity, rectal cavity and the pelvic flexure region of the large colon in all subjects. Each mucosal site (oral, rectal, colonic) featured a distinctive and unique vascular architecture that was conserved among subjects. Increased operator skill, stabilization, and mechanical support of the hand piece resulted in improved video quality and increased the ease of video collection. Handheld dark field microscopy can be used as a method to subjectively assess changes in peripheral perfusion subsequent to induced hypotension and pharmacological intervention. Additional research is needed to determine the clinical significance of changes in microcirculation, response to pharmacological intervention, the relationship between anesthetized and standing interpretations and the correlation of microcirculatory parameters to clinical outcomes. MicroScan Video Microscope, Microvision Medical, Amsterdam

Keywords: Dark field microscopy, microcirculation, MicroScan, perfusion

Factors Influencing the Occurrence of Eimeria Leuckarti in Horses

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Because of emergence of drug resistance, research in parasites has been tuned for exploration of non-chemical control strategies. Knowledge about factors influencing the prevalence of Eimeria in one area or in a certain type of equine population assists in their diagnosis and effective control by enhanced more targeted use of drugs. Little is known about life cycle, morphology, epidemiology and treatment of coccidiosis in horses. So, a cross sectional survey was planned. Faecal samples were collected from whole the district using two stage cluster random sampling method and analysed by standard parasitological procedures. Of the total 484 faecal samples examined for Eimeria, 244 (50.41%) were found infected with Eimeria leuckarti. Peak prevalence was observed in August (OR=1.156; $\chi 2=20.055$) indicating higher prevalence at higher humidity while least number of animals were found infected with Eimeria leuckarti in months of April to June, being the driest period of the year in Pakistan. Wet season was found favourable for propagation of Eimeria. Foals (124/197; 62.94%; OR=0.422; $\chi 2=20.825$) and mares (196/347; 56.48%; OR=0.512; $\chi 2=13.265$) had significantly higher prevalence ($P<0.05$) of Eimeria than adults (120/287; 41.81%) and males (48/137; 35.04%) respectively. Among management and husbandry practices; Farming type, feeding system and floor type strongly influenced the prevalence of Eimeria. Coccidosis was more prevalent in mix farming, ground fed, pond watered animals and non-cemented floor ($P<0.05$) as compared to single farming, tap watered animals, trough fed and partially cemented floor type respectively. Study reports in detail the risk factors influencing prevalence of Eimeria in horses.

Keywords: Eimeria leckarti, epidemiology, horse, Pakistan

Evaluation of Serum Copper, Iron, Zinc and Macro-Mineral Concentrations in Equines

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The concentrations of serum copper (Cu), iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K) and chloride (Cl) and their interrelationships were investigated to determine the mineral deficiency based on spices, gender and age variations in equines of Urmia, Iran. Ten ml jugular blood was prepared from 78 horses and 22 mules aged up to 21 years old from both genders and sera were analyzed for minerals by Atomic absorption, Auto-Analyser and flame photometer machines. The overall mean serum trace and macro mineral concentrations were 14.5, 40.2, 7.48 µg/dl, 2.71, 1.81, 0.73, 137.5, 3.35 and 102.7 mmol/l, respectively. The concentrations in mules were greater than in horses but were not different. With the exception of P and Na, the mean trace and macro-mineral concentrations in stallions were greater than in mares, but only Fe and Ca concentrations in mares were lower than in stallions ($P<0.01$). The lowest concentrations for minerals were in 7-21 years old except for Mg which was 1-6 years old. The age differences for minerals were not significant except for Cu, Fe and Mg which were close to significant difference ($P<0.01>0.05$). There was significant correlation between Cu/Fe, Cu/Zn, Fe/Zn, Ca/Mg, Na/Cl, P/K and K/Cl concentrations. The correlation between Cu/Zn, Ca/Mg and Na/Cl was stronger and higher than others. The concentration of minerals in Urmia equines were normal and do not influence by spices, gender and age, except for Fe and Ca which were low in mares at upper ages and Mg in lower ages. The correlations among minerals reveal their close corporations in the equine body. Therefore, Urmia equines did not suffer from mineral deficiency, although adult mares probably susceptible to Fe and Ca deficiencies as well as Mg in lower ages.

Keywords: Age, equines, gender, spices, trace macro minerals

Use of Synthetic Mesh to Ventral Hernia Repair in Pigs Using Laparoscopic Method

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Ventral hernias are common problem in animals. Due to this fact the fields of research and improvement of the materials using in hernia repair need to be done. Hernias may occur as congenital or the result of an injury or trauma. Hernia repair can be performed with classical method – by replacing of abdominal contents and closing the defect with stitches or new techniques such as laparoscopic surgery and applying synthetic mesh. Meshes for hernia repair gain raises in popularity owing to their special properties like optimal tissue integration, minimal shrinkage, tensile strength, the elasticity and bending resistance. Information found in literature indicates that surgical mesh may reduce the rate of hernia recurrence as well as recovery time of the patient. The aim of our experimental study was evaluation of usefulness various types of synthetic mesh used in ventral hernia repair. Hernia was experimentally induced by middle line incision. Observation of changes in tissues located around implanted mesh was made using diagnostic laparoscopy. This study was carried out on 9 pigs of polish large white breed weighting 25-30 kg and aging 3 months. Three mesh type for hernia repair were used: Optilene Mesh, Premilene Mesh and Omyra Mesh. Abdominal structures were observed and evaluated by diagnostic laparoscopy to confirm the presence or absence of adherences and other changes in tissues, especially in area of implanted mesh. In all pigs adhesions between implanted mesh, liver lobes, omentum, spleen or intestines were presented. Bleeding during ventral hernia induction has occurred, which presumably caused formation of fibrous bands. This study has shown that Optilene Mesh caused the least adhesions with surrounding organs. We are waiting for results of the histopathology of the material collected from pigs. In the presentation these results will be presented. We can conclude that meshes can be successfully used to hernia repair because of its safety – lack of interaction between mesh and organism, reduction risk of infection; easy implantation and shorter operation time.

Keywords: Hernia repair, laparoscopy, mesh, pigs

Overexpressions of ADAMTS-13, Neuronal Nitric Oxide Synthase and Neuron Specific Enolase Relate with Severity of Neuropathology in Streptozotocin-induced Type 1 Diabetic Rats

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Hyperglycemia plays a critical role in the development and progression of diabetic encephalopathy. Over the long term, diabetes leads to serious consequences in brain. A few studies have focused on A Disintegrin And Metalloprotease with Thrombospondin type I repeats-13 (ADAMTS-13) expression in the central nervous system (CNS), and its function continues to remain unclear. The purpose of this study was to evaluate the effects of ADAMTS-13, nitric oxide (NO) and astrocyte function in experimental streptozotocin-induced diabetic rats as well as the underlying pathophysiologic mechanisms. For that, twenty male Wistar albino rats were randomly divided into following two groups (10 for each group): control group Group I (normal control) received only vehicle 0.5% aqueous carboxymethyl cellulose. Group II diabetes was induced via a single intraperitonealy injection of streptozotocin (STZ) (65 mg/kg body weight). At the end of 20 days of experiment period, the rats were sacrificed, brain tissue samples were taken from rats for histopathologic and immunohistochemical analysis. Expression levels of ADAMTS-13, neuronal nitric oxide synthase (nNOS), glial fibrillary acidic protein (GFAP) and neuron specific enolase (NSE) in the brain tissues were immunohistochemically analysed. Results of the study revealed that the levels of ADAMTS-13 ($p<0.05$), nNOS ($p<0.001$) and NSE ($p<0.001$) expressions in the brain tissue markedly increased while GFAP activity decreased ($p<0.001$) in STZ-induced diabetic animals than in the control. The results indicate that the interaction of ADAMTS-13 and NO may play an important role in the regulation and protection of the CNS microenvironment in STZ-induced diabetic animals. The results also clearly indicated that increased levels of NO might contribute to neuropathology related with STZ-induced diabet. Furthermore, expression of NSE and inhibition GFAP might gives an idea of progress of the disease and critical for diagnostic significance of this disease. To the best of the authors' knowledge, this is the first report on ADAMTS-13 expression in the CNS of STZ-induced diabetic animals.

Keywords: ADAMTS-13, neuropathology, nitric oxide, type 1 diabetes mellitus

Embryological Development of Organs of Japanese Quail (Coturnix Japonica) During Incubation

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In present study some of the internal organs of Japanese quail (Coturnix japonica) embryo were selected for their pre-hatch growth and development. For this purpose 10 groups of eggs were made such that each group having five eggs. They were weighed, labelled and placed in incubator at relative humidity 65-70% and temperature 37oC. The average weight of the eggs was 9.58 grams. In order to collect organs, one group of the eggs was taken from the incubator on daily basis starting from eighth day of incubation. The 12 organs namely brain, eye, heart, kidney, tongue, esophagus, proventriculus, gizzard, intestines, liver, trachea and lungs were collected from each embryo. Weight of the embryo collectively and the weight of each organ individually; was measured till the hatching day (day 17). In addition to weights, the lengths of esophagus, trachea and tongue were also measured to the nearest millimeter. The Janoscheck growth curve function was fitted to age group means by the non-linear regression procedure of Paul (1975). The characteristic parameters of growth curve of all organs were also studied. The curves based on measured and predicted means and resulting growth curves for each organ studied have been graphically illustrated. The graphic presentation of the results revealed sigmoid and exponential growth curve for most organs.

Keywords: Embryo, growth curve, internal organs, Japanese quail

Hepatitis E: First Detection of the Virus from Wildlife in Greece and Phylogenetic Analysis of Virus Strains

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Hepatitis E virus (HEV) can cause acute liver disease in humans. The virus is an emerging non-enveloped positive strand RNA virus with worldwide distribution and the only member of the Hepeviridae family. Seven genotypes of the virus have been recognized. Genotypes 1 and 2 are found exclusively in humans, genotypes 3 and 4 are found in humans and animals, genotypes 5 and 6 have been detected in Japanese wild boar and genotype 7 has been isolated recently from dromedary camels. Wild and domestic animals have been identified as potential reservoirs of the virus. The study objective was to assess the virus circulation in the wild boar and cervid populations in Greece and to genetically characterize the isolated strains of the virus. In collaboration with local hunting associations, whole blood and tissue samples have been collected from hunter-harvested and/or found-dead animals, during the hunting seasons 2010-11 to 2014-15. In total, 73 wild boars, 23 roe deer, 2 red deer and 1 fallow deer samples have been collected and tested for presence of HEV RNA. RT-PCR and sequencing was performed using primers which amplify a part from the ORF2 region of the HEV genome. Molecular evolutionary analyses were conducted on nucleotide sequences of HEV strains detected in wild boar in Greece and on HEV sequences that were retrieved from the EMBL database, using the program MEGA 6. Phylogenetic distances were calculated by the Kimura-2-parameter method and unrooted trees generated based on the neighbor-joining method. A bootstrap analysis with 1000 replicates was included. Nine samples from wild boars (12%) were found positive and the presence of HEV nucleic acid was confirmed by sequencing. A different virus strain was genetically characterized from each of the nine wild boars and designated by the codes GRE-WB1 to GRE-WB9. The Greek strains from wild boars clustered with isolates from genotype 3. No virus antigen was detected in samples from wild cervids. The results of the study confirm that HEV is circulating among wild boar populations in Greece. To our knowledge, this is the first report of detection of HEV RNA from wildlife in Greece. Presence of HEV RNA in wild boar samples implies that wild boar can possibly may act as a potential source of HEV infection in humans. Further studies are needed to fully elucidate the epidemiology of HEV in wildlife and the foodborne zoonotic transmission risks.

Keywords: Hepatitis E, phylogenetic analysis, wildlife, zoonosis

The Role of Academic Veterinary Medicine in Combating Antimicrobial Resistance

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The Association of American Veterinary Medical Colleges (AAVMC) has joined with the Association of Public and Land-grant Universities (APLU) to form a task force to address antimicrobial resistance. The task force was established in response to the recent White House report from the President's Council of Advisors on Science and Technology, "Combating Antimicrobial Resistance." The task force is comprised of representatives from U.S. colleges of veterinary medicine and colleges of agriculture, as well as key representatives from the production animal agriculture community and the pharmaceutical industry. The goal of the task force is to advise the federal government on a research agenda and help to publicly disseminate information of the use of antibiotics in production agriculture. Officials from key federal agencies serve as observers and leaders from public universities in Mexico and Canada are serving as ex officio members. This presentation will provide an update on the activities of the task force in the areas of research, education, stewardship, outreach, surveillance, and global health.

Keywords: Antimicrobial resistance, education, stewardship

Genetic Analysis of Resistance to Nuclear Polyhedrosis Virus in Silkworm, *Bombyx mori* L

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The nuclear polyhedrosis virus, producing the fetal and destructive disease of grasserie in silkworm, is accounted as one of the most important reason for production loss in sericulture industry. In the current study, in order to evaluate genetic resistance of silkworm lines of 154 and 104 to grasserie, virus inoculation in various generations (i.e. Parental (P1 and P2), F1, F2, BC1, BC2, RBC1 and RBC2 generations) was performed. Collected data were analyzed through generation mean analysis method and applying individual and joint scaling test. Results indicated that beside additive and dominant effects, additive-aditive epistatic effects influence the genetic resistance of silkworms to grasserie. Dominance ratio of the studied trait indicated that resistance to grasserie is controlled by over dominance. Heritability of resistance to grasserie was variable from a low to medium degree. The results indicate the importance of dominant effects and therefore the importance of applying crossbreeding for genetic control and improvement of resistance to grasserieat in silkworm.

Keywords: Genetic, nuclear polyhedrosis virus, resistance, silkworm

Prevalence of *Escherichia coli* O157:H7 in Cattle and Slaughterhouse Wastewater; Virulence Genes and Antibiotic Resistance Profiles of the Isolates

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The aims of this study were to investigate the prevalence and seasonal distribution of *Escherichia coli* O157:H7+/H7- in cattle at slaughter and slaughterhouse wastewater between July 2011 and June 2013 over a two years period in Turkey, determine the virulence gene profile of the isolates by multiplex PCR, assess the minimal inhibition concentration (MIC) of 20 different antibiotics (ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, cefotaxime, ceftriaxone, cefaclor, aztreonam, gentamicin, amikacin, kanamycin, tobramycin, streptomycin, tetracycline, ciprofloxacin, norfloxacin, nalidixic acid, sulfamethoxazole, trimethoprim and chloramphenicol) on *E. coli* O157 isolates by the Epsilometer test (E-test) for the phenotypic resistance profiles and find out the antibiotic resistance genes of the isolates by PCR. For this purpose, a total of 744 samples belong to 240 cattle and 24 slaughterhouse wastewater were subjected to immunomagnetic separation (IMS) based cultivation technique to isolate *E. coli* O157. Cattle samples (720 samples) were categorized according to age, gender, breed and sampling site (240 rectoanal mucosal swap [RAMS], 240 carcass sponge and 240 bile samples). Verification (*rfbEO157*), identification (*fliCh7*), detection of major virulence factors (*stx1, stx2, eaeA, hly, lpfA1-3* and *espA*), intimin variants (*eae-α1, eae-α2, eae-β, eae-β1, eae-β2, eae-γ1* and *eae-γ2/ϑ*) and shiga toxin variants (*stx1c, stx1d, stx2c, stx2d, stx2e, stx2f* and *stx2g*) of the isolates were assessed by PCR. A total of 102 *E. coli* O157 colonies (99 sorbitol negative [NSF] and 3 sorbitol positive [SF]) belong to 10 (4.2%) RAMS, 11 (4.6%) carcass sponge and 5 (20.8%) slaughterhouse wastewater samples were isolated. Overall, 17 (7.1%) and 15 (6.3%) of 240 sampled cattle were shown to harbor *E. coli* O157 and *E. coli* O157:H7, respectively. Statistically significant differences between categories; season, age, gender and breed of cattle were not observed ($p > 0.05$). None of the isolated *E. coli* O157:H7+/H7- strains harbored any of the investigated intimin types other than *eaeγ1* or shiga toxin variants *stx1d, stx2e, stx2f* or *stx2g* while all were *lpfA1-3+* except 5 *E. coli* O157:H7- strains. Intimin variant *eaeγ1* and shiga toxin 1 variant *stx1c* was detected from all of the *eaeA+* (97/102) and *stx1+* (32/102) strains, respectively while from *stx2+* (80/102) isolates, both *stx2c* (68/80) and *stx2d* (12/80) variants were determined. Of 93 NSF *E. coli* O157:H7, 19 were resistant to tetracycline and sulfamethoxazole, 14 to trimethoprim, 13 to cefoxitin, 11 to streptomycin, 10 to ampicillin, 8 to chloramphenicol, 6 to cephalothin, 4 to cefaclor, 4 to aztreonam and 4 to nalidixic acid. In 6 of the *E. coli* O157:H7- isolates, tetracycline resistance was detected while 5 of them were also resistant to ampicillin, sulfamethoxazole and trimethoprim. In PCR analysis, 26.0% of the NSF *E. coli* O157:H7+ and all of the *E. coli* O157:H7- isolates harbored one or more antibiotic resistance genes. While *tetA, tetB, tetC, strA, strB* and *sulI* genes were detected from a number of the isolates, *tetD, tetE, tetG, cmlA, floR, sulII, aadA* and *ampC* genes were not detected in any of the isolates. Results showed that, slaughterhouse wastewater is a significant source of *E. coli* O157:H7. Isolation and molecular characterization of SF *E. coli* O157:H7 is a new finding. So future studies should be focused on the detection of the prevalence of SF *E. coli* O157:H7, if there is a necessity for a revision in the reference isolation method. Also *E. coli* O157:H7+/H7- isolates were detected as highly resistant to selected antibiotics. Most of the isolates' phenotypic resistance profiles, did not correlate with the presence of antibiotic resistance genes.

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Keywords: Antibiotic resistance, cattle, E. coli O157:H7, shiga toxin variants, slaughterhouse wastewater

Evaluation of Antibacterial Activity of Graphene, Graphene Oxide and Reduced Graphene Oxide in Food-Borne Pathogenic Bacteria

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The aim of the study was to evaluate the antibacterial effects of graphene (G), graphene oxide (GO) and reduced graphene (rGO) in selected Gram-positive and Gram-negative bacteria, considered being particularly important in food safety ensurance. The graphene oxide was prepared by modified Hummers method. After purification part of the material was then used in further biological experiments. The rest of GO was chemically and hydrothermally reduced. In this manner, partly reduced graphene and graphene were obtained. Scanning electron microscope (SEM) method was used in order to assess the size and shape of the flakes of graphene-based materials. To examine the quality of the materials Raman spectroscopy was applied. As Gram-negative and Gram-positive bacterial model, *Campylobacter jejuni* (ATCC 29428) and *Staphylococcus aureus* (ATCC 25923) were used respectively. Bacterial strains, in the stationary phase of growth, were treated with three different graphene-based materials (G, GO, rGO) in two different concentrations (200 and 400 µl/ml) each. Samples were incubated at room temperature under 200 rpm shaking speed for 2, 4 and 6 hours. Quantitative bacteriological analysis was carried out in order to determine the number of surviving bacterial cells and the log10 reduction level was calculated. In order to confirm the mechanical damage to the bacterial cells by an extremely sharp edges of graphene-based materials, measurement of the efflux of RNA was conducted. SEM observations and Raman spectroscopy studies proved the microstructural and structural changes taking place during the reduction of graphene oxide. As found, this differences have a crucial impact on the antibacterial activity. Results show that GO sheets, in both 200 and 400 µl/ml concentrations, present the greatest antibacterial activity among the three types of graphene-based materials – 1D reduction level. For both bacterial models, reduction was observed during the first 2 hours of nanoparticles-bacteria contact. The prolongation of the incubation time, did not increase the reduction. There was no evidence of increasing the loss of bacteria viability in a concentration-dependent manner. Measurement of RNA concentration in the reaction medium confirms the efflux of the cytoplasmic materials of the bacteria, which was assigned to the cell membrane damage caused by sharp graphene edges. There were only few studies on the bactericidal activity of graphene-based materials on foodborne pathogens so far. Presented results are consistent with those available in the literature. However, the conclusions presented by other researchers on the strong bactericidal activity of graphene, are too far-reaching. Since the maximum bacterial reduction level was not higher than 90% (1-log reduction), the practical use of graphene-based materials seems to be very limited. Nevertheless, graphene oxide in conjunction with other control measures may contribute to improve of hygiene and safety level.

Keywords: Antimicrobial activity, foodborne pathogens, food safety, graphene, graphene oxide, reduced graphene oxide

Surveillance for Blue Tongue Virus Detection in Wild Cervid Samples in Greece during the Current Outbreak: Results of an Ongoing Study

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Since May 2014, a bluetongue outbreak occurs in Greece and has spread across the country; in total, 73,806 sheep, 1,492 goat and 89 cattle have been affected thus far. Reported morbidity rates are 30.0%, 26.0% and 14.5%, respectively. The outbreak strain is considered to be a reassortant strain of Bluetongue virus serotype 4, containing genome segments from serotypes 1, 2 and 4 isolates that have been circulating in the Western Mediterranean and North African countries in recent years. Objective of this study was to determine presence of Bluetongue virus in wild cervids in Greece. In collaboration with local hunting associations, spleen and whole blood samples were collected from hunter-harvested and/or found-dead cervids during the hunting season 2014-2015. Samples from 23 roe deer, 2 red deer and 1 fallow deer have been processed for presence of Bluetongue virus RNA. A one-step RT-PCR was performed, according to an OIE prescribed protocol for international trade using primers to amplifying a 274 bp region of VP5 (NS1) gene of the virus. Data regarding location of each sampling site were analyzed with Geographical Information System (GIS) using the software ArcGIS Desktop 10.1, three applications (ArcMap, ArcCatalog, ArcToolbox) and Google Earth. BTV-4 presence was confirmed in 4 samples; this was confirmed by sequencing. Three samples were collected in Koziakas hunting area (total surface 483 km2) and one was collected in Drama in Northern Greece (dead animal). All 4 samples were collected from roe deer. In the Koziakas hunting area, according to GIS analysis, there are 545 permanent livestock farms, out of which 478 are farms with sheep and goats numbering 44,215 animals (range 8-415). Cattle population reaches 5,849 animals. These numbers are even higher, if taking into account presence of sheep flocks / goat herds in semi-nomadic state moving through the area. Within a buffer zone of three kilometers from the collection sites of the positive samples, there were 25, 24 and 73 livestock farms respectively. Mean distance of positive roe deer samples from livestock farms was 840±108 m. The findings provide evidence that BTV infects wild cervid populations in Greece and supports the potential of wild cervids as bluetongue maintenance and/or spill-over hosts, considering co-existence of domestic and wild ruminants, as shown by the GIS data presented. However, the possibility that wild cervids are BTV reservoirs in Greece warrants further and larger-scale investigation. The role of wild ruminants in bluetongue epidemiology is a source of perpetual confusion in disease understanding. Nowadays, researchers consciously avoid imparting a specific role in wild susceptible species. Current opinion suggests that wild ungulates interact with virus and the competent vectors, through complex and multiple mechanisms, for which little is known. The recent outbreaks of bluetongue in Europe have raised concerns regarding involvement of wild ruminants in bluetongue surveillance, specifically about the potential contribution of wild deer in virus expansion and annual re-emergence. Preliminary results in our study demonstrate exposure of roe deer to bluetongue in Greece, implicating wild cervid species in the outbreak currently ongoing in the country. Hence, it is of significant importance to include wild ruminant surveillance programs in strategies for controlling bluetongue in a region.

Keywords: Bluetongue, GIS, Greece, wild ruminants

Development and Standardisation of Elisa for Diagnosis Fascioliasis in Cattle of District Sargodha

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Control of bovine fasciolosis remains hampered by the limitations of the currently available flukicidal drugs and that there is no successful vaccine against fascioliasis. Most flukicides have low efficacies against immature stages of Fasciola and there is evidence for the development of drug resistance. This makes research into the prediction of risk periods, exploration of epidemiology of disease and thus the optimum application of available drugs more pertinent. Diagnosis of the disease through conventional methods is achieved at 14th week post infection but major hepatic damage due to migration of flukes has been occurred upto to that time. Furthermore, this method does not work in low parasitic burden. So, early diagnosis of the disease is helpful for control and treatment. Early detection of Fascioliasis with highly sensitive assay and enhanced epidemiological understanding of disease will improve the health status of livestock and thus result in poverty alleviation of livestock farmers. Serological and coprological survey of bovine fascioliasis was conducted in cattle of district Sargodha, Pakistan. In first phase, an indirect ELISA was developed and standardized using indigenous Fasciola (F.) gigantica excretory/ secretory (ES) antigens of cattle of district Sargodha, Pakistan. Livers and fecal samples of 146 cattle and 184 buffaloes were collected from slaughter house and examined for presence of any Fasciola in bile ducts and ova in feces respectively. Blood samples of same animals were collected in gel and clot activator vacutainers and serum was separated. ES antigens were prepared by incubating adult Fasciola in PBS for 6-8 hrs and then filtering through 0.22 µm syringe filter. Then CBT was performed and optimum concentration of antigen and serum was determined. Rabit antibovine IgG cojugated to HRP was used as secondary antibody and TMB as substrate. Seroprevalence was found to be 38.35%. Taking liver examination as gold standard, sensitivity of ELISA was found to be 100% in cattle as compared to that of coprological examination in cattle (54.54%). Indigenous ELISA was also proved to be highly specific with value of 98.90% in cattle. Positive predictive values were calculated as 98.21% in cattle while negative predictive values were 100%. After standardization of ELISA, serological survey of bovines of district Sargodha was executed. A total of 5580 fecal samples and 600 blood samples were collected from all six tehsils of Sargodha. Sedimentation-floatation technique was adopted to identify Fasciola egg in feces. Sera were screened for presence of antifasciola antibodies by indigenous ELISA kit and DRG kit. Both kits were equally sensitive while indigenous ELISA was found to be more specific. The highest prevalence was found during month of December in both serological and coprological examination. Higher prevalence was found in Bhalwal, Sahiwal and Shahpur tehsils as compared to Sargodha, Kot-Momin and Silanwali tehsils. Risk of fascioliasis was found to be negatively associated (OR=1.181; χ^2=105.6757; P-value <0.0001) with age categories being highest prevalence of fascioliasis in >2-4 years age group and then decreasing with advancement of age. Sex was found non-significantly associated with disease. Among management practices, higher prevalence was found in grazing group (χ^2=61.3443; P-value <0.0001), pond watered and river watered group (χ^2=89.7096; P-value <0.0001) as compared to stall feeding and tap watered group.

Keywords: Cattle, elisa, epidemiology, fasciola, Pakistan

Phylogenetic Analysis and Biological Characterization of Newcastle Disease Viruses Isolated in Turkey between 2012 and 2014

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Newcastle disease (ND) is a contagious and widespread disease which leads to important economic loss in poultry industry worldwide. The causative agent of the disease is Avian Paramyxovirus serotype 1 (APMV-1) which belongs to the Avulavirus genus of Paramyxoviridae. APMV-1 strains are classified as very virulent (velogenic), virulent (mesogenic) and avirulent (lentogenic) according to their pathogenicity. The disease caused by virulent APMV-1 is defined as ND. The objective of this study was to investigate the biological, molecular and phylogenetic features of the circulating ND viruses in Turkey. It was expected that the data obtained in this study would help to understand the epidemiology of ND and also it would contribute to the control and prevention programs in Turkey. In this study, 67 ND virus isolated in Veterinary Control and Central Research Institute from chickens, turkeys and pigeons and samples sent for confirmation from other Institutes between 2012 and 2014 were examined. For virus isolation, SPF embryonated eggs, for identification of the agent real time RT-PCR (rRT-PCR) and haemagglutination inhibition tests were performed. In addition, Intracerebral Pathogenicity Index (ICPI) and rRT-PCR methods were used for pathotyping of the ND virus. Partial sequence analysis of the gene encoding the fusion protein was also used for identifying the virulent strains of the virus. At the end of the study, all of the 63 ND virus strains isolated from chickens and 1 strain from a turkey were found to be virulent by ICPI and partial protein sequence analysis. All virulent strains isolated from chickens and turkey were found to be exhibit the same motif of amino acid sequence 112R-R-Q-K-R-F117 at the cleavage site of the fusion protein. Also, all of the strains isolated (64 strains) were detected as APMV-1 genotype VII by protein sequence analysis, were found to be similar to those seen in Far East, Asia and Middle East countries. The other APMV-1 strain isolated from a turkey in 2013 was identified as lentogenic by ICPI test. At the end of partial protein sequence analysis tests, the motif that belongs to the amino acid sequence at the cleavage site of the fusion protein was 112G-R-Q-G-R-L117 and identified as genotype II which is similar to APMV-1 clone30 vaccine strain. On the other hand, 2 viruses were determined as mesogenic and virulent isolated from pigeons in 2013 and 2014. The motif that belongs to the amino acid sequence at the cleavage site of the fusion protein was determined as 112R-R-Q-K-R-F117 in these 2 isolates. At the end of F protein sequence analysis, they were found to be exhibit genotype VI characteristics similar to those were isolated in Belgium and China. In this study it was demonstrated that the isolates that caused the outbreaks of ND in Turkey were mainly of genotype VII. Also genotype VI viruses caused ND in pigeons. This is the most comprehensive study ever performed in Turkey with APMV-1 viruses. Genetic characteristics of APMV-1 viruses circulating in Turkey was also put forth with this study. The molecular epidemiologic data obtained in this study is expected to contribute to the success of control and prevention programs in Turkey.

Keywords: Biotyping, Newcastle disease virus, phylogenetic analysis, Turkey

Seroprevalence of Specific IgG Antibodies against T. Gondii in Wild Boars in Different World Regions

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The actual knowledge about the toxoplasmosis in society is limited and frequently refers only to the pregnant women, who were informed by their gynaecologists about existing problem during pregnancy. It is commonly wrongly thought that the consumption of the oocysts in the feline faeces is the main route of the infection. There is a certain lack of information about the possibility of infection by eating raw or undercooked meat including venison. From 30 to 63% people in Europe, depending on the country, are infected with *T. gondii* by this way. The main aim of this study is to present the incidence of *T. gondii* specific IgG antibodies in wild boar sera from over 35 different countries in the world and the environmental factors influencing the results. What is more, due to bad hunting habits and leaving the alimentary system of gunned game in the hunting district, the hunters contribute to the spreading of the parasite in the environment, because wild felids and other scavengers could consume it and drag on remains on vast areas. The material of the study were the wild boar sera from different world regions, which was tested with one of the serological Methods: MAT, ELISA, IFAT, HI TEST, DIRECT AGG. TEST, LATEX AGG. TEST or biological trial. The muscle tissue was tested in some of the studis by RT-PCR method in order to determine the serotype of the *T. gondii*. IgG antibodies against *T. gondii* were found in wild boars in many countries of the world e.g. US, Argentina, Brasil, Japan, South Korea, Malaysia and most of the European countries. The occurence of the antibodies in wild boars in Europe varies from 6,7% in Switzerland (150 sera tested) up to 55% in Corsica, France (1399 sera tested). There is a certain lack of standarisation of the diagnosing techniques due to the geographical differences (e.g. terrain formation, altitude above the sea level, insular areas, farm density per km sq), different labeling techniques (e.g. ELISA, MAT, IFAT, LATEX AGG. TEST, biological trial), tested animal populations (wild and farmed) and the size of the tested group (from 1399 in Corsica to 8 in Portugal). Previous studies show that the occurence of the specific antibodies againt *T. gondii* is realted to the age of the individual, farm density and the presence of domestic cats and the altitude. It was proved that the presence of the oocysts was limited to the cat defecation area - in rural and urban areas in case of domestic cats, and in sylvan areas in case of wild felids (Europe - lynx and bobcat; Asia - tiger, panther, leopard). Due to the fast growth of the wild boar population, they much more often enter the urban areas where they have contact with oocysts defecated by cats. In Meditarranean countries such as Croatia, Spain and France the occurence of antibodies in wild boars is much higher, because in warmer climate the population of feral cats is much larger.

Keywords: Antibodies, toxoplasma gondii, toxoplasmosis, serum, wild boar

Major Histocompatibility Complex and Microsatellite Variation in 5 Breeds of Iranian Sheep

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In Animal breeding, selecting parents with more polymorphic disease-susceptibility genes, can elevates the disease resistance potential of populations. Diversity of the major histocompatibility complex (MHC) genes was assessed by within region Microsatellites marker. Polymorphism of this marker could be a good indicator of MHC region diversity. More diversity of genome at MHC may led to more disease resistance in domestic sheep (Ovis aries) or other species. Moghani, Balochi, Lori, Lori-Bakhtiari and Arabi sheep were investigated. 150 DNA sample were extracted from jugular vein blood. The PCR condition was calibrated for primers and samples. Alleles and genotypes were detected according patterns dissimilarity in polyacrylamide gel electrophoresis. The data was analyzed by Arlequin software. Totally 13 alleles were observed at the MHC microsatellite locus. Allelic size range was 28 bp and Garza-Williamson index vary between 0.276-0.310. Observed and expected heterozygosity were 0.18 and 0.82 respectively. Chi square test confirms a Hardy-Weinberg disequilibrium. Phylogenic survey of breeds according to studied locus of genome express a close relation of Arabi and Lori-Bakhtiari breed. Although there was a difference between the studied breeds but entirely all of them showed high diversity in MHC region. This difference may be correlated with the geographic origins of the breeds and the agricultural use of the breeds. These breeds are characterized by a local distribution, resist to poor condition, living in extensive breeding systems, grazing in the poor pastures, linked to traditional farming system. This may be the explanation for the high degree of genetic variability. Besides, the high value of heterozygosity may be result of application of empiric selection and different breeding objectives, as well as application of uncontrolled natural mating. The result indicates a deviation of Hardy-Weinberg equilibrium because of the potential impact of selective breeding by man, history of disease prevalence and finally our sampling errors. The close geographical region of breeds is proposed as a possible explanation for phylogenic pattern.

Keywords: Iranian sheep, MHC, microsatellite, polymorphism

Effects of Different Levels of Sorghum Grain on the Colon of Ghezel×Arkhar-Merino Crossbred Lambs

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Sorghum grains with variable concentrations of tannin are becoming an increasingly important source of energy in animal nutrition. A large intake of tannins may cause bowel irritation on gastrointestinal tissues. Experiment was conducted to study the effects of replacement different levels of barley grain with sorghum grain on the duodenum. In this study sixteen male Ghezel×Arkhar-merino crossbred lambs were used. Dietary treatment were contain alfalfa hay (20% total DM), as roughage part of the diets and grain part (80% total DM) had different levels of the barley grain substituted with sorghum grain. Lambs were randomly assigned to one of the four dietary treatments in a completely randomized design assignment, in which sorghum grain was used in the levels of 0, 60, 70 and 80 percent of total ration. Colon samples were removed and proceed by routine histological techniques. The gross examination of colon did not reveal any significant pathological changes. The microscopic results showed that, increases of the sorghum grain levels resulting histological changes as increase of goblet cells, dilatation of lumen of brunner's glands in order to degeneration of apical parts of secretory units surface cells in colon. Also degeneration of surface epithelium was observed. Hyperemia and sporadic hemorrhage was observed in lamina propria and tunica submucosa of high level sorghum diet.

Keywords: Colon, histology, sheep, sorghum grain

Efficacy of Chitosan, a Natural Polysaccharide, Against Cryptosporidium Parvum Infection in vitro and in vivo in Goat Kids

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Cryptosporidiosis is a zoonotic disease caused by a protozoan parasite, Cryptosporidium parvum. In animals, it is considered as an economically important disease with clinical signs and death in young ruminants. The usual clinical course is acute diarrhoea affecting animals from 1 to 3 weeks old. Today, no drugs are fully effective in the treatment of cryptosporidiosis in man and animals. Therefore the research for new therapeutic agents is crucial. We report here details of the adaptation of in vitro culture systems (HCT-8 and Caco-2 cell lines) for C. parvum to investigate the "anticryptosporidial" activity of drugs and the results obtained with two new molecules (Chitosan NAG and Chitosan Mix). Chitosan is a sugar that is obtained from the hard outer skeleton of shellfish, including crab and shrimp. It is used for medicine. Chitosan, a natural polysaccharide compound, has been found to be active against a variety of diseases including antimicrobial and antitumoral effects. We investigated the effects of Chitosan in our two in vitro models we established in the laboratory. Paromomycin, a classical drug used in veterinary medicine, was used as a positive control. Immunofluorescence technique was used for the identification and enumeration of the parasites. Our results showed a very significant reduction of viability of Cryptosporidium oocysts (>95%) after pre-incubation of 24h at 37°C with Paromomycin ($P < 0.001$), Chitosan Mix and Chitosan NAG ($P < 0.001$). On the other hand, Paromomycin, Chitosan Mix and Chitosan NAG inhibited significantly the development of C. parvum in HCT-8 and Caco-2 cell lines ($P< 0.005$). These effects are dose-dependent. Synergic effects were obtained when Chitosan Nag treatment was associated with Paromomycin. In conclusion, these findings provide for the first time the evidence of in vitro inhibitory activities of natural polysaccharides against C. parvum. The efficacy of Chitosan was evaluated in goat neonates experimentally inoculated with Cryptosporidium parvum oocystes per oral route. The preliminary results showed the efficacy of chitosan in reducing oocyst shedding and diarrhoea in goat kids cryptosporidiosis.

Keywords: Cryptosporidiosis, kids, prevention, small ruminants, treatment

Evaluation of the Th1 Immune Response in the Intestinal Mucosa of Baby Alpacas (*Vicugna pacos*)

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The aim of the study was to evaluate quantitatively the expression of the RNA messengers (mRNA) of the transcription factors T-bet, STAT-1 and STAT-4 and the cytokines interleukin (IL-) 12, IL-2, gamma interferon (IFN-γ) and alpha tumor necrosis factor (TNF-α) involved in the activation and development of intestinal mucosa Th1 lymphocytes in clinically healthy baby alpacas (Vicugnapacos) from three age-groups of 1-8, 10-21 and 22-47 days-old, which were breed in flocks with natural grazing in the Southern Peruvian highlands. Two centimeters-intestinal samples were obtained from the middle portion of healthy baby alpacas' jejunum from the IVITA MaranganiStation (Cusco, Peru), which were stored at -196°C and processed in the Laboratory of Veterinary Microbiology and Parasitology, Immunology section, of the School of Veterinary Medicine from San Marcos University (Lima, Peru). Total RNA were extracted and the reverse transcription-PCR real time (RT-PCR) was performed. Cycle threshold (Ct) values and melting temperature (Tm) of the RT-PCR products were measured. Subsequently, the mRNA expression of the factors and cytokine mentioned above regarding to the calibrator control (fetus) were relatively quantified based on the $2^{-\Delta\Delta Ct}$ method in the three age groups using GAPDH ashousekeeping gene and endogenous control. T-bet, STAT-1 and STAT-4 kinetic expression patterns were shown positive and directly proportional to the age, exceeding in thirty five, six and three-fold the calibrator control's expression in the 22-47 days-old group, respectively. Likewise, IL-2, IFN-γ and TNF-α kinetic expression patterns were shown positive and directly proportional to the age, exceeding in twenty six, more than a hundred and five-fold the calibrator control's expression in the 22-47 days-old group, respectively ($p<0.05$). IL-12 kinetic expression patternreached it maximum in the 10-21 days-old group showing a low expression in comparison to the calibrator's one ($p>0.05$). In conclusion, the expressions of both transcriptional factors activated by gamma interferon T-bet ($p<0.05$) and STAT-1 ($p>0.05$)increase with age, as well as the three cytokines involved in the Th1 immune response. Conversely, STAT-4 and IL-12 expressions were not statistically different in the three age groups.

Keywords: Cellular immune response, intestinal mucosa, South-American camelids, transcription factors

Evaluation of the Proportion of Pregnancies in Slaughtered Livestock and the Reasons for Slaughter of Pregnant Animals

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The EU has among the world's highest standards of animal welfare and the safety of the food chain is indirectly affected by the welfare of animals, particularly those farmed for food production. The welfare of food producing animals depends largely on how they are managed by humans. Harmonised EU rules are in place covering a range of food safety- and welfare-affecting issues but a regulatory framework which governs the slaughter of pregnant farm animals is still missing. A need for action on this part has not been seen by the European Commission yet, because it was assumed that pregnant animals are only slaughtered in exceptional cases. However, first own investigations on cattle show, that the proportion of pregnant heifers raised in different European member states amounted up to 10%. These results are very similar to those seen in the United States and Asia. For other animal species no data is available until now, but there are reasonable indications, that the problem ist not only restricted to cattle. In this context, the research project „S!IGN", funded by the German Federal Ministry of Food and Agriculture was started in February 2015. The project aims at the collection of representative data on the slaughter of pregnant livestock in Germany as well as on the evaluation of reasons that lead to the establishment of this practice. Besides that, the collection of key data with regard to the slaughter as well as the stage of gestation resp. the developmental stage of the fetus should be evaluated. In case of pregnant animals, their origin and the possible causes for their disposal to the abattoir should be determined. Here, it should be ascertained in particular, whether the farmers who have intended pregnant animals for slaughter, had knowledge of the pregnancy or if the pregnancy was irrelevant for their decision to slaughter the animals. If the farmer was aware of the pregnancy, the reasons or motivation for slaughter should be elicited.

Keywords: Ethics, food chain, pregnant animals, slaughter

Tail Docking in Ruminants: Evaluation of Current Practices and Welfare Aspects in Turkey

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In order to increase animal efficiency and performance, management practices such as tail docking, cauterization, castration and dehorning are followed in detail by public. Tail docking is applied in farm animals such as pigs in order to prevent biting their tails, in sheep for protecting fly strike, and also in cows to reduce the risk of mastitis and udder hygiene. Studies have indicated that practice of tail docking causes mild-moderate acute pain and behavioral responses in sheep and protective effect of tail docking against fly strike is controversial. It is debated that the process of tail docking on increasing sheep's live weight and carcass quality is still questionable. It is argued that tail docking in cows causes relatively little pain and excess quantity of fly strike on back of cows. Furthermore, dairy cows don't bear any beneficial effect of tail docking. Tail docking in cows is decreasingly practiced in the United State and it is totally banned around the world. Although it is banned by law in Turkey, some breeders are not totally convinced about the real benefits of tail docking; therefore, tail docking is still applied in practice. This application leads to pain and behavioral disorders in cows which adversely affect to animal health and welfare. In this study, it is aimed to identify the physiological and behavioral responses caused by the tail docking in ruminant, affirm the scientific evidence for the rationale, and evaluate animal welfare and current practices in Turkey.

Keywords: Animal welfare, ruminant, tail docking, Turkish case

Dietary Effect on Apparent Plasma Glucose Absorption and Disappearance in Healthy And Heretozygote Haflinger Mares Carrying One Copy Of The GYS-1 H-Allel

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The oral glucose tolerance test (OGT) in combination with the plasma insulin response is a suitable test to diagnose equine metabolic syndrome. In carriers of the GYS-1 H-allele, glucose take up appeared to be increased. The aim of the current study was to estimate absorption and disappearance of glucose in the central compartment of healthy and PSSM-11 heterozygote Haflingers using data obtained in the OGT. In a randomized block design with cross-over six heterozygote PSSM-1 mares and 5 healthy control mares were fed only hay (H) or a hay and carbohydrate rich (CR) ration for one month. Washout time was 4 weeks. At the end of each period an OGT was performed and plasma insulin was determined. The Tmax of the PSSM-1 group was reached later than that of the control group (137 vs 111 min.). The Kabs in the PSSM-1 group was hardly affected by diet (0.77 for H and 0.76 for CR), while the control group had a mildly reduced Kabs after having fed with a CR diet. The Kelim decreased in the PSSM group from -0.41 after a hay only diet to -0.33 after a CR diet. The control horses had a greater elimination independent on diet. Insulin appears not significantly (p=0.976) affected by the character of previous diets fed prior to glucose challenge for PSSM horses, however in non PSSM horses the diet characteristics had significant effect (p=0.006) on plasma insulin concentration during the 360 minutes post glucose challenge. Glycaemic indexes however were not significantly different between the groups. In this study, using 30 minutes blood samples, PSSM type 1 horses unexpectedly showed a slower glucose absorption and slower plasma elimination than control horse. Glycogen contend in the diet decreased the elimination rate of glucose from the plasma. This counterintuitive data suggest that estimation of tissue uptake of glucose by PSSM-1 horses cannot reliably estimated from samples taken at 172 hr frequency.

Keywords: Heretozygote, mares, PSSM

Comparison of Serum Amyloid A and C-Reactive Protein Concentration with Synovial Parameters in Horses with Joint Disease

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The aim of the study was to investigate whether serum SAA or CRP were sensitive parameters for diagnosing and differentiation of septic and aseptic arthritis. Furthermore it was studied if monitoring of the repair process in the joints was possible with these markers of inflammation. The SAA and CRP was analyzed in serum. Serum samples from 53 Horses with spontaneous septic (SA) or aseptic (AA) arthritis were analyzed for serum amyloid A (SAA) and C- reactive protein (CRP) concentrations with a commercially available ELISA test kit. SAA and CRP concentrations were higher in the SA group than in the AA group. ROC curves were used to determine the best upper levels for SAA and CRP concentrations in the serum to diagnose joint sepsis. At 4 µg/ml for SAA test sensitivity was 1.0 and specificity was 0.88. Test accuracy was 0.92 and positive predictive value (PPV) was 0.8. At a cut-off level of 8 µg/ml for CRP the test sensitivity was 0.80 and specificity was 0.44 Test accuracy was 0.58 and PVV was 0.55. Therefore, SAA appeared the better more specific and sensitive parameter for accurately diagnosing a septic process in the joint. Using CRP as diagnostic parameter, too few cases will be assigned as septic while they are not.. The conclusion of this study is the SAA and CRP levels in blood are useful parameters to support the diagnosis of joint disease; however these parameters alone are not suitable to determine the character of joint disease. These parameters are suitable for monitoring disease progression or repair activity by the extent of inflammation. Inflammation is more severe r in septic arthritis than in aseptic arthritis. SAA and CRP serum levels facilitate the differentiation of septic arthritis from aseptic arthritis. The beginning of the right therapy will reduce the blood SAA, CRP concentration, the WBC and neutrophil counts in synovia faster than other parameters and they return to baseline value within one week. Synovial fluid WBC is the more sensitive parameter than SAA or CRP, because after beginning of the therapy, synovial WBC count decreased faster than any other of the studied parameters. An increase of synovial mononuclear cells is associated with healing.

Keywords: Aseptic Arthritis (AA), C reactive protein (CRP), horse, serum amyloid A (SAA), septic arthritis (SA),

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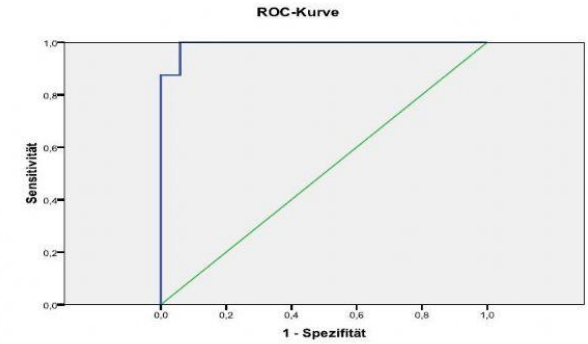
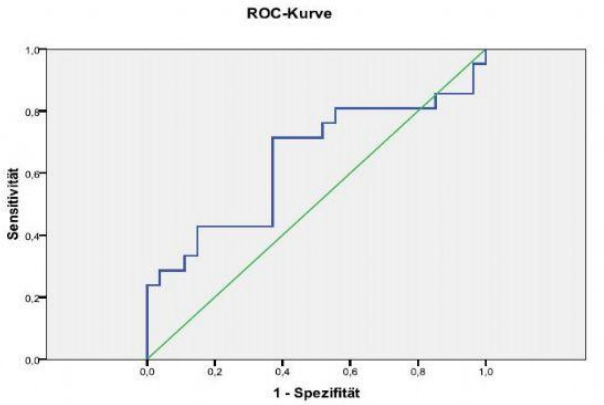
Roc curve for Serum amyloid A protein in septic groups



Sensitivity and Specifity of Serum amyloid for diagnostic value for horses in septic group

Roc-curve for C-reactive protein in septic group



Sensitivity and Specifity of C-reactive protein for diagnostic value in horses wit septic arthritis

Median serum concentrations and ranges of CRP in horses with septic and aseptic arthritis(µg/ml) on day 1,3,5,8

	Day	n	Min	Max.	Median
Septic arthritis	1	21	1	32	10.8
	3	10	1	30	10.5
	5	9	5	65	10.2
	8	4	3	21	9.2
Aseptic arthritis		27	1	18	8.8

the results of C-reactive proteins horses with septic joint disease.

Table 2: Median serum concentrations and ranges of serum amyloid A in horses with septic and aseptic joint disease on day 1,3,5

	Day	n.	Min.	max.	Median
Septic arthritis	1	8	6	10	7.4
	3	5	7	10	8.5
	5	4	6	10	7.4
Aseptic Arthritis	1	17	2	27	2.5

the results of Serum amyloid A horses with aseptic joint disease.

How to Diagnose Seminal Vesiculitis Using Color Doppler Ultrasonography in Stallions?

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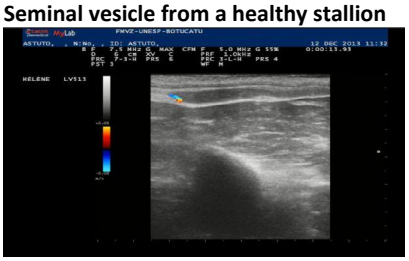
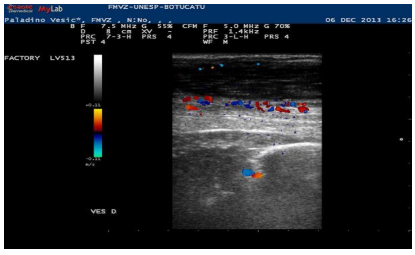
Stallions affected by seminal vesiculitis commonly present low semen quality, leading to reduced fertility. The final diagnosis is established by bacterial culture of the seminal vesicle content, performed by endoscopy. However, due this exam be invasive and not routinely used, the aim of this study was to identify possible vascular changes of vesicular gland wall from stallions with seminal vesiculitis, compared to health stallions. Six healthy stallions in reproductive activity, control group (CG), and seven stallions diagnosed with the disease, seminal vesiculitis group (GSV), were used (mean age ± standard error: 8.03±1.72 and 10.99±1.49, respectively). The diagnosis was based on semen alterations (presence of pus or blood) associated with endoscopic inspection of the glandular lumen and bacterial culture of vesicular flushing. Color Doppler assessment was performed in both groups using the ultrasound MyLabTM Five (Esaote do Brasil, São Paulo/SP). The probe was positioned by trans-rectal way, over each vesicle gland (right and left) and the images were archived. Vascular perfusion was subjectively estimated according to the percentage of gland wall presenting color signals relative to the total wall area, excluding the lumen. Data were analyzed by t-Student test and differences were considered significant when $p<0.05$. The control group showed (mean percentage±standard error) 10%±1,2^{a} of vascularization compared to 43,0%±4,9^{b} of the group with seminal vesiculitis. In conclusion, stallions with seminal vesiculitis present higher vascularization of glandular wall compared to healthy stallions. The vascularization of at least 38,1% of the glandular wall, assessed by color Doppler, may indicate seminal vesiculitis in stallions.

Keywords: Color doppler ultrasonography, diagnosis, seminal vesicle, seminal vesiculitis, stallion

Seminal vesicle from a healthy stallion



Seminal vesicle from a stallion with seminal vesiculitis



Immunologic Treatment with Dendritic Cells in Equine Squamous Cell Carcinoma

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The immunologic treatment with dendritic cell therapy in horses is well established for equine sarcoids. Today there are several other indications for the use of dendritic cells (DCs), e.g. squamous-cell carcinoma, fibrosarcoma or melanoma. The case of a 23 years old crossbred-mare with a widespread squamous-cell carcinoma dorsal of the anus is presented as an example for these indications. The chirurgical and clinical pretreatment resulted in aggres-sive and extensive recurrence of the disease. At the start of the DC therapy the tumor has a diameter of approx. 10 cm, is solitaire, partly keratinized, bad differentiated and highly infiltrative. The mare is in a bad clinical condition. She has reduced appetite in suc-cession of a gastric ulcer and cannot clear out feces by herself. 3 DC applications are produced in a 4-week-interval. In a clean room laboratory monocytes are isolated from full blood of the patient and differentiate to immature DCs. By addition of tumor lysate of the mare the cells mature. DC injection is applicated intradermally. After application, the DCs migrate towards the draining lymph nodes for presenting the processed tumor antigens to naive T-lymphocytes resulting in the induction of a tumor-specific immune response. The mare´s health is improving during the immunologic treatment drastically. She gains weight, defecation is possible without difficulty. The tumor is healing and left only a little scar.The immunologic therapy with dendritic cells can be used in complicated oncologic equine cases with a good clinical result.

Keywords: Dendritic cells, immunologic, squamous cell carcinoma, tumour

Clinical, Ultrasonographic and Bacteriological Investigations of Jugular Veins in 395 Horses During and After Intravenous Medication Using Indwelling Venous Catheters

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The aim of this study was to quantify the pathological changes in jugular veins of all horses that received an indwelling catheter during an 11 month period of time at the reporting clinic. Collected data was evaluated to assess potential associations of intravenous catheter complications in horses with various pathological problems, and to compare our results with the findings of previous studies. During an 11 month period, two types of indwelling venous catheters (Polytetrafluoroethylene, PTFE, n=272 and Polyurethane, PU, n=175) were used in a total of 395 clinical patients. Placement of both catheter types and routine examinations of the catheterised veins were performed according to standardised protocols. Ultrasonography of the jugular veins and bacteriological examination of the catheter tips were both carried out following catheter removal. In 271 jugular veins (60,9%) low grade pathological changes (swellings, haematomas) were diagnosed. Moderate to severe venous pathology (Periphlebitis, phlebitis and thrombophlebitis) was encountered in 24 jugular veins (5,4%). In 19 affected horses venous thrombosis was detected sonographically at the entrance of the catheters and at the catheter tips respectively. PTFE catheters remained in the jugular veins for a significantly shorter period of time (left: 1,1 days, right: 1,4 days) compared to PU catheters (left: 8,2 days, right: 8,7 days). Pathological problems were encountered in a higher number of veins with PTFE catheters (PU: 2,9%, PTFE: 7%). Analysis of the data revealed a trend indicative of increased risk for complications associated with the use of PTFE catheters, but the difference was not significant (p=0,059). The risk of venous complications increased significantly when PTFE catheters remained in the veins for more than 24 hours (p<0,001). A significantly higher number of right jugular veins with PTFE catheters was affected (7,5%) compared to left jugular veins (2,9%). Compared to other types of clinical cases, a higher, though not significantly increased risk of jugular problems was diagnosed in colic horses. Age and sex did not influence the risk for venous thrombosis. Icelandic horses were at significantly higher risk for venous pathology compared to other breeds. Although experienced surgeons had lower numbers of venous problems in their patients, the difference when compared to less experienced veterinarians was not significant. Bacteriologic examinations in 344 catheter tips revealed 82 positive results (23,8%). Coagulase negative Staphylococci (35%), Bacillus spp. (23,1%) and α-haemolytic Streptococci/Enterococci were the microorganisms most frequently cultured.The choice of suitable catheter materials, careful asepsis during catheter placement and meticulous management of indwelling catheters are the most important factors in the prevention of thrombophlebitis.

Keywords: Bacteriological examination, horse, jugular vein, thrombophlebitis, ultrasonography, venous catheter

Detection of Enteretoxin Production and Antibacterial Resistance of *Bacillus Cereus* Isolated from Spices Used in Meat Products

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Bacillus cereus is a Gram-positive, aerobic or facultatively anaerobic, motile, spore-forming, rod-shaped bacterium that is commonly found in soil and water. *B. cereus* is a species of bacteria which causes severe food poisoning. This bacteria produce a diarrheal syndrome induced by hemolysin BL (Hbl) and Non-haemolytic enterotoxin (Nhe). Spices are used to prepare various of meat products mainly because of their flavouring and seasoning properties. In this study, a total of 83 random spices samples, including each of 18 ground black pepper, 17 red pepper, 12 ground red pepper, 20 cummin, 16 pimenta obtained from various markets and retail shops located in Bursa province between January and December 2014. They were investigated to determine the prevalence of *B. cereus*. The samples were supplied in packaged and unpackaged forms. For the identification of *B. cereus* MALDI-TOF-MS (Bruker Daltonics, Bremen, Germany) techinque was used. Identified B. cereus isolates were also tested to detect the resistance to several antibacterial agents and toxin production. The result of the analysis showed that 40% (n=34) of spice samples had *B. cereus* count between $1x10^2$-$4.8x10^4$cfu/g. A total of 33 (97%) isolates were resistant to at least one antibacterial agent and 32 (94%) were resistant to two or more antibacterial agents. In addition, a total of 25 (74%) of the isolates reacted positive for both Hbl and Nhe, in the assay. These findings indicate that *B.cereus* was present commonly in various of spices used in meat products, accompanied by multiple antibacterial resistance and Hbl and Nhe enterotoxins. Our findings highlight the urgency for stricter hygiene strategies in spice production in pre and post harvesting period.

Keywords: Antimicrobial resistance, bacillus cereus, enterotoxin production, meat products, MALDI-TOF-MS, spices

Salmonella spp. in Raw Poultry Marketed at Ibague, Colombia

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The presence and impact of Salmonella in the poultry industry in the Tolima region is currently unknown and our recent studies reported the isolation of S. Shannon and S. Enteritidis from egg-laying hen farms (Rodríguez et al., 2015). In this paper a cross-sectional study was conducted to estimate the prevalence of Salmonella spp., in raw chicken marketed in different outlets at the Ibague city. A total of 270 samples (drumstick) of chicken of about 200 g each were randomly taken from meat stores and transported refrigerated in sterile airtight plastic bags to the Laboratory of Veterinary Diagnosis of the University of Tolima for Salmonella isolation. Salmonella was isolated by using standard microbiological tests, followed by biochemical characterization, and molecular confirmation by serotyping and PCR. A questionnaire was administered to the shop owner during an interview to evaluate potential risk factors associated with the presence of the bacterium. The prevalence of Salmonella in raw chicken was 17.41% (47/270), and 14 different serotypes were found. Serotypes Paratyphi B, Hvittingfoss and Muenster were the most prevalent and represented 65, 95% of all serotypes identified. Raw chicken as the only type of meat sold (Odds ratio: 2,157, $p<0.05$), and stainless steel as a contact surface (Odds ratio: 13, 29, $p<0.05$), were the risk factors associated with the presence of Salmonella in chicken meat. This work constitutes the first report of Salmonella spp., in chicken meat marketed at Ibague and together with our previous findings of Salmonella in egg surface, suggests that governmental institutions need to establish more rigorous control and contingency measures to minimize the presence of the bacteria in raw chicken. The presence of Salmonella in other poultry products or byproducts as well as its impact on public health of this region of Colombia need to be addressed in future studies to eventually control and prevent Salmonella contamination.

Keywords: Raw chicken, risk factors, salmonella

Antimicrobial Efficacy of UV Radiation on the Tiles Coated with Nanosized Titanium Dioxide Prepared by Various Methods

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The aim of this study was to compare the antimicrobial activity of UV radiation of wavelength 253.7 nm (used in typical germicidal lamps) against bacteria on the surfaces of conventionally produced ceramic wall tiles (matt and shiny) and the same tiles coated with titanium dioxide (TiO2) using three different Methods: RF diode sputtering, atmospheric pressure chemical vapour deposition (APCVD) and spray pyrolysis deposition (SPD).*Staphylococcus aureus* and *Salmonella* Enteritidis were used as test microorganisms. Before each experiment the test tiles, both control and coated with TiO2, were disinfected by immersion for 15 minutes in 70% ethanol, then rinsed with sterile distilled water and stored in darkness at approximately 20°C for 18-20 hours. From test tubes containing bacterial cultures in nutrient broth 0.1 cm3 was taken and placed in the geometric center of sterile tiles. Then the tiles contaminated with *Staphylococcus aureus* or *Salmonella*Enteritidis were exposed to UV radiation. Device equipped with 4 Philips TUV lamps T5 16W/G16 emitting radiation of UV-C of 253.7 nm wavelength was used in the experiments. Tiles were exposed to UV radiation for 0, 60, 90 and 120 seconds. After exposure bacterial suspension was collected from the surface of each tile using sterile swabs. The tips of swabs were cut off with sterile scissors and placed in test tubes containing 4.9 cm3 of diluent. From each test tube 1 cm3 of suspension was collected to prepare a series of decimal dilutions followed by plating onto Baird-Parker Agar to determine *Staphylococcus aureus* count, or plating onto Brilliant Green Agar to determine number of *Salmonella* Enteritidis. Plates were incubated at 37°C for 48 hours under the aerobic conditions. After incubation the colonies were counted, bacterial counts were multiplied by the appropriate dilutions and numbers of bacteria (colony-forming units) on the entire surface of each tiles were calculated. The bacterial counts were transformed into logarithms and statistically analysed using the General Linear Models supplied by PASW Statistics 18 Edition 18.0.0. All experiments were performed in three replications. Results indicate that the bactericidal action of UV radiation is much stronger on the surfaces of tiles coated with TiO2 than on the tiles uncovered. The strongest bactericidal effect of UV radiation was found for film prepared by APCVD method. Results of experiments for shiny and matt tiles did not differ statistically. The tiles coated with nanosized titanium dioxide present alternative to traditional disinfection methods, which are work and time consuming. The use of such ceramic wall tiles in slaughterhouses, food processing plants, cutting plants, catering outlets and wherever UV radiation is applied to disinfect the surface, should greatly improve the disinfection effectiveness and contribute to a radically improvement of hygienic conditions in those areas.

Keywords: Food hygiene improvement, nanotechnology, titanium dioxide, pathogens inactivation

Evaluation of Activity against *Listeria* of Newly Isolated Bacteriophages by *in Vitro* Efficacy Tests

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Listeria monocytogenes (*L. monocytogenes*) is a foodborne bacterial pathogen able to grow at refrigeration temperatures, tolerating a wide range of pH and salt concentrations. For these features even low contaminations at production level are dangerous if the shelf life of the product, especially Ready To Eat (RTE) foods, is longer than a few days. Moreover a serious problem is related to biofilm formation and persistent bacteria within food chain productions (1). To control this hazard in food, new strategies are needed. Among them, bacteriophages hold attributes that appear to be more and more attractive (2). Phages are bacterial viruses that invade specific hosts, disrupt bacterial metabolism and cause its lysis without compromising the viability of other flora in the habitat (3). The objective of this study was to investigate the *in vitro* efficacy of bacteriophages isolated from the environment in order to control *L. monocytogenes* on food and within food productions. In this study *L. monocytogenes* strain ATCC-7644 was used to propagate ϕIZSAM-1 while *L. monocytogenes* strain ATCC-19115 was used for the other four phages (ϕ72; ϕ73, ϕ010120/1 and ϕ10022/27). Each bacteriophage was assessed as a pool and as pure isolates from single plaques. The host strains were grown at 30 ± 1°C overnight in BHI. For the experiments, 24 well plates were used and in each well, a concentration of *Listeria* ranging from 10^2 to 10^7 ufc/ml was added. Each phage was then individually inoculated at concentrations able to cover a wide range of Multiplicity of Infection (MOI) (0.01; 0.1; 1; 10; 100; 1000; 10.000) and the plates were incubated at 30 ± 1°C for 24 h. Phage p100 and *Listeria* strains without phages were used as controls. Every 2 h optical density at 600 nm was measured and compared to control cultures to evaluate phage efficacy against bacterial growth. All bacteriophages as pool and pure lysates were efficient in controlling *L. monocytogenes*. In particular MOI 1, 0.1 and 0.01 gave the best results in terms of phage replication (increasing of phage titre) and of ability to keep*L. monocytogenes* at low levels. *L. monoctogenes* was shown to be kept 2-down when phages were applied. Potential *Listeria* resistant strains were still sensitive to other phages of the same pool. Phages showed a better lytic activity when applied at lower MOI. It would suggest that the best results *in vivo* are expected by applying low phage concentrations. Rotation of phage cocktails in a food industry environment could help in keeping *L. monocytogenes* at low levels, enhancing food quality and consumer's safety.

Keywords: Bacteriophage, foodborne pathogens, listeria monocytogenes, ready to eat

Prospective of Veterinary Education in Latin America

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Latin America is integrated for19 countries with a population of approximately 600 million inhabitants and a surface area of more than 22 million Km², which is equal to 13.5% of the planet's surface. In the field of animal production, is theworld's leading exporter of beef meat and poultry meat, and the thirdlargest exporter of pork meat. Consideringthat livestock products provide one-third of the protein consumed by humanity and that by 2050 the livestock production has been anticipated to be twice the one in 2000, there is no doubt of the importance that Latin America has and will have in the near future. The high development of countries such as China, India and Latin America will increase their income and the population of these countries will aim to have a better quality nutrition, including more quantity and better quality of animal protein. Latin America is recognizing the importance of the one health concept, mainly because of both, zoonotic diseases and epidemics, which have occurred in recent years around the world. An example of this is the Flu PandemicAH1N1 in humans and AH5N1 (avian/bird flu). Another important field for Veterinary Medicine is companion animals; the worldwide importance of pets and companion animals for society is out of doubt. In Latin America for example, Mexico has 18 million dogs and 5 million cats; in Argentina 38% of households have a dog. Only between Argentina, Brazil and Colombia, 3000 tons of pet food are exceeded. In this way, there are problems that have to be solved,like climate change and pollution caused by animal production;for example, livestock is responsible for 18% of greenhouse gas emissions, 37% of atmospheric methane is produced by rumen fermentation and,last but not least, the world is heading for a shortage of water. To analyze this and other problems Veterinary Medicine Education in Latin Americahas been focused onthe future of animal production, animal health, public health and food safety. Veterinary Education in Latin America: In 1992, under the auspices of the Pan-American Association of Veterinary Sciences (PANVET) an International organization was integrated named Pan-American Association of Veterinary Medicine Colleges, its membership includesnow a daysVeterinary Medical Colleges from 17 countries. The objectives of the Federation includes mainly: to promote periodic review of veterinary curriculain order to orient the veterinary education according to realities of the region. Analyze and recommend the Competencies of graduating veterinarians in Latin America. Two International meetings with Deans and representatives of countries from Latin America. The first meeting took place in 2013 in Peruvian University Cayetano Heredia in Lima, Peru, with representatives of 13 countries to stablish the professional skills of the graduating veterinarians in areas like Sustainable animal production, Public health, Food safety and Animal health, in accordance to the skills proposed by OIE. The second meeting took place in 2014 in the University of Buenos Aires, Argentina, with representatives of 14 countriesfrom Latin America to stablish the “Professional Veterinary Medicine Profile in Latin America - Vision 2030 –“; it considered the future of the veterinary education in Latin America. Veterinary Medicine Competencies provide an important pathway to envisioning the future ofthe Veterinary profession in Latin America.

Keywords: Education, Latinamerica, PANVET

Use of Social Media in Veterinary Public Health Teaching/Learning to Enhance Student Engagement and Experience

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Despite the worldwide recognised role of the veterinary profession in protecting human health, veterinary public health (VPH) is a subject which is normally perceived as difficult and non-highly relevant in the veterinary curriculum at Nottingham (SVMS). That misconception is partially linked to the wrong belief that VPH means post-mortem slaughterhouse inspection (as part of the official controls) only. However, the wider role of veterinarians in food safety issues as the well as the link between animal disease control and prevention, transboundary diseases etc. with VPH tends to be overlooked by students. Information in VPH topics is freely available on the internet, but students do not necessarily find it easy to find or they may struggle identifying which on-line sites may contain reliable information. Additionally, the access to images as teaching material in Veterinary Public Health (VPH) is not always easy. Issues arise due to copyright issues and because of confidentiality matters. The use of images for food safety teaching in VPH is especially critical as the risk of increasing the risk of cross contamination during visits reduces the chances of having access to these facilities, making difficult to offer a consistent exposure to the students in some areas. As most of our students rely on social media for interacting it was though that using social media in order to inform students of the new developments in VPH could be a useful tool to highlight the relevance of this particular topic. Two social media, flickr and twitter have been used at the SVMS in order to raise student awareness and encourage topics of VPH relevance worldwide. A twitter account was opened (@vetpubhealth) where updates from international bodies (World Organisation for Animal Health (OIE), World Health Organisation (WHO), European Food Safety Authority (EFSA), Federations of Veterinarians of Europe (FVE)) and national organisations (Food Standards Agency (FSA), DEFRA) are continuously posted. For visual material a pre-existing Open Educational Resource (OER) of the School of Veterinary Medicine and Science, University of Nottingham (https://www.flickr.com/photos/nottinghamvets/sets/) was considered to be the most suitable option to produce VPH photo albums that could be accessible for students and staff alike as an OER. With the agreement of Food Business Operators (FBOs) pictures from food several processing facilities were taken and uploaded to the website. Additionally, in order to give students a broader perspective of VPH images obtained from several countries have been included. It is not compulsory for SVMS students to open an account or to interact on twitter; however, currently several veterinary students at Nottingham choose this social media to clarify their doubts in VPH or to discuss VPH topics that appear in the media. The twitter platform has been also useful to identify colleagues in the UK and abroad willing to publish and discuss their cases, helping to create a useful working network. Additionally an international photo competition in VPH for veterinary students was organised and publicised through twitter. The pictures from the competition were also included in the flickr VPH albums. Pictures uploaded to the VPH albums on the SVMS flickr account are currently used by SVMS lecturers in several modules (not only in VPH) as well as by colleagues abroad. The VPH picture database is constantly increasing (pictures of VPH practical, zoonoses, animal production systems) becoming a valuable image resource for teaching/learning purposes. Activities carried out have received positive feedback from students and have led to further initiatives in collaboration with other institutions.

Keywords: Flickr, online resources, social media, twitter, veterinary public health

Should There be Award Scheme to Recognise Excellence in Animal Welfare During Veterinary Education?

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World Animal Protection is aiming to establish a system to recognise good practice in animal welfare within veterinary education. The aims of this initiative are to: 1. Establish ten key areas of teaching, research and organizational culture through which veterinary schools show excellence in animal welfare practices; 2. Embed these standards in all veterinary schools to improve animal welfare in education; 3. Influence future vets to have a thorough grounding in animal welfare. With the support of the World Veterinary Association and the World Organization for Animal Health, World Animal Protection conducted an online survey from April – October 2014 investigated the following four key themes: 1. The benefits of improving animal welfare education 2. The criteria for welfare standards of excellence award scheme 3. How to assess vet schools against the award scheme standards 4. What the awards scheme system should be called. The number of respondents was 2,614 from 97 countries. Over half of respondents were female (57%). Responses were analysed in terms of the global average, after which they were broken down by region and socio-demographic variables. Analyses so far indicate strong support for animal welfare education from the veterinary profession, motivated primarily by the desire to ensure better treatment of animals. The response was consistent across professional groups (vet educators, students, practising vets and other professionals) and regions of the world. More than 80% of respondents supported each of the ten proposed criteria for standards of excellence in animal welfare, and more than 50% were in strong agreement. The results of further analyses will be discussed. It is concluded that a system for recognition of excellence in animal welfare at veterinary schools is supported by veterinary schools and has potential to support and encourage demonstration of good practice in animal welfare by veterinary schools.

Keywords: Animal welfare, veterinary education

Veterinary Education on Animal Welfare Legislation: on Overview of Italian Students' Knowledge and Perception

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There is pressure from the global community to make animal welfare (AW) a fundamental part of veterinary education. Both the OIE and the World Veterinary Association recommend the compulsory teaching of animal welfare as a separate subject, using a multidisciplinary approach encompassing ethics, economics, and animal behaviour as these relate to animal welfare. The recently concluded EU-funded project AWARE drawn a clear picture of animal welfare education at university level in Europe, bringing evidence that more intensive education on AW is given in North West Europe than in any other region. To further investigate on this aspect, a survey was carried out to assess the knowledge of Italian veterinary students on animal welfare, focussing in particular on EU animal welfare legislation (AWL). A total of 440 students, all attending the second last year of course, were interviewed in twelve Italian Veterinary faculties. The second last year of course was chosen since at this stage usually Italian veterinary students have already approached the animal welfare topics within Ethology, Veterinary legislation and Clinical courses, therefore their level of knowledge is comparable and it is not biased by the specialisation courses usually attended during the last year of study. The survey was divided into two main sections. The first part investigated: on the personal knowledge on AW, asking students to self-evaluate their own on a five-point scale from no informed through to very informed (Q1); on the obligatoriness of AW teaching in the attended faculty (Q2); and on the perceived appropriateness, or need of improvement, of the AW legislative framework (Q3). The second part investigated about knowledge on AWL, using a set of 22 questions divided in four thematic groups, each referring to a set of norms: avian species (EC Directive 1999/74, EC Directive 2002/4, EC Directive 2007/43; Q4), farming (EC Directive 1998/58, EC Directive 2008/119; Q5), laboratory animals (EC Directive 2010/63; Q6), transport and slaughter (EC Regulation 1/2005, EC Regulation 1099/2009; Q7). Students were asked to answer specific questions about some prominent aspect of each piece of legislation and the percentage of correct answers was calculated. Students evaluated themselves as moderately informed on animal welfare (Q1 mean 3.3). Education on animal welfare appears mandatory for the 77% of them (Q2). A general perception of need to improve the current AWL emerged (Q3: strong agreement 36%, moderate agreement 49%). Concerning the level of knowledge on AWL, the mean percentages of correct answer to Q4, Q5, Q6 and Q7 were 55%, 45%, 42%, 63% respectively. The percentage of overall correct answer was 51%. The Wilcoxon signed rank test was used to compare the correct answers for each group, adjusting the significance level through Bonferroni correction. Significant difference were found for each comparison, except Q5 vs. Q6 (P=0.02). Chi-square was used to test for differences among students with different self-evaluation score (Q1) having or not mandatory AW course (Q2) and with different opinion about appropriateness on AWL (Q3) on the knowledge of AWL. The test was significant for Q1 (P=0.003) showing positive correlation between the self-evaluation score and overall % of right answers about AWL, while no significant differences were found in the other two cases (P=0.54 and P=0.14). Almost all Italian veterinary students received education on animal welfare that brought them to an awareness of their knowledge level. In students' opinion, AWL should be more restrictive, this opinion could be due to an average students' knowledge on AWL. In addiction differences emerged in students' skill among the normative fields, probably due to their personal interest in related animal species, a more homogenous education on AWL could reduce this difference.

Keywords: Animal welfare legislation, education, veterinary students

Risk Practices for the Spread of Highly Pathogenic Avian Influenza (H5N1) Along Poultry Value Chains in Kano State, Nigeria

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The outbreaks of highly pathogenic avian influenza (HPAI) H5NI which occurred previously for four years (2006, 2007, 2008 and 2015) in Kano State, Nigeria, have resulted in heavy economic losses to farmers and the Government. It was against this background that poultry value chains and their linkages with risks for the spread of HPAI in Kano were evaluated. A total of 15 poultry value chains (from producers to retailers) comprising ten Sector 3 producers, five sector 4 producers, 15 collectors and 30 retailers in live bird markets (LBM) were traced, studied and analyzed through administration of semi-structured questionnaires. The information generated was organized and summarized using simple statistics (tables and percentages). The result revealed the following risk practices: 90% of sector 3 farmers relied on intermediaries for the supply of day-old and point-of-lay birds; 80% of sector 4 farmers source their poultry breeding stock from live bird markets (LBM) and 80% mixes different species of birds in the same house; 60% of sector 4 farmers consume sick or moribund birds; 60% of sector 4, 20% of sector 3, 33.3% of collectors and 66.6% of retailers in LBM dispose dead birds in the environment; all sector 4 and 90% of sector 3 farmers do not report disease problems in their flocks because all sector 4 and 60% of sector 3 farmers experience difficulties in contacting animal health workers; 30% of sector 3 and 60% of sector 4 farmers may likely violet movement restriction and remove their chickens from culling zone during outbreaks. Trade practices revealed that: 30% of sector 3 and 60% of sector 4 farmers sell live chickens within farm and household premises; all collectors transport live poultry with 60% transporting multiple species together in the same compartment; 60% of collectors visit several farms and households per day; 80% of collectors use public transport to convey birds; 26% of collectors and 43.3% of retailers return home with unsold birds from LBMs. The findings indicate that the current containment measures to control the disease, especially in Kano State, are ineffective and as such there is a need to review the current strategies adopted. Regulations on live bird transportation should be enforced and monitored through regular veterinary inspection of the activities of the actors in the value chain. Awareness campaign on biosecurity at all levels of the poultry value chain should be re-emphasized in Nigeria.

Keywords: Avian influenza, Nigeria, risk practices, value chain

Multiplex-PCR for 2 *Mycoplasmal* Agents of Chicken Breeder Flocks and MG Vaccine Strain Differentiation by mgc2-PCR RFLP

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Due to the economic impact of *Mycoplasma* infection in poultry, it is essential to have a fast, reliable and accurate diagnostic test to diagnose the infection. Multiplex-PCR (mPCR) is advantageous in that a single swab can be used to identify the presence of either *Mycoplasma gallisepticum* (MG) or *Mycoplasma synoviae* (MS), testing can be completed in half the time, using fewer materials resulting in lower expense. The objectives of this study are two-fold: to optimize a mPCR for the detection of MG and MS from a single tracheal swab, and to differentiate the MG vaccine strains, ts-11 and 6/85 from field infection. A total of 900 tracheal swab samples were collected from nine chicken breeder flocks, three flocks each from Ankara, Bolu, and Eskisehir provinces. Swabs were pooled into groups of 5, (180 pools) and were examined for the presence of MG and MS by mPCR and bacteriology at the same time. The isolation of MG and MS from these tracheal swabs was done following the standard culture method as described by Kleven (2008). 16S rRNA (MG-14F and MG-13R) primers (Garcia et al 2005) for MG and Mspcl4 and Mspcl5 primers (Marois et al 2005) for MS were used for the detection of both Mycoplasmal agents by multiplex-PCR. For differentiation of MG vaccine and field strains, the primers detecting adhesin-encoding gene of MG previously described by Garcia et al. (2005) were used. Detection limit of mPCR with pure MG-MS culture and artificially spiked samples were determined. Restriction endonuclease enzymes *Sfa*N1 and *Hae*II were used to cut mgc-2 PCR products. Sensitivity of the mPCR was determined to be 6 colony forming units (CFU) ml-1 and 10 CFU ml-1, respectively, from pure MG S6 and MS WVU1853 cultures. In artificially spiked samples with pure MG S6 and MS WVU1853 cultures, sensitivity decreased to 60 CFU ml-1 and 100 CFU ml-1, respectively. Testing revealed, 1/180 (0.55 %) was found MS positive by both mPCR and culture. While 6/180 (3.88 %) were determined MS positive, solely by mPCR. Differentiation of 6/85 and ts11 MG vaccine strains from field strains was achieved by mgc2 PCR-RFLP using *Hae*II restriction endonuclease enzyme. The optimized mPCR can be used reliably because of its high specificity and sensitivity, as a confirmatory test, surpassing culture for timeliness and false negatives when primary screening tests are positive. mPCR may be another diagnostic tool in screening breeder flocks for MG and MS. Additional advantages are that it can detect the presence of MG and MS from a single tracheal swab, simultaneously, and with fewer reagents making it cost effective. The differentiation of MG vaccine strains, by mgc2 PCR-RFLP, using *Hae*II restriction endonuclease enzyme would be a useful diagnostic tool for commercial layer flocks.

Keywords: Chicken breeder flock, mPCR, mgc2-PCR-RFLP, mycoplasma

Infectious Laryngotracheitis Outbreaks in Layer Chickens in Turkey

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Infectious laryngotracheitis (ILT) is a highly contagious disease of chickens that causes severe economical losses in poultry industries worldwide and has a significant concern for animal health and welfare. The objective of this study is to investigate the presence of the infectious laryngotracheitis virus (ILTV) in the field outbreaks affecting the commercial layer chicken farms in different geographical provinces of Turkey between October 2014 and March 2015, using various diagnostic methods including histopathology, virus isolation, real-time reverse transcriptase polymerase chain reaction (RRT-PCR), and sequencing. The index case originated from a layer flock in Afyon province. The outbreaks spreaded soon to Konya, Bursa, Çorum, Isparta, Kayseri, Malatya and Niğde provinces by the pullet movements. None of the affected layer flocks had vaccinated against ILT. Based on the case history, clinical signs, and gross and microscopical lesions, the first presumptive diagnosis of infectious laryngotracheitis was made. This was confirmed by virus isolation, RRT-PCR and sequencing in samples from same farms. In this study, at least 10 samples were received from each of 15 layer flocks with clinical respiratory symptoms and mortality in different 8 provinces of Turkey. Necropsy was carried out recently dead chicken carcasses and sick chickens. Tissue samples including larynx, trachea, and lungs were collected during necropsy. Longitudinal or transversal sections of tracheal mucosa were taken from the larynx along the whole length of the organ. After processed by standart histological techniques, tissues were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin. For virus isolation, tissue extracts were processed and inoculated into 9 to 11 days old embryonated specific pathogen free eggs by chorio-allantoic membrane (CAM). All samples produced typical pock lesions in CAM after fifth days of incubation of the first and second passages. Lesions observed were yellowish pocks with opaque edges, distributed throughout the CAM. Serological confirmation using the Agar Gel Immunodiffusion test showed sharp precipitation lines reacting to a standard reference ILTV antisera. For RRT-PCR, DNA was extracted from the samples of clinical infected chickens in same flocks. Partial viral characterization was accomplished by amplicon sequencing. In affected flocks, histopathological examinations revealed fibrinonecrotic and hemorrhagic laryngotracheitis and similar inflammatory changes in the bronchi of the lungs. Mucosal lesions were characterized by multifocal to diffuse areas of epithelial necrosis, desquamation, and syncytial cell formations with frequently eosinophilic intranuclear inclusion bodies, accompanied with inflammatory exudations and hemorrhagies into the lumen. The results of virus isolation and RRT-PCR from the samples belonging to 15 layer flocks were also positive for ILTV in same flocks. Additionally, viral characterization was performed. This study confirms that the field outbreaks in the commercial layer chicken farms in different geographical provinces of Turkey between October 2014 and March 2015 were due to the ILTV and is the first official report of Infectious Laryngotracheitis in Turkey.

Keywords: Chicken, histopathology, Infectious laryngotracheitis (ILT), real-time RT-PCR, sequencing, virus isolation

Effect of Heat Stress on Fertility and Antioxidant Capacity of White or Black Karagouniko Ewes

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The aim of the study was to evaluate the effect of heat stress on fertility and antioxidant capacity of indigenous breed Karagouniko ewes during the summer period. During summer two trials (I and II) were conducted at the onset of the breeding season. Sixty six ewes were allocated into six groups according to the white (W) or black (B) color of their wool: WC (White color-Control group), BC (Black color-Control group), WIH (White color-I trial-Heat stress), BIH (Black color-I trial-Heat stress), WIIH (White color-II trial-Heat stress), BIIH (Black color-II trial-Heat stress). In both trials oestruses were synchronised by intravaginal progestagen sponges remained in situ for 14 days. At sponges removal all ewes received 300 IU of equine chorionic gonadotrophin. Ewes were then mated. Ewes of groups WC and BC were control, and were housed during the trial in room temperature less than 28° C. In trial I ewes of groups WIH and BIH were subjected to high environmental temperatures above 30° C daily for 4 hours during 14 days, starting at sponges insertion. Accordingly, in trial II ewes of groups WIIH and BIIH were subjected to heat stress for 8 days starting at sponges removal. During the heat stress period in both trials, body temperature and breathing rate were recorded from all ewes. Total antioxidant capacity (TAC) was assessed in blood samples collected from all ewes at the onset and the end of the treatment. Pregnancy diagnosis was performed by ultrasonography 45 days after sponges removal. The results shown that pregnancy rate did not differ between groups. However, the total number of lambs per ewe was lower ($P<0.05$) in groups WIH and WIIH compared with the black heat stress or white control groups. TAC declined within WH and BH group and was higher in the black compared with the white heat stress ewes at the start ($P=0.02$) and the end ($P=0.07$) of the treatment, but did not differ between control groups. This study indicates that heat stress exerts its effect on reproductive function probably at ovarian level by affecting follicular growth, dominance of preovulatory follicles or oocyte health status in ewes mainly of white color. The black Karagouniko ewes may show a heat tolerance indicative of their adaptation in greek hot climate during the onset of the breeding season of the breed.

Keywords: Ewe, fertility, heat stress, Karagouniko breed, total antioxidant capacity

32nd WORLD VETERINARY CONGRESS

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POSTER PRESENTATIONS

Selection of Optimal Degradation Agents for Hydrolysis of Animal Cadavers

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Many infectious diseases have emerged or re-emerged during the past 50 years in South Korea. There were three outbreaks of foot and mouth disease (FMD) in South Korea between January 2010 and March 2011. Over 3.45 million animals were slaughtered (33.3% of the existing pigs, 8.4% of dairy cows and 3.4% of cattle). To select optimal degradation agents of animal cadavers, degradation rates and fertilizer components of pig cadavers were investigated using hydrogen chloride (HCl), potassium hydroxide (KOH) and sodium hydroxide (NaOH) hydrolysis methods. Degradation rates of pig cadavers using HCl, KOH and NaOH were 81.1, 82.8 and 91.6%, respectively. Total nitrogen (T-N) concentration in degradation solution of pig cadavers using KOH hydrolysis method was higher than that in NaOH and HCl hydrolysis methods. Total phosphorus (P2O5) concentrations in degradation solution of pig cadavers in all hydrolysis methods ranged 0.14 ~ 0.28%. Total potassium (K2O) concentration for KOH hydrolysis method was higher than that for other hydrolysis methods. The concentration of T-N and K2O in degradation solution of pig cadavers by KOH hydrolysis method were higher than that in NaOH and HCl hydrolysis methods. Thus, to recycle animal cadavers in agriculture, the optimal degradation agent for hydrolysis was KOH.

Keywords: Animal cadavers, hydrolysis methods, infectious diseases, optimal degradation

The Producer Profiles and Socio-Economic Structure of Angora Goat Breeding in Turkey

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The aim of this study was to determine the characteristics of producers, current production and marketing problems, financial and socio-economic structure, herd scale and management features, the effects of price support policies, current marketing conditions of mohair and producer considerations related to organization and marketing system. The study included preliminary findings which were obtained based on the data from the field within the context of the project numbered 2013/AR-GE/36 supported by General Directorate of Agricultural Research and Policies of the Turkish Ministry of Food, Agriculture and Livestock. The research material consisted of data which were obtained as a result of face to face field surveys and interviews with producers from a total of 102 enterprises located in Ankara and its districts (Ayaş, Beypazarı, Güdül, Elmadağ, Bala, Polatlı, Sincan, Kızılcahamam, Nallıhan) and in the provinces of Bolu, Karaman, Konya, Aksaray, Çankırı, Kastamonu, Kırıkkale and Eskişehir. In this study which was carried out for the first time in terms of subject, extent and content in order to establish value-added, profitable and sustainable structure in mohair production, the results of enterprise outputs, socio-economic structure and producer remarks related sectoral problems were analyzed in detail. Within the study, the average age of producers in a survey carried out in enterprises was 54,72. In this study it was found that 81,37% of producers graduated from primary school and the average Angora goat breeding period was 27,83 years. In the survey, 90 of the 102 enterprises (88,23%) had no partners while 77 of them (75,49%) had activities other than angora goat breeding. Regarding products of animal origin, the ratios of enterprises which carry out sheep breeding, hair goat breeding and cattle breeding as well as angora production to the total number of enterprises were 49,01%, 15,68% and 22,55% respectively. The mean established capacity of Angora goat breeding enterprises was 979 animals/enterprise and the mean capacity utilization rate of enterprises according to the number of animals present was 55,98 %. In this study, the leading causes of reduction in angora goat breeding and mohair production that were mentioned by the breeders were; breeding being unprofitable, problem finding a herdsman, insufficiency of subsidies regarding mohair production, reduction in family labour, conversion of grasslands and pastures into agricultural lands, reduction of mohair yield and quality as a result of crossing with hair goats, low meat prices during goat and kid sales and problems related with breeding stock. In Angora goat breeding enterprises, labour (herdsman), feed, veterinarian-health (vaccine, medicine etc.), maintenance and repair, fuel-energy and transport expenditures are the main costs. On the other hand, the breeders proposed; to increase the subsidies to a level that encourages profitable production, to provide credits-funding during the investment and management period under suitable interest and expiry conditions, to reduce feed costs and to promote the use of pastures, to provide grants to entrepreneurs-investors, to emphasize quality-price relation while supporting mohair production financially and to support and to encourage mohair export. Satisfaction level of enterprises regarding the activities carried out by cooperatives and breeders associations for the solution of their problems was 81,11%. Considering the reduction in the Angora goat population presence of Turkey, increasing mohair production and the productivity level, particularly the implementation of incentives and the support for product processing, improving mohair quality, effective utilization of foreign trade potential have a great importance. Beside, for the sustainability of Angora goat breeding in Turkey, the policies which reduce the costs of mohair production, increase the benefits to producers and product sales revenues and enable the integration with industry should be priority targets.

Keywords: Angora Goat, marketing, mohair, producer, support, socio-economic structure

Evaluation of the Effect of Freeze-Thawing on 146S Antigen Amount in Vaccine Production

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Foot-and-mouth disease (FMD) is highly contagious infectious disease that affects all cloven-hoofed animals and is one of the most economically important diseases of livestock. The virus belongs to the genus Aphthovirus, in the family Picornaviridae. It has seven different serotypes, namely, O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2, and 3. FMD virus (FMDV) is a highly variable RNA virus, and in general, there is little or no cross-protection between serotypes and even between different strains of the same serotype. An inactivated FMDV vaccine is being used to control of FMD. In many areas of the world and our country inactive vaccine is used for the control of FMD. To create a high immunity in animals a good vaccine strains:
-Should adapt easily the system which was produced in,
-Should reproduce by giving high antigenicity and infectivity after the adaptation,
-Should be homologous structure with the origin of the virus during reproduction,
-Whose 146S particles should be stable during storage of vaccine?
Decrease in 146S values causes economic losses by disrupting the vaccine quality. In this study it is aimed to produce virus which has less cost and better quality by raising the 146S antigen value. For that purpose, by using the freeze-thaw method it is provided to be extracted existing virus in a cell. Cultivated virus samples whose 146S value has been previously calculated and which will be used in the production of the vaccines have been subjected to freeze-thaw operation 5 times; after each operation value of 146S antigen has been calculated and recorded. As a result of operations, an increase in 146S values is observed after each freeze-thaw operation and approximately 2-3 times increase is obtained between the initial and final values. Desired increase in the value of 146S has been obtained through acquiring viruses in the cells with freeze-thaw method. In this way, more cost effective and reliable information regarding the FMD has been obtained.

Keywords: 146S, FMD, Freeze-Thawing

Evaluation and Comparison of the Sensivity to Demonstrate FMDV in Cryopreserved Primary Cell Cultures and Established Cell Lines

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FMD is undoubtedly the highly contagious infectious disease of cloven-hoofed animals and causes by far the greatest economic loss. Currently the most sensitive cell culture system for FMDV isolation from vesicular materials is the primary bovine thyroid (BTY) cells. However, that cell neither can be passaged in vitro nor frozen without impairing its sensitivity. Therefore, most institutes or diagnostic laboratories use other cells which are less sensitive for detection of FMDV but more convenient to handle such as other primary cells (Bovine tongue and Bovine kidney) or established cell line such as BHK-21, IBRS-2 and PK-15 cells. In this study, we prepared primary bovine tongue (PBT) cell culture and primary bovine kidney (PBK) cell culture according to our institute protocol and established cell lines were from Cell Culture Collection (HÜKÜK). Primary cell cultures were cryopreserved and used 1, 2, 3 passages of them. Cryopreserved primary bovine tongue cell culture and primary bovine kidney cell culture and established cell lines was examined for susceptibility to FMDV. Cryopreserved primary bovine tongue cell culture was found to be a sensitive for the FMDV while cryopreserved primary bovine kidney cell culture and permanent cell lines as BHK 21, IBRS-2 and PK-15 cells were refractory. Several advantages of PBT cell cultures according to primary BTY cells are once prepared and frozen, they are readily available, their growth after retrieval from liquid nitrogen is consistent.

Keywords: FMDV, primary cell culture, sensitivity

Following of the Disease of Schmallenberg on the Abortions at the Responsible Provinces of Erzurum Veterinary Control Institute

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Schmallenberg Virus has been observed in Germany for the first time in November, 2011 (2, 6). EFSA (European Food Safety Authority) has reported that disease spread rapidly in many countries in Europe (18). Agent; In Orthobunyavirus genus of the family Bunyaviridae is a RNA virüs.(3, 5, 7). Schmallenberg Virus; BTV Serotip 8, Akabane virus (AKAV) are closely related.(4,14). The virus is sensitive to the enviromental impact and some disinfectants (3, 5, 24). SBV especially has been detected the presence of infection in cattle, sheep and goats. Also, disease is detected on red deer, bison, alpaca, deer and wild sheeps (4, 517). Morditity of the diseae in herds is variable and it may be between 20%-70%. Mortality was reported to be limited to abort the fetüs (24). The spread of the disease can be not only the vertical way from mother to infant but also Culicoides type of biting flies take an active role in transmission (2, 4, 5). Direct transmission isn't seen from animal to animal (17). The disease is mild in adult animals: general symptoms such as decreased milk production, diarrhea, fever and lost of productivity are observed (18). In young animals, AH (Artrogriposis-Hidranensefali) syndrome, ankylosis, torticollis (dizziness) and scoliosis (spinal curvature) can be seen. It is reported that the virus can cause infection on fetuses in 25-50 days of pregnancy of goats and sheeps, but 70-120 days of pregnancy in cattle (17, 20). The incubation period of disease changes from 1-5 days.(3, 11) Also, the diesae isn't zoonotic character, but it is known that there is ongoing researches about this subject (3, 12, 24). Molecular and virological diagnostic methods used in the diagnosis of the disease (4, 9, 10, 18, 22). This study was conducted with the aim of observing the presence Schmallenberg virus on the abortuses in our institute's responsibility province. For this study, The SBV suspect samples belonging to swap, internal organs the stomach contents coming from to responsibility in province and the samples between 2014 July and 2015 February,total 1041, were examined by the RT-qPCR (Real Time Reverse Transcription Chain Reaction) method and Schmallenberg Virus antigen isn't detected. As a result of, in samples examined by our Institue, Schmallenberg Virus antigen isn't detected and in European countries, the disease seen in 27 countries in a short period as 2 years, in our country seen in western region, but the absence of any known cases, indicates that a threat at a later time for our region. In this study and similar studies were showed that there was SBV and Schmallenberg virus at our area of responsibility. Our study clears up how to make program for protection and control and how to develop vaccine. Also this study guides other similar studies at this area.

Keywords: Abort, RT-qPCR, schmallenberg virus

A Study on the Determination of Lamb Meat Marketing Margins in Ankara Province

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In this study, it is aimed to estimate structural problems in the marketing system of lamb and lamb meat as well as to determine intermediary margins in case of livestock-wholesale, wholesale-retail and livestock-retail formed in the marketing system of lamb and lamb meat in Ankara, as an example. The materials of the study consist of records related to sales operation performed in the period of years between 2008 and 2011 in the livestock and meat stock exchange depending on Ankara Commodity Exchange. Through the daily average price data obtained (livestock, wholesale and retail lamb meat prices), monthly average prices and current intermediary margins are determined. In order to eliminate inflation effects, the intermediary margins in marketing are also calculated as percentage and with fixed prices based on producer price index and consumer price index of Turkish Statistical Institute in 2003. On the other hand, in the analysis carried out under research, correlation is evaluated between livestock and lamb prices and intermediary margins in case of livestock-wholesale, wholesale-retail and livestock-retail. In the scope of study in Ankara since 2008, livestock-retail current margins in lamb and lamb meat marketing are respectively 38.23%, 45.12%, 39.83%, 40.13%; lamb-livestock-wholesale margins are respectively 18.41%, 26.99%, 18.55%, and 20.08%; and finally wholesale-retail margins are respectively 27.72%, 27.89%, 29.70%, 28.50%. In the period of 2008-2011 in Ankara, the share transferred to the producer through current retail sales prices of lamb meat is 59.17% as average. The correlation is found significantly as r=+0.936 and r=+0.960($p<0.01$) between fixed and current intermediary margins for livestock-retail and fixed and current retail lamb prices in lamb marketing in Ankara. According to this value, it is determined a strong and significant relationship in the same direction between retail lamb prices and livestock-retail fixed and current margins. As a result, in the lamb marketing structure for the said period in Ankara, marketing margins are in high level within the retail lamb prices paid by consumer and the income transferred to the producer through sales prices is in lower case.

Keywords: Lamb, lamb meat, marketing, marketing margin, price

The Costs of Disease Control in Broiler Production in Turkey

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In this study, it was examined the cost structure of disease control and biosecurity measures in broiler production in Turkey was evaluated. Preventive medication (vaccination programme and the use of coccidiostats), hygiene and control materials (disinfectants and pesticides), cleaning (between production periods) and veterinary inspection were accepted as disease prevention inputs in production process. Previous research findings, veterinarians and producers opinions about cost analysis of these inputs were used. The calculation of our study was made for per chick and total population. Besides, the factors were classified as effective in biosecurity measures in broiler production. The current situation in Turkey was evaluated in cost of total and per chick of animal health insurance was determined. As a result, in this study, it was emphasized that animal diseases have negative input feature also importance of disease control and biosecurity measures decrease the risks of production (yield losses).

Keywords: Biosecurity measures, broiler production, cost, disease control

Eco-Climatic Study of Oltenia-Craiova Region (Romania) and Bio-Economic Implications of Dairy Cattle Adaptation to Climate Change

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By 1975, global warming has worsened, and 2007-2013 was the warmest in the history of the planet's climate observations. Warming manifested differently in Romania and even locally, in the Oltenia region: it had significant consequences for the entire biosphere and the environment in general. Dairy cows are very sensitive in terms of heat stress and its effects have a significant economic impact for animals and breeders through decreased productivity, changes in the milk quality (increase in somatic cells) and health problems. Caloric stress affects health and productive performance. A key finding is that the effect of heat stress at a certain temperature varies with relative humidity. Mechanisms by which the cow is trying to decrease caloric stress: increased respiratory rate (> 70 / min) is a primary modification; reducing physical activity; increased water intake - may increase by 20 to> 50%; seeking shade and cooler areas. The index temperature - humidity (THI) is calculated taking into account the temperature and relative air humidity. Materials and methods Our study was conducted over seven years on a number of 594 dairy cows purchased on the EU market from 5 countries. After 4-5 years, the accommodation crisis and adaptation disease were installed because heat stress, among other things, and it was followed by important economic losses. The severity of the thermal stress was related both to the ambient temperature and humidity level or the temperature-humidity index. In the case of dairy cows, THI discomfort occurs when the index exceeds 72 units comparing to 80 units in humans. For stress induced by acclimation we employed a systematic study using cross methods pursuing aspects, phenomena and processes at a time and longitudinal methods pursuing processes and issues during a period of time. Results and discussions The maximum monthly temperature recorded large fluctuations. The highest monthly thermal values were recorded in summer season and were between 34.0 and 42.6 ° C. The smallest monthly thermal values occurred in the winter months and were between 8.2 ° C and 16.8 ° C. The annual number of tropical days ranged between 49 in 2010 and 2011 and 86 in 2012. Tropical days were recorded also in the months adjacent to summer season. Temperature-humidity index (THI) >= 80 was originally developed for measuring human comfort, but later studies have shown that THI has a good meaning even for animal and vegetative processes. In our study, (THI) >= 72 ranged between 46 days in 2013 and 84 days in 2012. In terms of bio-economic issues, was recorded an increase of economic damage resulting from the slaughter of necessity, mortality, reform and recovery through the slaughterhouse. Clinically, the animals showed respiratory signs, salivation, decreased milk production, infertility, and loss of body weight. Conclusions In the period analyzed in the present study (2007-2013), climate variability represented the "peak of the warmest decade" in the history of meteorological observations. Animals purchased on the EU market and brought to a farm in Oltenia area suffered severe climatic stress disorder due to habituation and acclimatization.

Keywords: Bio-economy, cattle, climate change

Behavioral Observations of Poultry Exposed to Gas-Filled Water Based Foam as Euthanasia

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The highly pathogenic avian influenza is a great concern for the poultry industry and depopulation of the infected birds in accordance to animal welfare is important to prevent the disease transmission. Gas-filled foam euthanasia method was developed as an alternative way of mass emergency poultry depopulation. In the study, new type of gas-filled water-based foam containing nitrogen (N2) and carbon dioxide (CO2) was applied for euthanasia laying hens and broiler chickens. The loss of consciousness of chickens was induced shortly after headshaking and wing flapping as a result of anoxic anoxia in both N2 and CO2 gas-filled water-based foam. For N2 gas-filled water-based foam, the loss of consciousness was taken at 55 and 43 seconds in laying hens and broiler chickens, respectively while in CO2 gas-filled water-based foam, it was taken at 52 and 41 seconds in laying hens and broiler chickens, respectively. Unlike with N2 gas-filled water-based foam, gasping was observed in CO2 gas-filled water-based foam. In the postmortem examination, small mass of feedstuff was detected in entering area of trachea in CO2 gas-filled water- based foam, however, there was no observation in N2 gas-filled water-based foam. In conclusion, N2 gas-filled water-based foam was proven alternative method of chicken euthanasia.

Keywords: Carbon dioxide, euthanasia, nitrogen, poultry, water-based foam

Determination of Characteristics of Various Mediums for BHK-21 Cell Culture

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Foot and mouth disease (FMD) is the most contagious disease that can affect all cloven-hoofed animals. FMDV infects many different species and it is excreted at high levels. FMD is classified as an OIE List A disease, by Office of International Epizooties which by definition, means that it has the potential for rapid and extensive spread within and across the countries and may cause severe economic consequences. In our country, for prevention and to struggle against the FMD, animals are vaccinated. Vaccine production methods based on cultivation of primary cells or cell lines were also introduced. The cells multiply rapidly, and cultures can easily be scaled up to large volumes. This system has become the most familiar method of producing FMD vaccines. In FMD Institutes, BHK cells are grown with Glasgow Minimum Essential Medium (GMEM).Through this study, mediums, Alpha Minimum Essential Medium (AlphaMEM), Dulbecco's Minimum Essential Medium (DMEM), Dulbecco's Minimum Essential Medium-F12 (DMEM-F12), RPMI 1640 Medium and Minimum Essential Medium Earle's (MEM Earl's) that are different than the one used in production (GMEM) are used to produce more cells in a short period of time. This project aimed to show that in order to produce BHK-21, instead of GMEM cell culture alternative mediums can possibly be used. In order to determine the effects of Alpha MEM, DMEM, DMEM-F12, RPMI 1640 Medium and MEM Earl's mediums on BHK-21 cell culture production, 10% FCS is added into mediums. BHK-21 cell culture is produced for each medium separately in 25 cm2 flasks and 96 well plates. Cell cultures are incubated in 5% CO2 incubator under 370 C for 48 hours and cells, cell numbers, their morphologies, and cell viabilities (XTT cell viability assay was performed) were examined. In another phase of the study, effects of GMEM and 25% 50% 75% 100% mixed mediums of DMEM-F12, AlphaMEM, DMEM, RPMI1640 and MEM Earle's on BHK-21cell culture production are observed. BHK-21 cells were seeded at a density of 5x104/ml per well in 96 well plates in 100 µl medium GMEM. The cells had a control group (GMEM) and different mediums with different concentrations (25%, 50%, 75%, 100%) incubated for 48h. To measure whether new mediums treatment promotes cell proliferations XTT cell viability assay was performed. In this BHK-21 cell culture production, the effects of the rutin which is GMEM medium that includes 10% Triptose Phosphate Broth (TPB) and other mediums on BHK-21 cell culture are investigated. As a result, the cell numbers of the control group that is the cell cultures which are produced by using GMEM mediums are raised from 5x105/ml to 2,4x106 ml after 48 hours incubation. The BHK-21 cell cultures that are produced by using DMEM, RPMI 1640 and MEM Earl's mediums. Comparison of the production values, cell numbers, cell morphologies and cell viabilities of GMEM which is selected as the control group and other mediums that are DMEM, RPMI 1640, MEM Earle's mediums show that GMEM is a more suitable medium. The results show that the cell numbers that are produced by using 50 % DMEM-F12 is 21% higher than the numbers that are produced by using GMEM. Also, the cell numbers that are produced by utilizing 75 % AlphaMEM is 15 % higher than the numbers that are produced by utilizing GMEM. The cell numbers that are produced by applying pure (100%) DMEM-F12 are 11% increased, while the cell numbers that are produced by applying pure (100%) AlphaMEM 8% decreased. As a result, it is concluded that instead of GMEM which is used to produce BHK-21 that is used for routine, 50% DMEM-F12 and 75% AlphaMEM can be used.

Keywords: AlphaMEM, DMEM, DMEM-F12, GMEM, MEM Earle's, RPMI 1640

A Research on the Effects of Bacterial Endotoxins on Continuous Cell Lines Used in Viral Vaccine Production

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Determine the effects of bacterial endotoxins on various cell lines used in vaccine production and other biological products. For this purpose quantitative and morphological changes at various cell lines had been determined and compared. *P. aeruginosa* ATCC and *E.coli* O55:B5 endotoxins were used as endoxin source. Vero, MDBK and BHK-21 cell lines were used in the tests. Endotoxin detection was performed by gel cloth LAL test. In addition to morphological changes were observed by microscopical techniques, crystal violet staining by ELISA and trypan blue staining methods used for detection of the effects of endotoxins on cell lines. The effect of endotoxins on Vero, MDBK and BHK-21 cells were found to be variable whereas different endotoxins displayed various effects on different cell lines. The most effected cell line was Vero cells to different dilutions of *P. aeruginosa* ATCC and *E.coli* O55:B5 endotoxins among the cell lines used in the study. On the other hand, BHK-21 cell lines exhibited variability at the basis of cell viability and live/dead numbers independent from endotoxin concentration. Last but not least, MDBK cell line exhibited inverse ratio between the cell concentration and endotoxin concentration of *P. aeruginosa* ATCC. The same cell line showed various responses for various dilutions of *E.coli*O55:B5 endotoxin. When the data examined achieved at the end of the study, it is concluded that Vero cell line was the most susceptible to the endotoxins while other cell lines were found to be more resistant to the endotoxins since their response were irrelevant and showed variety to different endotoxin concentrations. The most interesting finding was that the no change of cell death parameter against endotoxin exposure. There was no change in cell death ratio but a decrease in cell number. Thus we conclude that endotoxins may affect the mitosis course of cells or may have an effect on triggering the apoptotic pathway of Vero cell lines.

Keywords: Endotoxin, e.coli, p.aeruginosa, BHK-21, MDBK, Vero

Behavioural Analysis of Meloxicam and a Topical Anesthetic Cream for Pain Mitigation in Piglets Undergoing Surgical Castration

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In North America, over 100 million neonatal pigs are subject to painful procedures each year, including tail docking and surgical castration. While these practices have been demonstrated to cause significant pain and distress to piglets, they are often performed without the use of appropriate analgesics or anaesthetics. This potentially may have a serious impact on the welfare of these animals. To assess pain in castrated piglets using validated behavioural scoring techniques and use these assessments to evaluate the analgesic efficacy of meloxicam (0.4 mg/kg, IM) and EMLA® (a topical anaesthetic eutectic mixture of prilocaine/lidocaine) given 30min prior to castration. This study has strong relevance to the field of piglet welfare, as providing piglets with an analgesic and/or anaesthetic to reduce castration-associated pain may significantly improve their overall peri-procedural well-being. Four litters of 5 day old piglets (n=19) were surgically castrated with treatments randomized across litters: meloxicam + EMLA®, meloxicam + non-medicated cream, saline + EMLA®, saline + non-medicated cream and no treatment (n=2-5 piglets/group). Each pen was videorecorded for 1h, 24h pre-procedure, immediately after castration for 7h and again for 1h, 24h post-procedure. Thirty behaviours and postures were scored continuously for the first 15min at -24, 0, 1, 2, 3, 4, 5, 6, 7 and 24h by an observer blinded as to time and treatment. Data was analyzed using a mixed model ANOVA with repeated measures and a post-hoc Tukey test. All piglets displayed significantly more inactive behaviours (e.g., lying, sleeping) than active behaviours (e.g., walking, running, playing, nursing) up to 6h post-castration. The use of meloxicam and EMLA® were not associated with a reduction in painful behaviours or postures compared with untreated piglets. Behaviours of all piglets returned to baseline at 7h post-castration and were maintained at 24h post-castration. Our findings indicate that the current recommended dose of meloxicam is not effective in alleviating castration-associated pain in neonatal piglets. Further, pain resulting from castration persists for at least 6h post-procedure. Piglet activity levels can be used to clearly differentiate animals in pain from those nor in pain.

Keywords: Animal welfare, castration, pain mitigation, piglet processing

Monoclonal Antibody Production and Characterization against FMDV A/Aydın 98

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Foot and Mouth Disease is highly contagious viral disease of domestic animals like cattle, sheep, goat, swine and wild animals like buffalo and impala. FMD is one of the most important problems of world livestock breeding due to economical losses and international trade restrictions. In our country fighting has been vaccine twice a year. Applying vaccine strains must be protective to field strains. Protective property of vaccine strains may be lost because of it has often got a mutation and egzotic strains comes in to Turkey from neighborn east countries. If these changes are not detected,fighting would break off. Fighting and protection from disease depends on the protection of vaccine strains to field strains, detection of changes in the field and determination of a different vaccine strain. These methods must be easy and fast. One of these method is Mab production against FMDV and detection antigenic relation between vaccine strains and field strains. Different FMDV type A had been found in our cuntry for 1997-1998. Because of vaccine strain was changed. The purpose of study is producing hibridoma cells of antibody screned against new FMDV type A (A/Aydın 98) intact particle 146 S and detecting properties of monoclonal antibodies produced. Briefly, the virus-infected BHK-21 cells were cultured. Viruses were harvested post-infection and clarified by centrifugation. (BEI) was used to inactivivate. For immunization, the inactivated A/Aydın 98 viruses were concentrated with PEG and purified with 15–45% sucrose density gradient ultracentrifution. 12 S particles, trypsin treated virus and denatured virus were prepared from purified 146 S particle. 5-6 weeks old BALB/C mice were immunized subcutaneously with inactivated 146 S particle of FMD serotype A/ Aydın 98 and equal volume FCA. Booster immunizations were given at 4 weeks interval with FICA. Mice were boosted with the same antigen in phosphate-buffered saline (PBS) by intravenous injection 3–4 days before fusion. Immunized spleen cells were then fused with NSO and SP2/0 myeloma cells. After two weeks, hybridoma supernatants were screened using S- ELISA described Crowther. Specificity of the mAbs for whole virus particle, subunit, denatured or trypsin treated virus were measured with Indirect ELISA. Monoclonal antibodies were tested for virus nötralization, plaque reduction,Western Blot, Ig subtyping and cross binding tests. In this study, nine Mabs are produced and characterized against FMDV type A strains.These Mabs are defined as non-nötralization. These Mabs recognized an epitope which is conserved between types A, trypsin resistant, discontinuous and conformational These Mabs are contain IgG1 and IgG2a isotypes. After fusions, we evaluated arithmetic mean of colony produce 31.2 %, standart deviation +- 9, coefficient variation 28 %, standart error +- 2.8, 95 % confidence interval 24.8-37.5. We acquired 30 % efficiency at the end of this study. Because the use of Mabs increases the specifity, accuracy and efficiency of diagnostic tests compared to polyclonal antisera, these nine Mabs are suitable for type A dependent diagnosis of FMDV, such as DAS ELISA or could be adapted to rapid test.

Keywords: Epitope, foot-and-mouth disease virus, monoclonal antibody

Effects of Olfactory and Auditory Stimulation on Separation Anxiety by Salivary Cortisol Measurement in Dogs

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Separation anxiety (SA) is a serious behavioral problem in companion dogs, as it causes stress to both dogs and owners. Recently, there have been many discussions about the methods used to evaluate acute or chronic stress in dogs, especially non-invasive method measuring stress have been more taken notice for not only animal welfare but also more reliable results. In particular, using salivary cortisol to evaluate stress has garnered much attention and has been considered very useful. The aim of this study was to assess stress levels by measuring salivary cortisol levels before, during, and after the dogs were separated from their owners in an unfamiliar environment and to determine whether olfactory or auditory stimulation originating from a dog's owner can relieve the stress caused due to SA in the dogs. Twenty-eight dogs with SA were divided into three groups: Group 1 (control group, n = 10), Group 2 (with owner's clothes during separation period (SP), n = 9), Group 3 (a recording of the owner's voice was played during SP, n = 9). The dog's saliva was collected after the owner and their dog were in the experimental room for 5 min (PRE). Then, the dog was separated from his or her owner for 20 min while saliva was collected four times at intervals of 5 min (SP1–4). Finally, the owner was allowed back into the room to calm the dog for 5 min, after which saliva was collected (POST). Salivary cortisol concentration was obtained using the ELISA and the results were statistically analyzed. There were no significant differences among groups at corresponding sampling times ($p > 0.05$). However, the ratio of the concentration at SP1 to that at PRE (SP1/PRE) was significantly different among the groups ($p < 0.05$). Likewise, the ratio of the concentration of SP1 to that of POST (SP1/POST) was significantly different among the groups ($p < 0.01$). In addition, when comparing the differences in concentrations between PRE and SP1 among groups (SP1 – PRE), these levels were significantly different ($p < 0.05$). In the same manner, the differences in concentrations between POST and SP1 among groups (SP1 – POST), there were significant differences ($p < 0.05$). These results indicated that stress induced by the owner's departure could be reduced physiologically by allowing the dog to sniff the owner's odor or by allowing him or her to hear the owner's voice recorded in advance. This method may be recommended to owners along with practical training and drug therapy as a way of treating dogs with SA. This is an easy to implement and basic method and allows for more efficient management of SA when combined with other techniques. Furthermore, it is worthwhile to improve the dogs' welfare; they may otherwise live the majority of their time at home, alone with their anxiety.

Keywords: Animal welfare, dogs, salivary cortisol, separation anxiety

Effects of Electroejaculation on Oxidant-Antioxidant Status in Merino Rams

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Electro ejaculation (EE) is an important and routine procedure in farm animal veterinary field this method is used for collection of semen from rams for many years, however EE method is probably painful and stressful. The aim of the study emphasized to evaluate the stress response and the oxidant /antioxidant levels against EE process in merino rams. The study was conducted above sperm collection in six 3-4 years old merino rams. Heart and respiratory rate, rectal temperature, white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) levels, hematocrit (Hct) value, blood malondialdehyde (MDA) and glutathione (GSH) concentrations, plasma cortisol, nitric oxide (NO), glucose cholesterol and triglyceride concentrations as well as plasma total antioxidant status (TAS), total oxidant status (TOS) were measured in six merino rams before EE procedure and immediately after. Heart and respiratory rate, WBC, Hct value, MDA concentrations, plasma cortisol, glucose, cholesterol and triglyceride concentrations as well as plasma TOS and cortisol concentrations were dramatically increased ($p<0.05$) compared with initial values after EE procedure whereas rectal temperature, TAS and GSH concentrations were significantly ($p<0.05$) depressed. These results demonstrate that EE was a stressful situation leading to an oxidative stres.

Keywords: Cortisol, electroejaculation, ram, total oxidant status, total antioxidant status

A Computational Fluid Dynamic Model for Assesing the Indoor Envrionment of Poultry Facilities in Greece

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Poultry breeding constitutes the most dynamic sector of Greek stock-raising representing 5% of the agricultural economy and 10% of the animal sector, with the number of raised birds increasing by 75% during the last three decades. The present economic situation in poultry production forces producers to focus on improving efficiency in order to increase their competitiveness. One of the important factors to succeed improved efficiency is the provision of an optimal indoor environment (i.e. acceptable air quality including gas, and microbial concentrations as well as controlled temperature, relative humidity and ventilation rates, etc.). The identification of the indoor flow patterns as well as the ability to predict the distribution of the basic climatic and pollution parameters could assist in improving the building designing and therefore in its operation. Indoor environment can be assessed either experimentally via direct measurements or numerically via models. Undoubtedly, physical experiments allow the real time monitoring of variables. However, a detailed investigation of physical parameters requires quite long time series and expensive equipment. Numerical techniques, like Computational Fluid Dynamic (CFD) can render efficiently and accurately the quantification of the variables compound the microclimate inside livestock buildings. Additionally, parametric investigation can be conducted studying the impact of the external wind speed and direction on the distributions of air flow, temperature and ammonia in specific points inside the building. Obviously some form of experimental validation of the CFD model is required prior to the generalisation of the results. In the present study a numerical CFD model will be developed, validated and used for numerical investigation of the indoor environment inside a poultry farm, as well as the releases of GHG to the outdoor environment, through a set of parametric studies. The presenting results shows the influence of external wind speed and direction in the distribution of the main climate parameters which are characterize the indoor environment of poultry facilities.

Keywords: Ammonia, emissions, microclimate, welfare

Effects of Dietary Supplementation with Extracts of Oregano and Sage on Microclimatic Conditions and Antioxidant Status of Raw and Cooked Breast and Drumstick Meat of Broiler Chickens

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In this study, we used the essential oil of Greek endemic plants such as Origanum vulgare hirtum and Salvia triloba as a source of functional ingredients with antioxidant properties after dietary supplementation of chicken diet. The second objective was to investigate the effects of oregano and sage essential oil on gas emissions in poultry house. Nine thousand day-old broiler chickens were randomly divided into three dietary treatments, and kept in floor pens. The experimental period was 6 weeks and birds were fed either a basal diet, or a basal diet supplemented with 500 or 1000 ppm extract that contained 5% oregano essential oil [containing 77,9% carvacrol and 3,02% thymol] and 0,5% sage oil [containing 47% 1,8 cineole] obtained by PANAROMA, SA, Herso Kilkis. All birds were reared in a commercial farm (Agricultural Poultry Farmer Cooperative, Arta, Greece). Feed and water were offered to birds ad libitum. At the end of the trial, all birds were slaughtered under commercial conditions, their carcasses were processed, and samples were taken and stored at -20ºC for further analysis. Breast and drumstick meat were analyzed (FoodScanTM Lab, FOSS Denmark) for moisture, fat and protein content. Moreover, samples of breast and drumstick muscle were assayed for malondialdehyde levels, DPPH, total phenolic content, and meat colour. For gas emissions, ammonia, H2S and total suspended particles in poultry house were measured every week. Statistical analysis was performed by one-way analysis of variance using the IBM SPSS Statistics 20 statistical package. Tukey's range test was used, with differences considered to be significant at $P < 0.05$. The results of this investigation revealed no differences on moisture, fat and protein content of breast or drumstick meat from the three experimental groups. Results on oxidation showed that dietary oregano and sage oil at both supplementation levels were well accepted by the broiler chickens and improved oxidative status compared to control diet, the effect being dose dependent between the two levels of supplementation. Ammonia and total suspended particles were not affected by any dietary manipulation, kept in acceptable levels in control and herbal supplemented chickens. In conclusion, a combination of dietary oregano and sage exerted an antioxidant protective activity in both raw and cooked chicken breast and drumstick meat

Keywords: Broiler chickens, gas emissions, meat antioxidant status, meat chemical composition, oregano, sage

Evaluation of Management and Health Services for Free-Ranging Urban Animals in Turkey

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The aim of this study was to evaluate the situation and legal rights of free-ranging urban animals in Turkey. In the process of transition from agricultural population to industrial population, alterations in relationship structure between humans and animals started to occur and emerging variable opinions led the legal status of animals reach a debated position in the last decades. Regulatory principles regarding daily lives of urban animals are basically determined with “Law for Protection of Animals” in Turkey. The aforementioned law, executed by the Ministry of Forestry and Water Affairs enforces local authorities to build temporary nurseries to take all the responsibility for stray and weak animals. Nevertheless, most of the municipalities have not yet built temporary nurseries since 2004, when the code has been effective. Malfunctions in performance of the law together with the lack of supervision inhibit securing such fundamental rights of animals. The most substantial deficiency of the law in terms of content is in regard to health services. Even though the terms such as “protection, compensation, supervision and rehabilitation” are mentioned in the law and execution directive, definitive provisions addressing medical needs of such animals are lacking. More importantly, there is no reference to animal hospitals in which minimum technical conditions are determined by the Ministry of Food, Agriculture and Livestock. This results in transient nurseries trying to provide animal health service with inadequate number of staff and in conflict with standards, which are actually founded by law for sheltering, neutering/spaying and vaccination purposes. Current Directive partially disregards animals’ needs for health services, or limits them to applications for protection of human population health. Health services for free-ranging urban animals should be evaluated in accordance with the basic patient right “to be able to profit from service” and the directive must be renovated accordingly. However, in order to avoid animal rights breaches, necessary regulations must be issued or in short term, inter-corporation/institution service supply agreements must be encouraged.

Keywords: Animal welfare, veterinary medical ethics

Detected Contagious Bacterial Agents and Antibiotic Sensitivities during Foot and Mouth Disease (FMD) Vaccine Production by Biofermentators

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Control of Foot and Mouth Disease (FMD) in Turkey is carried out by programmed vaccination. Contamination is the biggest problem in the production of FMD vaccine. These contamination are viral, fungal or bacterial. The origin and ways of the contamination must absolutely be researched. It is very difficult to solve the problem without searching the origin and ways of the contamination. Cell cultures are one of the methods used in the researches, vaccine productions and diagnosis of some diseases. Bacterial contamination of cell cultures are common and this contamination is a serious problem that causes the disruption of cell physiology and metabolism. It causes immediate death of cells used in the production. At the end, it causes to cease the production. To end the production leads to many difficulties in morally and economically. The contamination of cells in cell cultures produced in bioreactors is detected by inoculation of the samples collected at different stages of cell production and/or viral culture to basic media after 24 hours incubation. For bacterioscopic examination smears are prepared and they are dyed by gram stain. By this examination, gram positive or gram negative bacteria are diagnosed among the cells. Blood Agar, Nutrient Agar, Mc Conkey Agar, Sabouraud Dextrose Agar, Fluid Thipyglycollate Medium (FTM) and Tryptic Soy Broth (TSB) are used while performing sterility tests for samples collected from Cell Bank, Suspansion Cell Culture Laboratory and Cell Production Laboratory. Media used for inoculation are prepared as two pairs. One group is incubated in incubator at 37 oC and second group at 22 oC. Changes in the incubated samples such as changes in color, turbidity, precipitation, deposition, layer occurrence are seen in 2-3 days later. Most common contamination sources in this area are contaminated laboratory materials, equipments and media, rarely virus pool or monoclonal antibodies. It is seen that laboratory staff also contaminate cell cultures. In this study, an attempt was made to 436 units samples. As a result of this work was identified with contaminated samples of 18. Identification was carried out by VITEK 2 method in the Bacteriology Department of National Food Reference Laboratory Directorate. From these 18 samples, 1 samples Citrobacter freundii (5.56 %), 3 samples Citrobacter amalonaticus (16.66 %), 8 samples Stenotrophomonas maltophilia (44.44 %), 1 samples Dermacoccus nishinomiyaensis/Kytococcus sedentarius (5.56 %), 1 samples Rhizobium radiobacter/Agrobacterium radiobacter (5.56 %), 2 samples Ralstonia (Pseudomonas) pickettii (11.11 %) and 2 samples Spingomonas paucimobilis (11.11 %), 18 different strains in total were identified. Antibiotic sensitivity tests were carried out to select the antibiotics in controlling of these agents. It has been detected that Stenotrophomonas maltophilia is resistant to Ampicilline, Cefotaxime, Gentamycin, Trimethoprim, Sulfamethaxazole-Trimethoprim, Chloramphenicol, Streptomycin, Kanamycin, Sulfonamides, Ceftraxone, Penicillin and Lincocin; but sensitive to (not resistant) Tetracycline and Ciprofloxacin. Some strains produce grey color in some special media like deoxycholate media. They do not cause hemolysis in blood agar. Citrobacter amalonaticus is found to be sensitive to Ceftazidime and Nalidixic Acid; but resistant to Ampicilline, Ciprofloxacin, Gentamycin, Sulphamethaxosole, Tetracycline, Trimethoprim and Cefoxitine. This bacterium is gram negative and opportunist in the cases when the immune system is weak. They are found in soil, air, water and in the intestines of many animals including bat and human. Spingomonas paucimobilis, gram negative bacterium was found to be resistant to Cefotaxime, Gentamycin, Ceftazidime and İmipenem but sensitive to only Ciprofloxacin. Rhizobium radiobacter/Agrobacterium radiobacter and Ralstonia (Pseudomonas) pickettii were found to sensitive to all antibiotics tested in this study. Dermacoccus nishinomiyaensis/Kytococcus sedentarius was not to able to produced in Brain Heart Infusion Agar used in the antibiogram test carried out disc diffusion method.

Keywords: Antibiogram, bacterial contamination, bacterial identification, cell cultures

The Use of Baytril and Micospectone in BHK-21 Cell Culture

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Antibiotics are frequently added to cell culture media; the greatest benefit to this is that it suppresses contamination. However, routine use of antibiotics is associated with the development of resistances, the tendency to forgo aseptic working procedures, and the repression and carryover of cryptogenic contamination. The most common antibiotic for cell cultivation is a combination of penicillin and streptomycin. The aim of this study was to determine the availability of Enrofloxacin and lincomycin hydrochloride and spectinomycin sulfate in proparagation of BHK-21 An30 cell cultures. For this purpose, trade name Baytril 10% enjectable solution (Enrofloxacin 100mg/ml) and Micospectone (50 mg Lincomycin hydrochloride and 100 mg Spectinomycin Sulphate/ml) antibiotics were used in this study. Baytril and Micospectone were added into GMEM (Glasgow Minimum Essentiale Medium) with 10% BAS (Bovine Adult Serum) and 5% TPB (Triptose Phosphate Broth) as 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 µg/ml separately. BHK-21 An30 cell cultures were grown with GMEM added antibiotics in different dilutions. Cell cultures were incubated at 37 C with CO2 5% for 3 days. Morphology, numbers and duration of growing period of BHK-21 An30 cells were investigated along the incubation periods for each antibiotic. In the cell cultures containing 0.1 µg/ml Baytril there was not any morphologycal changes and cell numbers incrised from 5x10^5 to 1.1x10^6/ml and 1.9x10^6/ml at 24 and 48 hours. At the cell cultures added Baytril at 0.2 µg/ml and higher doses, there was no proliferation in cells and seen degeneration and morphologycal changes like giant cells. In the BHK-21 An30 cell cultures containing 0.1,0.2 and 0.4 µg/ml Micospectone there was not any morphologycal changes and cell numbers incrised from 4x10^5 to 1.3x10^6/ml and 2.3x10^6/ml at 24 and 48 hours. At the cell culture added 0.8 µg/ml Micospectone cell numbers reached from 4.5x10^5 to 1.4x10^5 at 48 hours. There was no proliferation in cells used Micospectone as 1.6 and 3.2 µg/ml and seen morphologycal changes like giant cells. As result, it was concluded that the micospectone containing 50mg Lincomycin hydrochloride and 100mg Spectinomycin Sulfate/ml can be used to prevent bacterial contamination at 0.4 µg/ml medium on BHK-21 An30 cell culture. On the other hand, it was found that Baytril is toxic for BHK-21 An30 cell culture if used upper of 0.1 µg/ml doses.

Keywords: Antibiotic, BHK-21 An30 cell culture, enrofloxacin, lincospectine

Comparative In-Vitro Anti bacTerial Activity of Many Herbal Plants with 2 Antibiotic Drugs against Mastitis Caused Bacteria

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Mastitis in dairy cattle is the persistent, inflammatory reaction of the udder tissue. Mastitis, a potentially fatal mammary gland infection, is the most common disease in dairy cattle. Mastitis may be classified according two different criteria: 1. Clinical symptoms: A) Clinical mastitis. B) Sub-Clinical mastitis 2. Mode of transmission: A) Contagious mastitis. B) Environmental mastitis. Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. The aim of this study is Comparative anti bacterial activity of herbal plants with 2 antibiotic drugs against mastitis caused bacteria. In this research effect of two herbal plants (Artemisia absinthium and Salvia officinalis), (water extract, essential oil, ethanolic extract) and 2 antibiotics sulfamethoxazole and ciprofloxacin against 4 bacteria (E. coli, S. areus, B. cereus, S. agalactie) mastitis caused was examined. Bacteria were prepared from Iranian Research Organization for Science and Technology (IROST). After preparation this agent was sub cultured. Susceptibility tests were performed by the disc diffusion method of Bauer et al. (Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1996). The data of this study was analyzed by SPSS 18 Versions. ANOVA exam was done. The antimicrobial activity of plants was determined by the disc diffusion method. All products of these plants (essential oil, water extract and ethanolic extract) have antibacterial activity but essential oil of salvia has more than effect on mastitis caused bacteria. Further, bacteriostatic concentration was determined for each strain that evidenced sensivity to the essential oil of Salvia officinalis. The results of this article showed that Salvia officinalis and Artemisia absinthum essential oil have more than antibacterial activity against bacteria. Salvia officinalis effect on B.cereus 17 mm, S.areus 18mm, S.agaslctiae 19mm and E.coli 14 mm. Artemisia absinthum effects on e.coli 12mm, S.agaslctiae 13mm, S.areus 17mm and B.cereus 16.5 mm have a more than effect compare with ethanolic extract and water extract. sulfamethoxazole diameter in b.cereus 26mm, S.areus 28mm, S.agaslctiae 23mm and E.coli 25mm and ciprofloxacin diameter in b.cereus 21mm, S.areus 29mm, S.agaslctiae 26mm and E.coli 23mm. The results of this article showed that Salvia officinalis and Artemisia absinthum essential oil have more than antibacterial activity against bacteria. Salvia officinalis effect on B.cereus 17 mm, S.areus 18mm, S.agaslctiae 19mm and E.coli 14 mm. Artemisia absinthum effects on e.coli 12mm, S.agaslctiae 13mm, S.areus 17mm and B.cereus 16.5 mm have a more than effect compare with ethanolic extract and water extract. In 2007 G.P.P. Kamatou et al. by research with title Antibacterial and antimycobacterial activities of South African Salvia species and isolated compounds from S. chamelaeagnea showed that Salvia officinalis (African species) have effect on E.coli, B. cereus and S.areus. In another research Elham Mosafa et al in 2014 with title In-Vitro Antibacterial Properties of Sage (Salvia officinalis) Ethanol Extract against Multidrug Resistant Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae showed that salvia have antibacterial activity against E.coli and Staphylococcus aureus. We can conclude salvia and Artemisia have antibacterial effects against mastitis caused bacteria. All products of these plants (essential oil, water extract and ethanolic extract) have antibacterial activity but essential oil of salvia has more than effect on mastitis caused bacteria.

Keywords: Antibacterial activity, mastitis, in vitro

Quinolone and Multiple Drug Resistance in *Escherichia Coli* Isolated from Animals

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The aims of this study were to characterize quinolone and multiple drug resistance (MDR) in *Escherichia coli (E. coli)* isolates of animal origin. A total 116 clinical *E. coli* isolates from food-producing and companion animals were collected between January 2011 and June 2012 from Uludag University Animal Hospital of Faculty of Veterinary Medicine (Bursa, Turkey). Random amplified polymorphic DNA (RAPD) analysis was used to determine the genetic relatedness of the *E. coli* isolates. Broth microdilution testing was carried out to determine the MICs of the antimicrobials (nalidixic acid, enrofloxacin, ciprofloxacin, orbifloxacin, gatifloxacin, ampicillin, cefotaxime, ceftriaxone, cefepime, sulphametoxasol, trimethoprim, gentamicin, tetracycline, oxytetracycline, erythromycin and chloramphenicol) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). QRDR and transmissible genes were amplified using specific primers and PCR products of QRDR genes were sequenced to detect the mutations conferring quinolone resistance. Twenty four different RAPD banding patterns were identified in the 116 *E. coli* isolates of animal origin. MICs of *E. coli* isolates ranged from 0.008 µg/ml to 64 µg/ml for enrofloxacin, danofloxacin, ciprofloxacin, orbifloxacin and gatifloxacin, from 0.256 µg/ml to >= 256 µg/ml for nalidixic acid. Enrofloxacin, danofloxacin, gatifloxacin, cefotaxime, ceftriaxone and cefepime were found to be most effective against *E. coli* isolates. *E. coli*isolates were most frequently (96%) resistant to gentamicin. All isolates but *E. coli* E264 (24/25) were resistant to three or more antimicrobials. The resistance to 3 to 8 antimicrobials was observed in 88% of isolates. *E. coli* E245 and *E. coli* E306 were resistant to nine of the antimicrobials. The number of *E. coli* isolates with mutations in *gyrA* was three (E222, E245 and E246). The number of *oqxB*- containing *E. coli* isolates was one (E306) and *qepA* was not detected in any of the *E. coli* isolates. This study is the first to identify *oqxB* in *E. coli* isolates of animal origin in Turkey. Detection of quinolone resistance determinants and presence of MDR phenotype in *E. coli* isolated from animals indicated that this is an important public health issue and can create a high risk for the treatment of infectious diseases at the recommended available dosage regimens. Therefore, optimization of dosage regimens and/or combination therapy can be useful to prevent selection and emergence of resistance.

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Keywords: E. coli, MDR, quinolone

Molecular Characteristics of Extended-Spectrum β-Lactamases Producing *Escherichia Coli* Isolates from Healthy Broiler in Turkey

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The aim of this study was to detection prevalence and clonal typing of extended spectrum beta lactamase producing (ESBL) *Escherichia coli* in healty broiler in Turkey. 300 broiler cloacal samples will be collected from various broiler slaughterhouses and will be inoculated to the Levine agar plates supplemented with 2µg/ml cefotaxime. Suspect strains were identified by BBL™ Crystal™ Enteric/Nonfermenter ID Kit (Becton Dickinson/ USA) and ESBL production was confirmed by the double-disk synergy test. For 16 different antibiotics, antimicrobial susceptibilty of cefotaxime-resistant*E.coli* isolates will be performed by the agar disk diffusion methods. ESBL type were analysed by used PCR and sequencing. Pulsed-field gel electrophoresis (PFGE) was performed with XbaI to clonal typing of ESBL producing*E. coli* isolates. In total, 33 phenotypic ESBL-producing E. coli isolates were analyzed by to examine the presence of the beta-lactamases-genes. Eight of all *E. coli* isolates only harbouring the blaTEM-1 (n=8). These studies results in broilers, 25 isolates ESBL-producing 23 (92 %) of them CTX-M (20 CTX-M-15, 1 CTX-M-1, 1 CTX-M-9, 1 CTX-M-16), 4 (16 %) of them OXA-1 and 2 (8 %) of them were found in the SHV-12. In Turkey, broiler origin of *E. coli* ESBL producing isolates is the first study to investigate and determine. Increasing antibiotic resistance problem of in bacteria is a highly significant finding in terms.

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Keywords: Antimicrobial resistance, broilers, CTX-M, escherichia coli, ESBL, PFGE

Research of Antimicrobial Resistance and Extended Spectrum Beta- Lactamase Production in *Escherichia Coli* Isolates of Equine Feces Origins

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In this study, it is aimed to research the existance of ESBL and determine the resistance of *E. coli* strains isolated from feces examples obtained from horses located in Ankara Race Track and from show jumping horses in Ankara, against various antibiotics. 100 *E. coli* isolates are analysed against 16 antibiotics by using the Disc Diffusion method and also the ESBL existance has been analysed phenotypicly. The antibiotic resistance prevalence has been compared between race track group horses and show jumping group of horses. In this study, in 63 of *E. coli* isolates isolated from show jumping horses, the highest resistance against tetracycline is determined as 20.6% (13), whereas in 37 *E. coli* isolates isolated from race track horses, the highest resistance against tetracycline is determined as 81.1% (30). In 6 isolates among 100 *E. coli* isolates obtained from horses, the ESBL production has been determined by using fenotypic confirmatory test. The six isolates which are ESBL positive have been isolated from the horses in race track. The ESBL production ratio in race track horses has been determined as 16,2% (6). ESBL production was determined in the isolates obtained from fecal flora of the horses used for this study. The contamination of these agents in ecosystem causes a potential risk factor for public health.

Acknowledgements: The study was prepared from the first author Msc thesis.

Keywords: Antibiotic resistance, disc diffusion, E. coli, ESBL, fenotypic confirmatory test

Isolation of *Lactococcus Garvieae* from Rainbow Trout Farms and Examination of Antibiotic Susceptibility Profiles

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In this study, *Lactococcus garvieae* that isolated from rainbow trout cultured in fresh water were examined in terms of with Molecular Methods and Culture Techniques and Antibiotic Susceptibility Profile. Eight fish samples taken from a commercial Rainbow trout farm at the outbreak of infection in the Black Sea Region were examined as bacteriologically. Disease agent was detected and investigated of antibiotic susceptibility profile. Growing colonies at solid medium were performed for gram-staining, oxidase, catalase, motility, growth at respectively 10oC - 30oC - 45oC, beta-galactosidase, arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, H2S, alpha hemolysis, 0129 sensitivity (150 mg), glucose, mannitol, L arabinose, inositol, rhamnose, sorbitol, maltose, xylose, lactose, fructose, inulin, adonitol, sucrose and esculin tests with conventional methods. Antibiotic susceptibility of this strain was performed with Kirby-Bauer method. DNA extraction of the strains was performed with boiling method. Molecular identification of these strains were tested by PCR methods using specific primer set (LgF 5'-CCA ACT TCC GTG GTG TGA CG-3', and LgR 5'-AGT GGC TCA ACC ATT GTG TGC-3') for *L. garvieae*. Following amplification and electrophoresis, *L. garvieae* was considered as positive by observed PCR product at 857 bp. Sharp edges, white – cream colored, round and smouth character, colonies formed on the three different media. Four *L. garvieae* isolate were obtained on Tryptic Soy Agar, McConkey Agar and Shotts-Waltman Agar. It was detected that direct streaked from diseased organ and tissue on Shotts-Waltman Agar which was a selective medium for *Yersinia ruckeri* can be isolated and growth. PCR can resulted in short time and this method is advantageous for laboratory staff and fish farmers.

Keywords: Antibiotic susceptibility, isolation, lactococcus garvieae, rainbow trout

Investigation of Tetracycline Residues in Fishes Hunted from Surrounding Fish Farms in Muğla District

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In this study, it was aimed to investigate the tetracycline antibiotic (oxitetracycline, tetracycline, chlortetracycline, and doxycycline) residues in fish samples hunted around fish farms in Mugla district. Fish samples were provided from surrounding of 70 different fish farms from Göltürkbükü, Torba, Güvercinlik, Güllük Bay to Kuşadası around Muğla district by hand-line hunting method. Seventy pieces of fish samples, which consist of Sparus aurata, Sparus pagrus and Maena smaris species, were used for analysis. Fishes living in the natural environment around the farm are considered as bioindicator of environmental pollution for the aquatic environment. For this reason, it was aimed to analyze tetracycline residues, which is frequently used for the purpose of protection and treatment, in fishes hunted around the fish farms. Analyses were performed by the LC MS/MS method at Bornova Veterinary Control Institute, İzmir. Validation was performed at the critera of specificity, linearity, recovery, precision at sensitivity. Limits of detection (LOD) was determined as µg/kg: Oxytetracycline 11, Tetracycline 13, Chlortetracycline 7,4 and Doxycycline 9,4. Recovery values were determined as Oxytetracycline 100,5%; Tetracycline 101,3%; Chlortetracycline 99% and Doxycycline 100,5%. At the end of the analyses, no tetracycline antibiotic (oxytetracycline, tetracycline, chlortetracycline, and doxycycline) residues were found in the samples analyzed. LC MS/MS is a suitable method for the detection of tetracycline antibiotics residues in fish samples. It can be expressed that the fish samples have no tetracycline antibiotic residues at the detectable limits is a satisfactory result in terms of environment and public health.

Keywords: Fish, LC MS/MS, residue, tetracycline

Research on Zinc and Cadmium Concentration in Muscle of Indian Halibut Fish in Boushehr Province Waters

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Heavy metals may accumulate in various aquatic animal tissues and organs, e.g. liver, gills, muscles. Because of the consumption of aquatic animals by human, it matters in public health. This research was done to determine the concentration of Zinc (Zn) and Cadmium (Cd) in muscle of Indian halibut (Psettodes erumei; Bloch & Schneider, 1801) that is one of the most man-consuming fish in Iran. It is found on bottoms and mainly piscivorous. This fish is sold fresh and also utilized smoked and frozen. In this research, 35 Indian halibut fish samples were randomly collected from the Boushehr province waters. Then 100 gr pectoral muscles (between lateral line and dorsal fin) of each sample was dissected and prepared for measuring Zn and Cd by atomic absorption technique. The results showed that the maximum and minimum concentration of Zinc were 0.196 and 0.039; and for Cadmium were 0.088 and 0.025 mg/kg (ppm) respectively in pectoral muscles of Indian halibut fish. This study has not been reported yet in this water source Indian halibut for Zn and Cd muscle accumulation. Water quality (physicochemical parameters, temperature, pH …) and even season can effect on toxicity of heavy metals. For example, in waters with high salinity, the toxicity of heavy metals may be reduced. The results were totally in safe limits of international Organizations ranges (table 1).

Keywords: Boushehr, halibut, fish,

Table1.The safe limit of Zinc and Cadmium concentration by valid international organizations.

Heavy Metal (mg/kg) → / Int'l Organization ↓	Zn	Cd
WHO	100	0.2
FDA	-	1
UK	50	0.2
FAO	50	0.3

Study of Accumulation of Lead and Chromium in Muscle of Indian Halibut in Iran South-West Waters

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Heavy metals may accumulate in various aquatic animal tissues and organs, e.g. liver, gills, muscles. Because of the consumption of fish muscles by human, it matters in public health. According to the public health aspect, this research was done to determine the concentration of two heavy metals Lead and Chromium in muscle of Indian halibut (Psettodes erumei; Bloch & Schneider, 1801) that is one of the most man-consuming fish in Iran. It is found on bottoms and mainly piscivorous. This fish is sold fresh and also utilized smoked and frozen. In this research, 35 Indian halibut fish samples were randomly collected from the South-west of Iran waters. Then 100 gr pectoral muscles (between lateral line and dorsal fin) of each sample was dissected and prepared for atomic absorption measurement. By this technique, concentration of Lead and Chromium were measured in muscles. The results of this study showed that the maximum and minimum concentration of Lead were 0.136 and 0.047; and for Chromium were 0.050 and 0.028 mg/kg (ppm) respectively in pectoral muscle of Indian halibut. This is the first research on South-west Iran waters region Indian halibut fish for Pb and Cr muscle concentration. The quality of water (physicochemical parameters, pH, Alkalinity, and even season to name but a few) can effect on toxicity and accumulation of heavy metals in aquatic animals. For example, in waters with high salinity, the toxicity of heavy metals may be reduced. The results were totally in safe limits of WHO, FAO, FDA, etc. standards (table 1).

Keywords: Chromium, halibut, muscle

Table1.The safe limit of Lead and Chromium concentration by valid international organizations.

Heavy Metal (mg/kg) → / Int'l Organization ↓	Pb	Cr
WHO	0.5	10
FDA	5	-
NHMRC	1.5	-
UK	2	20
FAO	2	-

Quality of Bovine Embryos Produced Using Chemical Packets That Regulate CO_2 and O_2

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We previously reported that blastocysts were successfully produced without gas phase control during in-vitro maturation (IVM) and in-vitro fertilization (IVF) and using chemical packets that regulate CO_2 and O_2 only during in-vitro culture (IVC) (Saeki and Fujiki, IETS, Versailles, France, 2015). In the present study, we examined the quality of the blastocysts by TUNEL assay. Bovine cumulus oocyte complexes (COCs) were collected from slaughterhouse ovaries. The COCs were matured in 25mM HEPES-buffered TCM-199 (Gibco, Invitrogen Life Technologies, Tokyo, Japan) supplemented with 10% fetal calf serum (BioWest, Paris, France), 0.02AU/mL FSH (Antrin: Kyoritsu Pharmaceutical, Tokyo, Japan) and 1µg/mL estradiol-17β (Sigma-Aldrich Japan, Tokyo, Japan) for 22h, and fertilized in medium IVF100 (Research Institute for the Functional Peptides (IFP), Yamagata, Japan) with Japanese Black bull frozen-thawed sperm ($4x10^6$cells/mL) for 6h (IVF). Sperm and cumulus cells were removed from the oocytes. The denuded oocytes were cultured in IVD101 (IFP, 20 to 30embryos/50µL) for 8 days. Medium droplets (50 or 100µL) for IVM/IVF and IVC covered with mineral oil (Nacalai Tesque Inc, Kyoto, Japan) were placed on the bottom of a 60-mm plastic culture dish (Falcon-1007, Corning life Sciences Japan, Tokyo, Japan). These dishes were placed in a small plastic container (2.5L, Mitsubishi Gas Chemical (MGC), Tokyo, Japan). The container contained approximately 20mL of water for maintaining high humidity. For IVM/IVF, no chemical packets were used. For IVC, we used two different chemical packets, One packet (AnaeroPack (AP)-CO_2, MGC) that maintains a CO_2 level of about 5% and consequent O_2 level of about 15%, and the other (AP-MicroAero (MA), MGC) that maintains a CO_2 level of 5-8% and an O_2 level of 6-12%. Then the containers were placed in an incubator without gas control (100%-air, 39°C with high humidity). For a control group, the oocytes were matured and fertilized in a CO_2 incubator (5%CO_2, 39 °C with high humidity), and cultured in a CO_2/O_2 incubator (5%CO_2, 5%O_2, 90%N_2, 39 °C with high humidity). Four different combinations of gas conditions used for incubation were used (Table 1). Maturation, normal fertilization, cleavage and blastocyst rates were examined at each endpoint. Trophectoderm (TE) and inner cell mass (ICM) cells of blastocysts were differentially stained as described previously (Thouas et al., 2001) by staining with Hoechst 33342 (Sigma-Aldrich Japan) and Propidium Iodide (Sigma-Aldrich Japan). A TUNEL assay kit (in situ Cell Death Detection Kit, Roche Diagnostics, Penzberg, Germany) was used to detect the presence of apoptotic cells in the blastocysts. Experiments were repeated 3 times. A total of 933 oocytes were used in this study. Data were analyzed by ANOVA followed by a Tukey-Kramer test. Maturation and normal fertilization rates for Groups 2, 3 and 4 (100%-air) were 63 and 65%, respectively, and they were the same as those for group 1 (control, 73 and 62%, P>0.05). Cleavage and blastocyst rates for Group 1 (control, 76 and 42%), Group 2 (CO_2/O_2 incubator, 81 and 42%), Group 3 (AP-CO_2, 83 and 24%) and Group 4 (AP-MA, 81 and 31%) were the same (P>0.05) among groups. However, the total cell numbers of blastocysts for Group 3 (108) and for Group 4 (116) were lower than those for Group 1 (control, 140) and Group 2 (144) (P<0.05). The rates of TUNEL-positive cells in blastocysts for Group 1 (control, 20%), Group 2 (24%), Group 3 (29%) and Group 4 (28%) were the same (P>0.05) among groups. These results indicate that gas phase control is not needed for IVM/IVF of bovine oocytes for their subsequent development.

Keywords: development to the blastocyst stage, gas phase control, in-vitro production of bovine embryos, TUNEL assay, quality of blastocysts

Table 1. Gas conditions for incubation

Group	IVM/IVF	IVC
1 (Control)	5% CO2	5%CO2, 5%O2, 90%N2
2	100% air	5%CO2, 5%O2, 90%N2
3	100% air	AP-CO2
4	100% air	AP-MA

Comparison of the Effects of Intraocular Pressure with Phacoemulsification and Extracapsular Cataract Extraction Methods in Dogs with Cataract

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Cataracts are the most frequent causes of blindness in dogs. The remove of the lens for the repair of vision lost due to a cataract has become an ordinary procedure and technique in veterinary medicine. Cataract extraction procedures are known to cause of increase in intraocular pressure (IOP) within the first few hours after surgery in dogs. The reason of ocular hypertension in these cases that was to blockage of the iridocorneal angle by lenticular remnants, soluble proteins, pigments and vitreous humor, trauma during the surgical procedure. It was breakdown of the blood aqueous barrier and the presence of inflammatory cells, presence of viscoelastic material, emorrhage and synechiae The aim of this study was to investigate the effects of intraocularpressure (IOP) in extracapsular extraction (ECCE) and phacoemulsification (PHACO) methods with or without intraocular lens (IOL) on dogs with cataract for a 28-day period. Materials-Methods: Twenty adult dogs of both sexes with cataracts, weighing between 10 to 30 kg and at different ages were used as materials. Cataracts were diagnosed by direct and indirect ophthalmoscopy, ultrasonographic examination and biomicroscopy. Cataracts were classified as immature (7 animals), mature (7 animals) and hypermature (6 animals). Dogs were divided into two groups each consisting of 10 animals. Ten dogs with cataract were operated on for ECCE and the other group of 10 dogs underwent a phacoemulsification procedure. In the two groups, 10 animals were used for 41 dioptry single-piece acrylic intraocular lens. Results: Intraocular pressure was felt at the lowest level 14 days after the operation in the ECCE without IOL implanted group. The IOL implanted group showed irregular levels. Intraocular pressure level was the lowest on the 21st day, without IOL implanted group in phacoemulsification. However, all values remained within the reference values at the end of a 28-day period postoperatively. Conclusions: Both surgeries could be used for cataract cases in terms of IOP effects. Selection of the patient, correct surgical technique and adequate equipment are important for the success of a surgery. But, it has been also concluded that success of phacofragmentation surgery increased when the animals are in immature stages.
Keywords: Cataract, dog phacoemulsification, intraocular pressure

Table 1 - Intraocular pressure (IOP) of the dogs that underwent surgery according to the follow-up periods (mean ± SD)

	Pre-operative	7. Day	14. Day	21. Day	21. Day	References IOP Value (mmHg)
ECCE (-)	38,72 ± 5,51	27,84±4,03	16,52±1,56	23,96±2,23	21,08±1,72	15-30
ECCE(+)	15,36 ± 0,92	23,56± 3,69	23,44±3,68	13,72± 1,06	16,76±1,37	15-30
PHACO (-)	26,76 ± 1,87	12,20±1,30	11,52±1,41	9,28 ± 0,87	14,32± 1,33	15-30
PHACO(+)	29,12 ± 3,95	18,52± 1,90	17,56± 1,65	18,80± 1,47	17,76± 1,57	15-30

Prepartum Serum Ischemia Modified Albumin Levels (IMA) and Certain Biochemical Variables in Dairy Cattle and Their Associations with Dystocia, Abort and Retained Fetal Membrane

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Recently regarded as a sensitive marker of cardiac ischemia, the Ischemia Modified Albumin (IMA) was claimed to be increasing also in healthy pregnancies in human. In various studies conducted with human subjects, it was observed that IMA levels increased in preeclampsia, recurrent pregnancy loss, cesarean and in pregnancies when babies cannot grow sufficiently and it was also suggested that IMA may be beneficial in the early detection of complications. In veterinary, early detection of pregnancy complications is highly important for it results with huge economic losses with a decrease in milk productivity and infertility along with prenatal mortality. In this study, the maternal serum IMA and Albumin (Alb) levels of healthy pregnant cows and pregnant cows with complications (abort, dystocia, prolapsus) were measured. IMA levels of non- pregnant cows were also measured and the effects of physiological changes upon IMA levels were identified. In order to understand the possible increases in IMA, the levels of Total Antioxidant Status (TAS) and Total Oxidative Stress (TOS) were also evaluated. From 252 Holstein cows, serum samples were taken in the 2nd and 6th months of their pregnancy and these samples were frozen and kept until the labor. Then the serums of the cows developing complications throughout pregnancy were separated from those developing none and serum IMA, Albumin, TAS and TOS levels of each were compared. Moreover, from 20 non-pregnant cows blood samples were taken and were compared with those of pregnant ones. IMA and albumin-adjusted IMA (IMA/Alb) levels were relatively higher when compared to healthy pregnant ones, both in 2nd and 6th months of pregnancy ($P<0.01$). In the second month of pregnancy, both IMA and albumin-adjusted IMA levels were found to be higher than non-pregnant group ($P<0.05$). In complicated pregnancies, both in 2nd and 6th months TOS levels were found not to be statistically significant but higher than uncomplicated pregnancies ($P<0.01$). On the other hand, TAS levels were lower in complicated pregnancies when compared to uncomplicated ones ($P<0.01$). IMA serves as an early marker for clinicians in diagnosis of complicated pregnancies. The increase in IMA might be associated with oxidative stress. By detecting possible complications in advance and provide special care service for the patient, the side effects might be lessened and accordingly economic losses might be avoided to a degree. However, since the present study is the first in this field, further studies are required to confirm our findings.

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Keywords: Abort, cattle, dystocia, ischemia modified albumin (IMA), retained fetal membrane (RFM), serum chemistry

In-vitro Maturation of Bovine Oocytes without Gas Phase Control: Effects of Media on their Subsequent Development

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Although a gas phase of 5% CO_2 is usually used for in-vitro maturation (IVM) of oocytes, we recently produced bovine embryos without gas phase control using oocytes collected from slaughterhouse ovaries (Saeki and Fujiki, IETS, Versailles, 2015). In this study, we investigated the effects of different maturation media during IVM of bovine oocytes on their subsequent development to the blastocyst stage after in vitro fertilization (IVF). Bovine cumulus oocyte complexes (COCs) were collected from slaughterhouse ovaries. COCs were matured with four different media, 25mM HEPES-buffered TCM-199 with Earle's salts (Gibco, Invitrogen Life Technologies, Tokyo, Japan, H199E), TCM-199 with Earle's salts (Gibco, 199E), 25mM HEPES-buffered TCM-199 with Hank's salts (Gibco, H199H) and TCM-199 with Hank's salts (Sigma-Aldrich Japan, Tokyo, Japan, 199H) under 100% air at 39°C with high humidity for 22 h. All media were supplemented with 10% fetal calf serum (FCS) (BioWest, Paris, France), 0.02 AU/ml FSH (Antrin: Kyoritsu Pharmaceutical, Tokyo, Japan) and 1 µg/ml estradiol-17β (Sigma-Aldrich Japan). Ten medium droplets (50 µl) covered with mineral oil (Nacalai Tesque Co., Ltd, Kyoto, Japan) were placed on the bottom of a 60-mm plastic culture dish (Falcon 1007, Corning Life Sciences Japan, Tokyo, Japan). Ten COCs were introduced into each medium droplet. For a control, COCs were matured in H119E under 5% CO_2 in air at 39°C with high humidity for 22 h. After IVM, The pHs of the medium droplets were determined with a pH meter (Laqua twin, B-71X, Horiba, Kyoto, Japan). Then the oocytes were fertilized in medium IVF100 (Research Institute for the Functional Peptides Co, Ltd (IFP), Yamagata, Japan) with Japanese Black bull frozen-thawed sperm ($4x10^6$ cells/ml) under 5% CO_2 in air at 39°C with high humidity for 6 h. Sperm and cumulus cells were removed from the oocytes. The denuded oocytes were cultured in IVD101 (IFP, 20 to 30 embryos/50µL) under 5% CO_2, 5% O_2 and 90% N_2 at 39 °C with high humidity for 8 days. Experiments were repeated 4 times. A total 1,364 oocytes were used in this study. Data were analyzed by ANOVA followed by Tukey-Kramer test. Maturation, normal fertilization, cleavage and blastocyst rates were examined at each endpoint. The pHs of H199E (control), H199E, 199E, 199H, 199H were 7.3 ± 0.1, 7.8 ± 0.1, 8.1 ± 0.05, 7.0 ± 0.2 and 6.7 ± 0.1, respectively. Only 199H had a pH less than 7.0. The maturation rates with H199E (control), H199E, 199E, H199H, 199H (78, 71, 78, 74 and 49%, respectively) were not significantly different (P>0.05). Normal fertilization rates with these media (63 to 66%) and cleavage rates (75 to 86%) were not significantly different (P>0.05). However, the blastocyst rate with 199H (17%) was significantly lower than the rates with control (45%) and H199E (44%). The rates with 199E (42%) and H199H (39%) were similar (P>0.05) to those of control and similar to those with H199E and 199H. These results indicate that gas phase control is not necessary for IVM when using HEPES-buffered TCM-199 with Earle's salts. However, TCM-199 with Hank's salts is not suitable for bovine IVM even under 100% air.

Keywords: Bovine oocytes, development to the blastocyst stage, gas phase control, in-vitro maturation, maturation, medium

The Use of Formaldehit before Filtration Process of Foot-and-Mouth Disease (FMD) Vaccine Virus Production and Its Effect on FMD 146S Viral Particles Amount and Stability

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Foot and mouth disease (FMD) is the most contagious disease of mammals and caused by a virus of the genus Aphthovirus, family Picornaviridae. There are seven serotypes of FMD virus (FMDV), namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Infection with one serotype does not confer immunity against another. Formaldehyde (FA) and binary ethylenimine (BEI) are used for inactivation of FMDV vaccine strains in the preparing of FMD inactivated vaccines against FMD. In the FMD vaccine production process with BEI+FA, losses are seen in the amount of 146 S particules of FMD after adding of BEI+FA chemicals because of using together in the same time. The aim of this study was to reduce of losses of 146 S particules of FMDV using of FA in the different stage in the FMDV vaccine prosses. A-TUR-14 ve 0 TUR-07 FMD virus vaccine strains and two different methods were used and compared in this study. BHK21-An73 suspanded cell culture was proparaged at 37°C in Glasgow Minimum Essential Medium (GMEM) with Bovine Adult Serum (BAS), %10 Triptose Phosphate Broth'lu (TPB) fermenter with 30 L capacity. After reaching of cell numbers to 2-3x10^6/ml, GMEM was removed and added fresh GMEM without serum. A-TUR-14 ve 0 TUR-07 FMD virus vaccine strains (0.04MOI) were put in to cell culture (0.04MOI) incubated at 37°C for 16-18 hours. In the first method, at the end of the incubation period of FMDV vaccine strains in suspensed BHK21-An73 cell culture after observing %100 CPE, virus suspension was filtered in to the new fermenter and inactivation process was carried out by adding BEI+FA in the same time at 26°C. The inactivation was made for 24 hours. In the second method, at the end of the incubation period after observing %100 CPE of FMDV vaccine strains in BHK21-An73 suspended cell culture, virus suspensions were added FA at 37°C and waited for one hours. Virus suspension added FA filtered in to the new fermenter and inactivation was kept on by adding BEI at 26°C for 24 hours. In the first method, the 146 S particule amount of A and O type FMD viruses were found as 2.09 and 2.82 µg/ml before filtration and 1.52 and 2.24 µg/ml after filtration. after edding of BEI+FA in to virus suspensions for inactivation, the level 146 S particules of A and O type FMD viruses were detected as 1.22 ve 1.53 µg/ml at the end of the inactivation period. In the second method, the 146 S particule amount of A and O type FMD viruses were found as 2.32 and 2.95 µg/ml before filtration and 1.65 and 2.00 µg/ml after filtration. after edding of BEI+FA in to virus suspensions for inactivation, the level 146 S particules of A and O type FMD viruses were detected as 1.12 ve 1.47 µg/ml at the end of the inactivation period. As result, in order to reduce losses of FMDV particules during virus filtration and inactivation period performed by BEI, the edding of FA at the end of the virus incubation period at 37°C when observed 100% CPE in cells is very effective route in the production of FMD vacine strains.

Keywords: BEI, inactivation, formaldehyde, FMDV, production, vaccine

Investigation of Biokinetic Parameters for Batch Production Process of BHK 21 An_{30} Cells for FMD Virus Production

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In this study, the influence of glucose, glutamine, lactate and ammonium on biokinetic parameters of BHK 21 An_{30} cells used in the production of foot and mouth virus were investigated. Experiments were carried out in two main sections, cell and virus cultures, with in batch operating mode bioreactors. In the cell culture studies, simultaneously change intervals in initial concentrations of glucose, lactate, glutamine and ammonium are selected as 12-44 mM, 0-30 mM, 2-12 mM, and 1.4 to 7.3 mM, respectively. The design of experiment sets was carried out using Design Expert® 7.0.0 and these runs were accomplished in accord with the design. In designed experiments, cell growth, and viability, glucose, glutamine and energy metabolism and oxygen consumption metabolism were investigated. Cell growth was inhibited by high ammonium concentrations. 72% of the carbons of glucose converted to lactate. Lower ammonia and higher concentrations of glucose and glutamine has been attributed to increased contribution of OP to ATP production as 82% and this contribution was found as 68% in the average of all experiments. Specific ATP production rate was 0.81 ± 0.12 µmol 10^{-6} $cells^{-1}$ h^{-1}. High metabolite concentrations increased ATP production rate. In the metabolism of glutamine, contribution of the OP to the ATP production was 40%. Higher concentrations of ammonium and lactate and lower concentration of glutamine has been attributed to increased contribution of OP to the ATP production as 93%. Oxygen consumption rate was determined by a dynamic method and cell-specific oxygen consumption rate was found as 0.092 ± 0.017 µmol 10^{-6} $cells^{-1}$ h^{-1}. Cell specific glucose uptake rate and apparent lactate production rate were estimated as 0.181 ± 0.047 µmol 10^{-6} $cells^{-1}$ h^{-1} and µmol 10^{-6} $cells^{-1}$ h^{-1} 0.262 ± 0.083, respectively. Apparent specific glutamine uptake and ammonium production rate were found as 0,0555±0,0178 µmol 10^{-6} $cells^{-1}$ h^{-1} and 0,0526±0,0229 µmol 10^{-6} $cells^{-1}$ h^{-1}, respectively. Cells produced in cell cultures used in cultivation of O type FMDV. Independent variables of the system were determined as initial concentrations of glucose, lactate, glutamine, and ammonium. In response to these, specific growth rate, the final cell concentration, 146S, infectivity titer, antigenic titer, and the total amount of protein were determined. After the independent variables were introduced into Design Expert ® 7.0.0 software with surface response method, experimental planning was created and then responses for proposed experiments were modelled. ANOVA test of the proposed models were described by the statistical results. Proposed quadratic models for dependent variables with a low p value (<0.0001) were found statistically significant. Effects of glucose, lactate and ammonium concentrations on the specific growth rate were found statistically significant. The regression coefficient (R^2) of the model for 146 S was found to be 0.72. Effects of glucose, glutamine and ammonium concentrations on 146S were found statistically significant. Effects of the independent variables on the dependent variables were investigated by the proposed models, and model optimization was done to determine the optimum process conditions. Consequently, the proposed models four independent variables showed not only the effect on the biokinetic parameters of cell culture, but also the effect on the viruses produced from these cells. Such approaches give useful results about the total resultant effects on the complex processes which include living organism such as cell and virus cultures. It is thought that the achieved results are useful for process control, simulation and optimization purposes.

Keywords: Ammonium, BHK 21, batch culture, biokinetic parameters, FMDV, modeling

Detection of Inner Real Time Temperatures of Commercial Adult Bovine Serum During Heat Inactivation Process

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Adult Bovine Serum (ABS) is generally used to improve the growth cells. Generally, the heat exposure inactivation at 56C for 30 min. is a crucial process to destroy complement activity in the serum without affecting the growth-promoting characteristics of the product. Additionally, commercial sera may contain some viruses. During the inactivation period, serum inner real time temperature must be at least 56C so that some serum derived viruses can be destroyed. This study was established to determine the required inactivation time for serum inner temperature to be at 56C and to demonstrate the importance of serum inactivation before using in the cell culture. Commercial ABS and bovine blood serum freshly obtained were used. To indicate the prominence of serum inactivation period, 4 tubes of 250 ml blood were taken from 16-18 months calves and their sera are obtained and divided into sterile falcons. Two falcons of fresh sera were placed into water bath at 56C. The other falcons constructed the control group. Both groups were separately added as a 10% concentration into GMEM (Glasgow Minimum Essential Medium) with 5% TPB (Triptose Phosphate Broth) and antibiotics. BHK-21An30 cell line was three times passaged by using the prepared mediums. During cell growth, the morphology of cells, the duration of cell growth and the cell propagation were investigated. Commercial ABS was used for the detection of the duration of inner temperature to approach at 56C. Holes are made onto lids of 10 falcons and through these holes, thermometers were passed. Commercial ABS was filled into prepared falcons with thermometer. Falcons with commercial serum were placed into water bath at 56C. The temperature measurements were taken in each 5 minutes. Three grouped were established; non-inactivated sera, sera with 30 minutes exposure of inactivation and serum which was immediately taken as soon as inner temperature approaches 56C. To detect the effect of distinct inactivation over cell growth, two sera inactivated for 30 minutes and till inner temperature at 56C were separately added as their concentration 10% into GMEM with 5% TPB and antibiotics. BHK-21An30 cell line was three times passaged by using the prepared mediums. During cell growth, the morphology of cells, the duration of cell growth and the cell propagation were investigated. When the serum obtained from fresh blood with inactivation was used in the cell culture, BHK-21An30 cells could efficiently attach cell flasks. The initial cell number was adjusted as 4.8x10^5cell/ml. After 48 hours, the cell number was 2.6x10^6cell/ml. It was observed that there was no deterioration of cell morphology. Concerning the other group without inactivated serum, although initial cell number was same as inactivated one, cells couldn't attach cell flasks for 2 days. The required duration for inner temperature to approach at 55-56C was determined as 85-90 minutes. Additionally, for 30 minutes, internal temperature approaches at just 41-42C. Concerning the appearance of serum, there was no clear difference between 30 minutes and 90 minutes heat exposure. The attachment of cell, the morphology of cells, the duration of cell growth and the cell propagation didn't show significant differences between 42C and 56C. In conclusion, due to lack of inactivation of complements, the cell attachment and cell growth were negatively affected. However, after inactivation at 56C, as a result of inactivation of complements, cells could attach flask and show the proper cell propagation. There was no adverse effect of the inner temperature at 56C over BHK-21An30 cell culture. Instead, additional benefits might be gained. Serum derived viruses and extra-agents might be overcome via inactivation period at 56C.

Keywords: Adult bovine serum, inactivation

Cultivation and Comparison of BHK-21 Cells in Serum Free Medium by Using Two Adaptation Methods

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Serum is commonly used as a supplement to cell and tissue culture media. Serum has been shown to provide all the essential nutrients for cell growth and productivity, and attachment factors that serve multiple functions for the cells. But Serum is also a potential source of bacterial, mycoplasmal and viral contamination has important drawbacks, such as batch to batch variability, leading to lack of process and product consistency, complex downstream processing to remove serum contaminants from the final product, and fluctuating cost. For these reasons, current biotechnological approaches to cell culture avoid the use of serum. Furthermore, regulatory authorities in Europe and in the United States have encouraged biological manufacturers to reduce or eliminate the use of substances of animal origin in their manufacturing process. Recently, focus on the use of protein hydrolysates, especially origin of plant, in SFM.One of them is soybean which contains high percentage of protein In this study, the BHK-21 cell line was adapted to growth suspension culture with serum free medium (SFM) with soybean peptone by using different adaptation methods such as sequential adaptation method and inside or direct adaptation method. After adaptation growth studies of A serotype of foot-and-mouth disease virus (FMDV) were done with that cell culture. Characterization of the harvested virus was carried out by 146S antigenic particle quantification as well as determination of the antigenicity and infectivity titers. In conclusion, 1% soybean peptone can be used instead of serum supplement to grow BHK-21 cell line for FMDV production and switch adaptation method was useful and suitable for adaptation of BHK 21 cells to SFM medium because of reducing the time and number of cell passages. These results are significant mainly economic point of view for the large scale vaccine production plants.

Keywords: Adaptation methdods, BHK-21 cells, serum free medium, soybean peptone

The Employment of Cell Culture Adapted Pathogen Virus for the Immunogenic Study

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Foot and Mouth Disease (FMD) is a viral endemic disease of cloven-hoofed farm animal species. To struggle with FMD, inactivated FMD vaccines are commonly used. To determine the vaccine potency, strains of FMD virus with known titration of infective dose, adapted to the epithelial cell culture from calf's tongue, are injected into calf's tongue. Due to challenge of virus propagation in the calf's tongue and the hardness of determination of infective dose titration, BHK An31 cell line adapted virus is employed in the same cell line so that the level of immune response against FMDV and Calf's infective dose can be established. FMDV A and FMDV Asia 1 obtained from calf's tongue were inoculated into BHK An31 cell culture by adjusting inoculation as 1 ml ID50 10^4/ml. FMDV infected cell lines were innoculated at 37C, 5% CO2 for 3 days. Under same condition, FMDV A and FMDV Asia 1 were passaged three times. The propagation time of both virus strains and CPE formation were detected. The porduced viruses by following above procedure were diluted from 10^-1 to 10^-10. Two inoculation from each dilution were applied into calves via intralingual as 0,1 ml. For a period of 8 days, calves were investigated. 10.000ID50 of FMDV A and FMDV Asia 1 with known infective titrations via calf's tongue were determined. 8 calves already vaccinated with trivalant vaccine containing both strains were taken. 10.000ID50 FMDV A strain was inoculated into 4 vacinated calves and 10.000ID50 FMDV Asia 1 strain was done into the other vacinated calves via intralingual as 0,1 ml. As a control group, each strains with the same dose were inoculated into 2 unvaccinated calves separately. Inoculated calves are kept under observation for 10 days. Each day, lesion investigation was applied over their foot and tongues. Findings: 1. At the end of first passage, FMDV A and FMDV Asia 1 strain inoculated into the BHK An31 cell line drove to 70-75% CPE while in the third passage, 100% CPE formation was observed. Moreover, titrations of FMDV A and FMDV Asia 1 strains were found respectively as 10^7,7 ve 10^7,2. 2. FMDV A and FMDV Asia 1 strain propagated in the BHK An31 cell line didn't make lesion on foot and tongues of vaccinated calves while in the control group, they caused lesion coming from FMD. In conclusion, it has been clarified that cell culture adapted virüs strains can be employed for immunological studies on calves and challenge of adapted viruses.

Keywords: Adaptation, BHK 21 cells, FMD virus, patogenicity, vaccine

Comparision of Cytotoxicity of Antifoam by Different Methods on L929 Cells and BHK-21 Cells

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Antifoam agents can be an organic or inorganic chemical agent or a combination of both. They are used as little as possible to control the foaming in fermentation processes. Processes differ and selection of the proper antifoam often depends on the culture' metabolic activity as well as the desired end product. The ideal antifoam agent is one that is completely metabolized by the cells or easily removed by downstream processing. Cell culture can be used to screen for toxicity both by estimation of the basal functions of the cell or by tests on specialized cell functions. General toxicity tests, aimed mainly at detection of the biological activity of test substances, can be carried out on many cell types. A number of parameters including vital staining, cytosolic enzyme release, cell growth and cloning efficiency are used as end-points to measure toxicity. Cytotoxicity testing is a rapid, standardized, sensitive and inexpensive means to determine whether a material contains significant quantities of biologically harmful extractable. The purpose of this study is to evaluate and compare the cytotoxicity of antifoam agent which is silicone emulsion on L929 cells and BHK-21 cells by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), SRB (Sulforhodamine B sodium salt) and NRU (Neutral Red Uptake) methods. L-929 cells and BHK-21 cells were treated with ten different concentration of antifoam agent and incubated for 24h. Cell viability was determined by MTT, SRB, NRU methods and trypan blue staining. The present study showed that the optimum antifoam concentration for the remarkable proliferation and the best viability of cells was found at the dilution of 106. All the methods gave good correlation with viable cell number in toxicity assays but MTT assay were found to be very sensitive under the conditions examined.

Keywords: Antifoam, BHK-21 cells, cytotoxicity, L929 cells

Demonstrations of BVDV Antigen on Ear Skin Notchs of the Persistently Infected Cattle with BVDV

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Despite of vaccination of cattle aganist BVD virüs, it is still a widespread pathogen in cattle herds in Turkey. Disease leads to diarrhea, acute and chronic mucosal disease, immunosuppression or immuntolerance, congenital defects, reproductive failure and subclinical or persistent infection in cattle. Persistently infected animals are the most important cause of the spread of disease within a herd. In this regard, detection of persistent and subclinically infected animals in the herd is prerequisited to implement for the elimination of the disease. In our country, ELISA and RT-PCR techniques are widely used for the determination of BVDV infected animals. In these techniques, in order to diagnose persistent infection, blood should be collected from the suspected animals two or more times. In the current study, in addition to these techniques, the reliability of immunohistochemistry which is faster, practical and low cost was investigated from ear notch samples as a routine diagnostic technique. The blood and ear notch samples were taken with an interval of 3 weeks from 211 cattle under 1 year of age. The presence of antigen or antibodies was revealed with Ag-ELISA and Ab-ELISA in the collected blood samples. Ear notches taken from the same animals were examined for BVDV antigen by immunohistochemistry in parallel with other routine diagnostic techniques. According to the ELISA test results of the analyzed blood samples, 5 persistent infected animals were identified. Blood serum from one animal was evaluated as acute infection. Fluorescence and immunohistochemical images of BVDV antigen were captured from the ear skin biopsy samples of persistently infected animals. The results of the current study showed that IHC of formalin fixed and paraffin embedded ear tissue samples is a reliable method for the diagnosis of persistent infection with BVDV in cattle.

Keywords: BVDV, ear notch, ELISA, immunohistochemistry, persistently infected

Investigation of Pathogenesis of Different Sheep and Goat Pox Viruses in Cattle

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The aim of this study was to revealed of the pathogenicity of sheep and goat pox viruses in cattle. Bakırköy sheep and goat pox virus (BKSGP), Denizli goat pox virus (DGP) and Kenya 0240 sheep and goat pox virus (KSGP) strains were propagated on vero cell cultures and determined of their $TCID_{50}$. For this study, 12 cattle aged between 10-13 months were used and divided in to 3 group. 4 cattle were injected with every strain of Capri poxviruses via intradermal route for 1 ml. After inoculation, all cattle were screened for local and clinical symptoms for 28 days and blood samples were collected from all cattle at 0,4,7,12 and 28 days. Tissues samples were taken from the inoculation side at 28th day. All bloods and tissues samples were analysed to determine of BKSGP, DGP, KSGP viruses by PCR(OIE,2010). Titres of Bakırköy sheep and goat pox virus (BKSGP), Denizli goat pox virus (DGP) and Kenya 0240 sheep and goat pox virus (KSGP) strains propagated on vero cell cultures were determined as 10^4/ml, $10^{4.5}$/ml and 10^4ml $TCID_{50}$ respectively. In the pathogenicity studies, swelling were began to appear at 3rd day and disappear at 12th day in all cattle after inoculation of all Capri pox strains. The cattle given BKSGP and DGP strains, swellings were disappeared at 24-26th day. In the other hand, local necrotic lesions occurred about 5-7 cm diameter in cattle given KSGP virus at 28th day. Using PCR test, KSGP virus was detected only from skin samples inoculated KSGP virus via intradermal but BKSGP, DGP and KSGP strains were determined from all bloods samples at 7th and 12th day. At the end of the this study, it was determined that Bakırköy sheep and goat pox virus (BKSGP), Denizli goat pox virus (DGP) and Kenya 0240 sheep and goat pox virus (KSGP) strains infected all cattle and it was seen that KSGP virus strain was found to be more pathogenic than BKSGP and DGP virus strains in cattle. As a result, KSGP virus can be used as pathogenic strain to determine of immunity levels of cattle vaccinated with BKSGPV vaccine strain in Turkey.

Keywords: Cattle, goat, pathogenecity, poxvirus, sheep

Investigation of Presence of Bacterial Endotoxins in Media Used in Production of Foot and Mouth Disease Vaccines

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The aim of this study was to investigate the presence of bacterial endotoxins (BET) in the cell and virus cultures media, prepared as a part of foot and mouth disease (FMD) vaccines production process, and evaluate the potential adverse effects of the BET presence on the BHK-21 cell cultures and FMD virus cultures. Samples were taken during the media preparation at different stages of the process including water samples taken before and after collecting of the water as the first stage, samples taken after adding each group of media ingredients and at the last stage media samples were taken before and after sterile filtration of the prepared media. In order to detect and quantify the BET concentrations two methods of limulus amebocyte lysate (LAL) test, gel-clot method and kinetic turbidimetric method, were used. When needed, tests were performed on sample dilutions in order to avoid interference as well as to obtain BET concentrations falling into the working range. From the obtained results it was concluded that there were two main possible sources of the BET presence in the media. The first one was equipment cleaning. Cleaning method or frequency of the cleaning of the equipment during the process had direct effect on the BET levels. The second one was the sera used in cell cultures media. The contribution of the sera on BET levels in cell cultures media could be very changeable. Depending on the batch of the sera used, final concentrations of BET in the media changed dramatically. It was evaluated that final sterile filtration could be effective in reducing BET presence, but in some cases when BET concentrations were too high it failed to reduce BET concentrations under certain level. It suggested that relying only on final filtration was not a good strategy in avoiding BET presence. As a conclusion of the study it was evaluated that present BET levels in prepared media could have potential adverse effects on cell and virus cultures. However, in order to determine the acceptable level of the BET, further studies on BHK-21 cells cultures-BET as well as FMD virus cultures-BET interactions need to be performed. It was suggested that BET monitoring and BET acceptance criteria should be included in the cleaning validation procedures of the equipment and monitoring of the sera for BET should be in a stricter manner.

Keywords: Bacterial Endotoxins, BHK-21, medium, sera, FMD

Removal of Viral Particles Remaining Inside the Cell at the End of the Reproductive Period of Foot and Mouth Disease Virus

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Foot and Mouth Disease (FMD), with the restrictions on international trade hoofed animals that caused yield loss in causing huge economic losses, is an infectious viral disease. Protection against on FMD depends of virus neutralizing antibody depends on the expression levels. So, following reduction of the immunizing antibody levels increases sensitivity. Because of that protective immunity is required to achieve levels of vaccination at regular intervals. This study was established to realize of virion in the cell cytoplasm of unlysed cells seen CPE (cytopathic effect) with infected FMD vaccine virus strain at the end of the virus propagation cycles in the FMDV Vaccine production studies. This study was realized in bioreactor with 30 L working volume, automatic magnetic stirrer and computer-controlled. BHK-21 An 73 suspended cell culture was propagated in GMEM (Glasgow Minimum Essential Medium) with %10 BAS(Bovine Albumin Serum) and %10TPB (Tryptose Phospahate Broth) at 37°C in 2.2-2.5.0x106 cells / ml. O,A and Asia- 1 FMDV Vaccine strains were inoculated at 0.04 MOI (Multiplicity of infection, virus / cell) levels. After virus inoculation, incubation period was completed between 16-20 hours when CPE was seen100% infected cells, 350 g carbonate added at 37°C and kept on incubation for 1 hour and taken virus suspension samples before and after adding carbonate. FMDV particles were measured by combination of sucrose density gradient centrifugation method continuous-flow UV spectrophotometer. The titer of infectivity titer was calculated using plaque assay method according to Karbers methods The amount of the FMDV 146 S particles were obtained, 87.13 % for Asia- 1 strain, 104.41 % for A strain, and 268.44 % for O strain. The infective titers were found at the same value between before and after adding carbonate. The data obtained from this study proved that, in the FMDV virus production after the end of the incubation period observed in 100% CPE addition of same amounts of carbonate into the infectious virus suspension. Large amounts of Foot and Mouth Disease virus146S viral protein can be obtained therefore that high amounts of vaccine can be prepared in short time.

Keywords: 146S, carbonate, CPE, foot and mouth disease virus, vaccine

Production of FMD Virus in Micro Bioreactors Supported Carriers

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The purpose of this research is to be produced seed virus used in the FMD vaccine virus production supported on micro carriers in bioreactors. In light of the findings obtained in the project it is targeted that seed virus production is to be done in packed bed bioreactors rather than the roller bottles which are still used. In the present project, FMD virus in cell cultures of micro -carrier generation and availability padded bed bioreactor was investigated. The study, purchased commercially nonwoven polyester fabric (NWPF) produced on micro carriers BHK-21 cell, with 6M medium containing 10% bovine serum, were incubated in 30 L working volume bioreactor packed bed 24-48 hours at 37 ° C. The number of cells in the incubation period, the carrier concentration and the amount of virus inoculation (MOI), pH and dissolved oxygen was kept constant, the medium changes that may occur in the medium during the process was monitored using analysis and oxygen consumption rate. When the number of BHK-21 cells produced in bioreactors reached 1.8-2.0x106 cell / ml, O TUR-07 master seed virus were inoculated to be 0,04 MOI (Multiplicity of infection, virus/cell). At the end of the incubation period of 16-20 hours following the formation of 100% CPE cells Samples were taken to determine the reproducing virus 146S and infective titers. Repeated 20 times, the results of production activities in packed bed bioreactors were determined as, the highest 146S and infective titers, respectively, as 4.02 µg / ml and 8.44 pfu/mL, the lowest titers 146S and infective titers, respectively, as 2,89 µg/mL and 7,98 pfu/mL, in monolayer cultures were determined as, the highest 146S and infective titers, respectively, 3,62 µg/mL and 8,08 pfu/mL, the lowest titers 146S and infective titers, respectively, as 2,24 µg/mL and- 7,16 pfu/mL. As a result, in contrast to the monolayer culture the padded bed reactor studies have demonstrated to show an increase in amount of 146S 0.3 times and in infective titers 0.3 log times. In future, this for virus seed production system has been shown to be a viable alternative monolayer system. At the end of the study, in the light of all the results obtained, operating system of packed -bed bioreactors by converting into the perfusion system instead of discrete culture. for BHK- 21 cells and for the production of seed FMD virus, with less labor and material costs can be operated easily, with a low risk of contamination and advantageous system consumes less energy the realization of high yield concluded that provide optimal conditions

Keywords: BHK-21 cell culture, foot and mouth disease virus, micro carriers, oxygen consumption rate, packed bed reactors

Efficiency of Dithiothreitol and Sucrose on Bull Semen Cryopreservation

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The aim of this study was to assess the effects of supplementation of dithiothreitol (D) or sucrose (S) as antioxidants on the sperm parameters, plasma membrane integrity, antioxidant activities, sperm molility characteristics and DNA integrity in Tris extender for cryopreservation of bull semen.Totally 24 ejaculates were collected from the three Holstein bulls with the aid of an artificial vagina twice a week. A Tris-based extender (T) and 5% Glycerol (G) was used as the base for the experimental extenders. Each ejaculate was split into three equal aliquots and diluted using both of 5 mM D or 25 mM S, and without additives (control; C). The extended samples were equilibrated slowly to 4°C for 4 h and then froze using a digital freezing machine. Frozen straws were thawed individually in water bath at 37°C for 30 s to analyse motilities, acrosome and total abnormality, plasma membrane integrity, antioxidant activities and sperm motility characteristics. The volume of ejaculates was measured in a conical tube and sperm concentration was determined by Accucell photometer. Sperm motility was indicated using phase-contrast microscope (200x). Sperm motility characteristics were determined by sperm analysis system. DNA integrity was evaluated by comet assay using image analysis system. When compared to the control, D had greatest motility ($P < 0.05$). However, addition of D and S did not significantly increase the percentages of post-thaw sperm progressive motitility, acrosome and total abnormalities and plasma membrane integrity ($P > 0.05$). Control group gave the lowest MDA but this result was not supported with the GPx activity ($P < 0.01$; Table 1). Sperm motion characteristics such as VAP, VCL and BCF gave significantly different results except for VSL, ALH and LIN ($P < 0.05$). D and S were showed better DNA integrity than C (Table 2). In conclusion, it may be stated that, using D and S may improve the DNA integrity. In addition D gave greater motility result than S and C in T extender with 5% G.

Keywords: Antioxidant activity, bull semen, cryopresrevation

Table 1 - Mean (±SEM) CASA progressive motility, CASA sperm motility, acrosome and total abnormalities, plasma membrane integrity, glutathione peroxidase (GPx) and malondialdehyde (MDA) in frozen–thawed bull semen

Groups	Progressive Motility (%)	Motility (%)	Acrosome (%)	Total Abnormality (%)	HOST (%)	MDA (nmol/ml)	GPx (U/ml)
Control 5% G	25.50±3.70	49.50±3.94b	2.13±0.55	10.50±1.41	41.13±2.79	1.73±0.15a	10.34±0.48bc
5% G+5mM D	25.38±2.97	52.25±2.78a	2.38±0.42	11.38±1.29	39.00±3.39	1.85±0.13b	9.46±0.53ab
5% G+25mM S	18.38±2.57	44.75±4.41b	2.50±0.27	11.38±0.65	36.63±2.17	1.83±0.14b	10.82±0.38c
P	N.S.	<0.05	N.S.	N.S.	N.S.	<0.05	<0.01

Table 2 - Mean (±SEM) CASA sperm motion characteristics and DNA integrity in frozen–thawed bull semen

Groups	VAP (µm/sec)	VSL (µm/sec)	VCL (µm/sec)	ALH (µm)	BCF (Hz)	LIN (%)	Tail moment (µm/s)
Control 5% G	99.53±4.79a	80.34±4.87	173.00±8.96a	7.64±0.36	22.06±0.79b	47.88±1.79	9.58±0.22a
5% G+5mM D	102.16±4.43ab	82.05±4.15	178.80±9.11ab	7.76±0.32	22.24±0.82b	47.38±1.08	7.33±0.13b
5% G+25mM S	107.20±3.39c	83.95±2.70	186.71±7.60c	8.33±0.33	17.45±1.25a	46.13±1.41	7.78±0.27b
P	<0.05	N.S.	<0.05	N.S.	<0.05	N.S.	<0.05

Effects of Lycopene and Cysteamine on Bull Sperm Quality, DNA Integrity, Oxidative Stress Parameters and Fertility Results

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The objective of this study was to compare the effects of adding antioxidants; lycopene (L) and cysteamine (CY) on the sperm parameters, plasma membrane integrity, chromatin damage, antioxidant activities as well as fertility results in Tris extender for cryopreservation of bull semen. Ejaculates were collected from the three Holstein bulls using an artificial vagina twice a week. After collection, the ejaculates were immersed in a water bath at 35°C until their assessment in the laboratory. The volume of ejaculates was measured in a conical tube and sperm concentration was determined by means of an Accucell photometer. Sperm motility was estimated using phase-contrast microscope (200x). Tris-based extender (T; 189.5 mM Tris, 63.2 mM citric acid, 55.5 mM fructose, 20% v/v egg yolk, 7% G and 1000 ml of distilled water at a pH of 6.8) was used as the base extender. Ejaculates were split into three aliquots and extended to a final concentration of 15x106 spermatozoa/per straw (0.25 ml) with the T containing 500 µg/ml L, 5 mM CY and no additive (C). The extended samples were equilibrated slowly to 4°C for 4 h and then froze using a digital freezing machine. Frozen straws were thawed individually in water bath at 37°C for 30 s to analyse progressive motility and sperm motion characteristics as well as plasma membrane integrity. Biochemical assays were performed in a spectrophotometer using commercial kits. Chromatin damage was evaluated by comet assay using image analysis system. Fertility results based on 60-day nonreturns after rectovaginal insemination. When compared to the control, addition of L and CY did not significantly improve the percentages of post-thaw sperm progressive (22.00±1.46, 24.38±3.17, 8.75±1.19 respectively; $P<0.001$) and CASA motitilities (44.75±2.32, 49.13±3.52, 20.88±1.69 respectively; $P<0.001$), total abnormality (13.00±1.36, 12.25±0.77, 19.00±0.53 respectively; $P<0.05$) and plasma membrane integrity (47.50±0.28, 42.00±2.17, 34.50±1.63 respectively; $P<0.001$). In terms of chromatin damage, L exhibited lower tail intensity (9.78±0.94) compared with other groups (11.47±1.10 in C and 12.70±0.79 in CY respectively; $P<0.05$) however, these results were not supported with the fertility results ($P>0.05$). In conclusion, the suplementation of L or CY did not have any influence on fertility results in T extender with 7% G.

Keywords: Antioxidant activity, bull sperm, DNA integrity, fertility, oxidative stress, sperm freezing

Polymorphisms Study in the ITS rDNA Regions for Differentiating Strains of *Trichophyton Verrucosum* Complex in Sfax-Tunisia

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Trichophyton verrucosum is the most frequent etiologic agent of cattle dermatophytosis. Throughout the world it was the second most common agent of zoophilic dermatophytes in human. Strain identification is important for identification of strain-related differences in infectivity potential or transmissibility and epidemiological studies. The aim of our study was to evaluate the efficacy of the PCR-RFLP and PCR-Sequencing methods for the identification and differentiation of *T. verrucosum* strains. Thirty six clinical strains identified by morphological characteristics as *T. verrucosum* were isolated from patients referred to Parasitology-Mycology laboratory of Sfax University Hospital. Identification of our strains by conventional methods was confirmed by molecular methods in 94.4 % of cases. Two strains were reclassified as *T. violaceum*. PCR products digested with *Hinf I* produced three profiles and two profiles with *MvaI*. ITS sequence analysis revealed a polymorphism in the ITS1and 5.8S regions. Analysis and alignment of consensus sequences has distinguished two types of genotypes among our *T. verrucosum* strains. The type I was the dominant genotype (93.7%). Phylogenetic study showed that the first clade contained *T.verrucosum* strains with ITS type I and species of *T.mentagrophytes* complex. The second cluster contained two *T.verrucosum* strains with ITS type II. PCR-RFLP of ITS regions provided excellent tool for identifying *T. verrucosum* but also for detecting intra-species polymorphisms. PCR sequencing was useful for distinction of strains. In this study, most of*T.verrucosum* isolates were type I, dissimilar to others rare studies where type II has been the most common. Strains differentiation is relevant because it helps in prescribing the correct treatment and determining the source of the infection.

Keywords: ITS regions, PCR –RFLP , phylogeny, sequencing, trichophyton verrucosum

Bovine Brucellosis: Advantages of Using Vaccine RB51

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Bovine brucellosis is present worldwide and still causes severe problems, due to abortions, placental retention and transmission to humans. To control the disease there are three parameters to obey: Diagnosis, vaccination and elimination of positive animals. In cattle two vaccines are available, S19 and RB51. Both vaccine can be used because induce similar protection, however, RB51 do not induce antibodies which interfere the diagnostic and can be applied more than once without cause such inconvenient. In America, countries that use RB51 (USA, Chile, Uruguay) have lower prevalence than the others that use both vaccines (Ecuador, Venezuela, Mexico, Colombia, Brazil) or only S19 (Argentina). Years ago, RB51 was authorized for use in Argentina just for adult animals. We show here some examples of the contribution of RB51 for controlling brucellosis in infected farms. Two dairy farms where brucellosis was present were selected for doing this study Farm A: 2224 animals with the initial prevalence of 1. 75%, Farm B: 510 animals with an initial prevalence of 3. 1%. Strain 19 was applied to all female calves between 3 and 8 months of age in both farms. Serology: Buffer Plate Antigen Test (BPAT), Complement Fixation (CF) and Fluorescent Polarization (FPA) were the test used for diagnostic of brucellosis. RB51 was implemente in both farms to S19 vaccinated animals. Table 1 and Table 2: Animals were bleed and those positives were eliminated from the herd. Abortions were collected and burned as soon they were found. Serology indicate a strong infection. *Brucella abortus* biotipe 1 was isolated. After 6 months of iniciate the test and slaughter method the strategy have not good results. For this reason, RB51 was applied in both farms to the whole herd, including pregnant animals. In consequence, serology went down to 0 and no record of abortions was observed. Both tables indicate that after RB51 vaccine implementation, brucellosis was controlled and eliminated from both herds. Discussion Although vaccination (either S19 and/or RB51) is implemented, outbreaks due to brucellosis are still present in bovines in Latin America. As example, we studied two dairy farms, a " large" and a “medium” herd (in number of animals) where animals who were S19 vaccinated as calves, have an outbreak with abortions, positive animals by serology and initiated a tested and slaughter program. In both cases, positive animals were eliminated from the herd as soon they were detected. After 6 months of doing this program, results were very poor. For this reason, we decide to apply RB51 vaccine to the whole herd. Interestingly, no abortions were seen after application to pregnant animals. In other studies, we demonstrate that RB51 when is applied to animals previously vaccinated as a calf either with S19 and/or RB51 rarely abort after being vaccinated as adults. Data from tables 1 and 2 shows a progressive diminution of new cases after RB51 vaccination. Management of the herds continues the same as before in both herds and no other tool was applied. In both farms, due to the successful of the RB51 vaccination we decide to do a second RB51 dose to the whole herd to prevent futures complications with the disease. Serology continues to be negative in both cases after the second RB51 application. Conclusion, S19 and RB51 are both good vaccines, however the importance is to know how and when those inmunogens should be applied. The advantages of RB51 are that this tool can be used anytime without complicate diagnostic and it doesn’t induce abortion in pregnant animals previously vaccinated as a calf, but more important it is an excellent tool to control bovine brucellosis.

Keywords: Brucellosis, bovine, RB51

Table 1. RB51 vaccine applied to Strain 19 vaccinated animals

Number of Animals	Negatives	Positives CF	Nº New Cases	Time (days) between bleeding
2224	2185	39 (*)	39 (**)	60
2206	2151	55	55 (***)	29
2014	1994	20	20 (***)	38
1878	1850	28	28	45
1840 (RB51)	1813	27	27	35
1760	1743	17	17	66
2179	2168	11	11	45
2077 (RB51)	2071	6	6	45
2110	2110	0	0	40

*Positives CF: Complement Fixation * Initial prevalence (1,75%) ** 5 abortion were detected ***5 abortions were detected. RB51: Day of application of vaccine RB51 No abortion were observed after RB51 application. The farm continue been negative for brucellosis*

Table 2. RB51 vaccine applied to Strain 19 vaccinated cattle Dairy Farm B

Number of Animals	Negatives	Positives CF	Nº New cases	Time (days) between bleeding
510	494	16 (*)	16 (**)	62
488	480	8	7	59
501	502	9	7	61
495 (RB51)	489	6	6	45
494	489	5	5	45
502 (RB51)	497	5	4	40
499	496	3	2	43
506	506	0	0	40
517	517	0	0	52

*Positives CF: Complement Fixation * Initial prevalence (3,1%) **4 abortion were detected RB51: Day of RB51 application NO Abortions were observed after RB51. The farm continue been negative for brucellosis*

Implantation Technique of a Long Term Port-a-Cath Epidural Catheter System in Cows

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To describe the implantation technique of a long-term port-a-cath epidural catheter in cows. This study was approved and supervised by the institutional animal care and use committee (Protocol number: 024902/14). For this study, 8 cows with 4 ± 1 year of age and weighting 413±105 kg were used. At the morning of the study, each animal was restrained in a hydraulic stock. As follows, the sacrococcygeal region was submitted to hair clipping followed by surgical antisepsis. An association of 0.04 mg/kg acepromazine 1% with 0.01 mg/kg xylazine 2% was administered intramuscularly for purposes of sedation. Local anesthetic was infiltrated subcutaneously in the sacrococcygeal region (10mL) and in the mid-third of the gluteus medium (10mL). Fifteen minutes following local anesthetic infiltration, a semicircle incision of 8 cm in length was centered over the sacrococcygeal space. A 16-gauge Tuohy needle was than inserted into the respective epidural space and used as guide for implantation of a 17-gauge long-term port-a-cath epidural catheter, which was advanced 10 cm cranially. Tuohy needle position within the epidural space was confirmed by Gutierrez's and/or Dogliotti's tests. Tuohy needle was removed immediately following catheter implantation. As follows, a second 4 cm linear skin incision was performed over the right mid gluteus muscle, approximately 15 cm apart from catheter insertion. The catheter's distal extremity was than transposed to the second skin incision by subcutaneous tunneling. The excess extension of the catheter was excised and the catheter distal end coupled to an access portal which was concealed in the subcutaneous space. Catheter patency, leakage and obstruction were tested by administration of 1 mL of sterile distilled water through the access portal. Skin incisions were sutured with 1-nylon in simple separate pattern. Antibiotic therapy was performed with benzathine penicillin (20.000 IU/kg) every 48 hours in a total of three applications. The animals were kept in a paddock and examined daily until skin sutures removal 15 days following catheter implantation. Radiographs of the sacrococcygeal region were carried out to verify the correct position of the catheter into the epidural space and catheter patency was tested every 15 days by the administration of 3 mL of 2% lidocaine hydrochloride, followed by 1 mL of sterile distilled water. Mean patency of the catheter has been of 60 days so far. Following lidocaine administration the animals were evaluated for tail relaxation and perineal sensitivity. Surgical implantation technique of a long-term port-a-cath epidural catheter is of medium complexity. All animals presented relaxation and loss of perineal sensitivity and tail relaxation following lidocaine administration through the catheter. The mean period of observation following catheter implantation has been of 60 days. So far, there hasn´t been signs of implant rejection as well as signs of complete or partial catheter obstruction in any of the studied animals. Conclusion: The epidural port-a-cath system implantation technique has shown to be a feasible procedure, allowing drug administration within the epidural space for at least 60 days without the need for multiple punctures.

Keywords: Epidural anaesthesia, epidural analgesia, surgical technique

The Treatment with Locking Plate of Fracture of Distal Metacarpus in Calves

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Objective of study: to evaluate the effect of locking compression plate for the treatment of distal metacarpal fractures in calves. Material-Methods: twenty calves with distal metacarpal fracture in different breed, age and sex were constituted in this study. In all calves, xylazine HCL (0.2 mg/kg) and ketamine HCL (2.2 mg/kg) were injected intramuscularly for anesthesia induction. Following oro-tracheal intubation procedure, general anesthesia was provided by 3% inhalation of isofluorane. Operation was started by an "S" type skin incision on the dorsal median line of metacarpus. The fractured area was reached by blunt dissection of subcutaneous tissues. The limb was aligned and fractured bone was positioned. Metacarpus was stabilized using stainless steel LCP (locking compression plate,2 mm thicknes,20 mm width and 90-110 mm length) with fully threaded locking head screws (3.5 Ø, 30-35 mm length cancellous for distal fragment; 3.5 Ø, 26-32 mm length, cortical for proximal fragment). The plates were contoured to the cranial/dorsal surface of the bones. The shape of LCP was designed as letter T. The overhead of plate (2 mm thickness, 3 width and 2mm length) has got 3 holes. The shaft of plate has got 6-8 holes and both overhead and shaft holes were aligned as cross-sequential. All edges of plate were rolled. Statistical analysis was performed using Shapiro-Wilk test for normality hypothesis; levene's test for variance homogeneity. Logarithmic transformation was used for abnormal distribution data. Two-way variance analysis in repeated measurements was used to evaluate time and group factors. $P < 0.05$ was considered significant. Results: All fractures were occurred due to forced extraction during dystocia and were closed, comminuted and located in the distal diaphysis. One metacarpus was assigned to secure a 20 mm broad locking compression plate with 6-8 roughened corticalis screws on the proximal diaphysis and 3 cancellous screws on the distal diaphysis. The calves started partial weight bearing after the first postoperative day and resumed complete weight bearing after the 10th day. The callus formation was obtained at the end of 3rd week, completed end of 8th week and seen complete healing in 12th week in radiograms. We did not encounter with loss of obvious bone density on radiograms. Follow-up radiographs 4 months postoperatively revealed complete osteotomy healing and remodeling. The limb was in good alignment, the calf was fully weight bearing, and client satisfaction was very high. Telephone follow-up with the owner 12 months after surgery revealed continued full use of the operated limb. The average metacarpal length, diameter of cortex and diameter of medulla were measured as 163±1.98 mm, 22.57± 0.50 mm, 15.91±0.31 mm; 163±2.26 mm, 26.39±0.55 mm, 16.56±0.31 mm; 182±1.80 mm, 30.21±0.46 mm, 17.29±0.31 mm; 199±1.62 mm 34.53± 0.50 mm, 18.08±0.26 mm in fractured metacarpus, in postoperative 1st, 45th, 120th, 365th day respectively. The average metacarpal length, diameter of cortex and diameter of medulla were measured as 167±1.81 mm, 22.54±0.50 mm, 15.96±0.31mm; 174±1.75 mm, 26.81±0.52 mm, 17.44±0.31mm; 195±1.70 mm, 30.57±0.43 mm, 17.62±0.31 mm; 207±1.56 mm, 35.68±1.03mm, 18.25±0.27 mm in non-fractured metacarpus, in postoperative 1st,45th, 120th, 365th day respectively. The measurements of wrist and foot axis are calculated as 149,3°; 152,5° in fractured and non-fractured limbs (n= 6), respectively, after one year. There was significant difference in metacarpus length between fractured and healthy limb in 120th and 365th days. Conclusion: As a result, due to the characteristics of juvenile bones do not provide sufficient physical strength for implants. We decided that locking compression plate is associated with a good prognosis for surgical repair of distal metacarpal fractures in newborn calves.

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Keywords: Calf, fracture, locking compression plate, metacarpus

Experimental Infection of Mice with Bovine Viral Diarrhea Virus

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Bovine viral diarrhea virus (BVDV) is an important pathogen that can affect cattle productivity and lead to substantial economic losses in the livestock industry worldwide. BVDV is known to infect a wide range of wild and domesticated ruminant and porcine species. The objective of this study was to infect mice with bovine viral diarrhea virus (BVDV) and then to establish BVDV infection in mice. We inoculated mice with three different BVDV strains (ncp BVDV1, ncp BVDV2, cp BVDV1) by two different routes, intraperitoneal (IP) injection and intranasal (IN) inoculation. The challenge was performed by IP injection or IN inoculation of 0.4 mL of tissue culture fluid containing 5 x 105 TCID50 of each virus. All mice (ncp BVDV1-, ncp BVDV2-, cp BVDV1-infected, and mock-infected) were euthanized with CO2 gas to collect blood and tissues. The tissues were routinely processed and embedded in paraffin for hematoxylin and eosin (H&E) staining. Immunohistochemistry was performed using tissue samples. RNA was extracted from blood using the PureLink® Total RNA Blood kit (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed in a one-tube system using the pan-pestivirus primer pair V324-326 (INtRON Biotechnology, Inc.; Daejeon, Korea). All mice were euthanized at day 7 pi, and none of the infected mice exhibited any clinical signs of illness; however, the tissues harvested after BVDV challenge showed significant histopathological changes. Blood samples from five mice that were injected IP and one mouse that was inoculated IN were positive for BVDV by RT-PCR. IHC was used to assess the presence of viral antigen in the organs of mice infected with three BVDV strains. In IP-injected mice, BVDV antigen was detected in the spleen (5/6), mesenteric lymph nodes (4/6), lymphatic tissue of the lung (3/6), lung (1/6), and stomach (1/6) of the infected mice; however, it was not detected in the liver (0/6) or kidney (0/6). In IN-inoculated mice, BVDV antigen was detected in the lung and mesenteric lymph nodes of one BVDV-infected mouse, but was not detected in other tissues. Our study demonstrates that mice can be infected by BVDV. Even though none of the infected mice showed clinically apparent disease, the presence of viremia provided evidence that the mice were infected with BVDV. The results of this study suggest that the spleen is the most reliable tissue for BVDV antigen detection using IHC in the IP-injected group. This is the first report of BVDV infection in mice.

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Keywords: Bovine viral diarrhea virus, immunohistochemistry, intraperitoneal, intranasal, mouse

Investigation on Etiology of Subclinical Mastitis in Jersey and Hybrid Jersey Dairy Cows

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The aim of the study was to investigate the etiology of subclinical mastitis (SCM) in dairy Jersey cows with the use of bacteriological and molecular identification methods. A total of 337 dairy cattle (221 Jersey and 116 hybrid Jersey) at 25 family and agricultural type farms were investigated in Samsun district of Turkey. 121 Jersey and 78 hybrid Jersey cows with SCM were used for bacteriological examination. California mastitis test (CMT) positive milk samples (n=411; 225 and 186 were from Jerseys and hybrid Jersey, respectively) were examined bacteriologically. Afterwards, suspicious colonies evaluated for cultural characteristics (haemolysis, pigmentation), microscopy (Gram staining) and biochemical characteristics (catalase, oxidase, coagulase, aesculin hydrolysis, CAMP tests). Antibiotic susceptibilities of the strains isolated from the samples were determined by Kirby-Bauer Disc Diffusion Method according to National Committee for Clinical Laboratory Standards (NCCLS, 2003). DNA was extracted by boiling method for direct PCR of intact bacteria. The PCR protocols were used for bacteria commonly isolated from SCM. Data were exacuted with SAS (2009) statistics suit to summarize for some statistics such as means, frequencies and also their standard error of means. Prevalence of subclinical mastitis was 54.75% and 67.2% in Jerseys and hybrids, respectively. In bacteriological examination, a total of 92 strains were isolated from 411 milk samples. Of sixty-four strains (69.56%) identified as *Staphylococcus spp.*, 24 strains (26.08%) were identified as *S. aureus* according to their colony morphology (hemolysis, pigment production) and biochemical reactions (coagulase, DNase, mannitol fermentation). Of 92 strains, 25 were (27.17%) *Streptococcus spp.* and three of them (3.26%) were *S. agalactiae* and 22 (23.91%) were *S. dysgalactiae*. Three of all the isolates were identified as *Enterococcus spp.*(3.26%). *S. uberis* was not isolated. While all *S. aureus* and other *Staphylococcus spp.* strains were susceptible to rifaximin, rifaximin+cefacetrile, they showed resistance against spiramycin. These strains showed a variety of resistance against other antibiotics. Namely, >50% of the *Staphylococcus spp.* strains (other than *S. aureus*) showed resistance against penicillin (87.5%), neomycin (87.5%), vancomycin (67.5%), ampicillin (65%) and oxytetracycline (52.5%). While all *S. aureus* strains were resistant spiramycin, penicillin and neomycin, 91.66% of them showed resistance against ampicillin. Besides, *S. aureus* strains showed relatively high resistance against vancomycin (75%) and oxytetracycline (66.6%). *S. aureus* and other *Staphylococcus spp.* strains showed lower resistance against ampicillin+dicloxacillin, amoxicillin+clavulanic acid, teicoplanin and cefaperazone.

Keywords: Etiology, jersey, subclinical mastitis

Concentration of Haptoglobin (Hp) and Serum Amyloid A (SAA) in Cattle Naturally Infected with Bovine Viral Diarrhea and Infectious Bovine Rhinotracheitis Viruses

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The aim of this study was to evaluate Haptoglobin (Hp) and Serum Amyloid A (SAA) concentration in cattle with naturally infected with Bovine Viral Diarrhea (BVD) and Infectious Bovine Rhinotracheitis (IBR) Viruses. In this study, 43 serum samples of cattle with anorexia, diarrhae, weight loss, cough and nasal discharge were used as material. The cattle were different breed, gender and over 1 years old and not vaccinated against these diseases. The detection of BVDV (Bovine Viral Diarrhea Virus) antibodies, was made with commercial BVDV antibody ELISA test kit. For the detection of antibodies IBRV (Infectious Bovine Rhinotracheitis Virus) commercial Infectious Bovine Herpes Virus-1 (BHV-1) g B antibody test kit was used. The levels of Hp and SAA in serum were determined by use of the commercial test kits. Tests were performed according to procedures reported by the manufacturer. In this study 17 samples against for BVDV, 6 for IBRV and 20 for both viruses antibody-positive were determined. The value of serum Hp concentration (min-max) in cattle infected with BVDV 0,000-1,867 mg/ml, mean 0,543 mg/ml; with IBRV 0,001-1,086 mg/ml, mean 0,487 mg/ml; co-infected with BVDV and IBRV 0,000-1,436 mg/ml, mean 0,535 mg/ml were recorded. Serum SAA concentration (min-max) in cattle infected with BVDV 15,715-202,675 µg/ml, mean 128,536 µg/ml; with IBRV 5,715-165,355 µg/ml, mean 79,357 µg/ml; co-infected with BVDV and IBRV 12,685-203,04 µg/ml, mean 116,529 µg/ml were found. The result of the study indicated that mean serum Hp an SAA concentration in cattle infected with only BVDV virus were higher than infected with alone IBRV or both viruses.

Keywords: Bovine viral diarhea, haptoglobin, infectious bovine rhinotracheitis, , serum amyloid A

Rumen Bacterial Diversity in Nellore Steers in Feedlot by Sequencing Using Ion Torrent PGM Platform

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The rumen is characterized by high microbial population density, high diversity and complexity of interactions. The aim of this study was to investigate the rumen bacterial diversity in Nellore steers (*Bos indicus*) in feedlot by the Ion Torrent PGM next generation sequencing technology. Rumen contents were collected from four ruminally-fistulated Nellore steers (BW 501.3 ± 18 Kg), before the morning feeding of the animals. The diet contained 30% of Tifton 85 hay as forage source, and 70% concentrate (Table 1). The study period consisted of 14 days for adaptation and one day for sampling ruminal content. Rumen samples (about 50 g) were accurately weighed, frozen at -85°C within 30 min of sampling, and freeze-dried. DNA extraction was conducted in 250mg of sample using the extraction kit "Fast spin kit for soil" from MP Bio® according to the manufacturer´s instructions. The integrity and quantity of the DNA was checked by electrophoresis on agarose gel (0.8%) and were measured fluorometrically (Qubit® 2.0). The amplification of the V4-V5 hypervariable region of the 16S rRNA gene was performed by a PCR reaction using the forward 515F (5'-GTGNCAGCMGCCGCGGTAA-3') and reverse 926R (5'-CCGYCAATTYMTTTRAGTTT-3'). The PCR fragments were purified using the Zymoclean ™ Gel DNA Recovery Kit following the manufacturer's instructions. After purification the product was quantified by fluorometry (Qubit® 2.0) and the samples were united at equal molarities. The sequencing was on the Ion Personal Genome Machine (PGM, Ion Torrent/Life Technologies). The sequencing data we constructed a triangular matrix for *Mothur* software. The diversity index Shannon-Weaver and Simpson with reference to the phylogenetic classification of reads the database SILVA were calculated by Mothur software. After sequencing were obtained 840.682 reads, with 352 bp of mean read length. The diversity index Shannon-Weaver and Simpson were 4.710489 and 0.048215 respectively. We identified eight phyla present in the rumen microbiome analysis and 4% of the sequences were identified as not classified in the phylum level. Among the classified phyla a predominance of Firmicutes (62.7%), Bacteroidetes (24.8%) and Proteobacteria (3.6%) which represent 91% of the identified phyla in the bovine rumen. The other five identified phyla were Tenericutes (2.0%), Fibrobacteres (1.1%), Spirochaetes (1.0%), Actinobacteria (0.4%) and Planctomycetes (0.1%). In the class level 23.4% of the sequences were identified as not classified. Three classes were identified for the phylum Firmicutes: Clostridia (54.0%), Bacilli (38.5%) and Negativicutes (0.2%). Among the phylum Bacteroidetes only the class Bacteroidia (49.1%) was identified. Two classes were identified for de phylum Proteobacteria: Gammaproteobacteria (93.1%) and Alphaproteobacteria (3.5%). In the genera level we identified 22 genera and 57.7% of the sequences were identified as not classified. The genera predominance were *Kurthia* (14%),*Roseburia* (8.9%), *Prevotella* (7.1%), *Anaeroplasma* (2.0%), *Ruminobacter* (1.8%), *Succinivibrio* (1.6%), Fibrobacter (1.1%), Aerococcus (1.0%), Treponema (1.0%), *Pseudobutyrivibrio* (0.9%), *Butyrivibrio* (0.8%), and*Mogibacterium* (0.5%). By the Ion Torrent GM was possible to characterize the rumen bacterial diversity using V4V5 hypervariable region of the 16S in Nellore steers in feedlot with 4% of the sequences identified as not classified in the phylum level. The predominant phyla were Firmicutes, Bacteroidetes and Proteobacteria.

Keywords: 16S gene, bos indicus, microbiome, phylogenetic

Large Bone Sample by Rib Biopsy in Cattle

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The aim of this study was to evaluate the effectiveness of rib biopsy technique using a saw cup attached to an electric drill for obtaining bone tissue in Nellore bulls. A total of 27 rib biopsies were performed in 27 Nellore bulls. The animals were subjected to fasting 48 hours of food and 24 hours of water before the biopsies. Immediately before the procedure, patients were physically restrained in the squeeze chute and sedated with Xylazine 0.05 mg/kg IM. The surgical area was delimited between the caudal margin of the tenth left rib, the cranial edge of the paralumbar cavity, the lateral side of the transverse apophysis and the costochondral joint, this area was washed with a mild detergent and widely trichotomized, then was made local antisepsia, alternating 4 times of chlorhexidine 2% and alcohol 70%. An anesthetic blockage was made in the eleventh and twelfth left intercostal space by infiltrating 10 ml of lidocaine hydrochloride 2% and later in the surgical area was infiltrated superficialy and profoundely, lidocaine hydrochloride 2% along the side face of the twelfth rib. Two parallel incisions perpendiculars to twelfth rib body was made approximately 6 cm of lenght, and one transverse incision approximately 5 cm allowed unite the parallel incisions, forming a flap or rectangular window right on top of the rib. After hemostasis by avulsion of the abdominal oblique muscle external was exposed to the lateral face of the rib and removed remaining soft tissue with a scalpel. With saw cup adapted to a manual variable speed electric-drill (properly sterilized in an autoclave) which in whose center had a steel drill of 3mm in diamete was collected a bone circular sample 2.5 cm in diameter. Later the rib periosteum and debris were removed, and the external abdominal oblique muscle and subcutaneous tissue remaining were repositioned over the defect made in the rib and the skin edges were approximated with interrupted suture pattern using nylon # 2. Postoperative treatment consisted in the intramuscular application of penicillin (sodium, procaine and benzathine 12,000 IU/kg), streptomycin (5 mg/kg) and piroxicam (0.5 mg/kg) every 48 hours for 5 days. The animals were clinical evaluated daily for 15 days for pain signals, cicatrization and geral state, and support measures if necessary were applied. After 15 days withdrew from the points. The bone material obtained was satisfactory and with quality for mineral and radiographic evaluation, in all animals. Discomfort signals were not observed, however inflammation and wound infection were detected in two animals and satisfactory treatment with local cleans and antibiotics was applied. Postoperative, bone and soft tissues cicatrization were effective and without complications.

Keywords: Electric drill, mineral evaluation, nellore, saw cup

Evaluation of Clinical Mastitis Caused by Staphylococcus Aureus and Streptococcus Agalactiae in Farms of West Azarbayjan-Iran

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Bovine mastitis is the single most common cause for antibacterial use in lactating dairy cattle (18, 24). Treatment of this disease is also the most common cause of illegal antibacterial residues in marketed milk (10). Staphylococcus aureus are common causes of bacteraemia in humans (19). Among the different strains involved, the sources of major concern are the methicillin-resistant S. aureus (MRSA) and vancomycin resistant S. aureus (VRSA). MRSA strains emerged as early as 1961, and became a major concern for hospital epidemics in many countries. VRSA strain emerged in 1996 from MRSA strains a few years after introducing vancomycin in human therapy (16, 19). MRSA strains often express another resistance gene called emr which induces resistance to erythromycin. This gene has been identified in many isolates of poultry origin, but never from bovine or milk sources (38). Transmission of MRSA seems to be mediated by person-to-person transmission, and to the best of our knowledge, transmission of MRSA from bovine to humans has not been reported. A Belgian study identified MRSA resistance types in 1 to 3% of S. aureus isolates in the 1970's. However, these resistance types were not found in later years (1980's through 1990's) and they were also determined to be of human origin (9) The dynamics of environmental streptococcal mastitis in an experimental herd over a 7 yr period were reported (51). The dry period was identified as the time of greatest susceptibility to new environmental streptococcal IMI. The rate of environmental streptococcal IMI was 5.5-fold greater during the dry period than during lactation. Rate of streptococcal IMI was.00312/cowday during the dry period and.00054/cow-day during lactation. The aim of this study is Evaluation of clinical mastitis caused by Staphylococcus aureus and Streptococcus agalactiae in farms of West Azarbayjan-Iran. From 1296 cows that had clinical signs of mastitis, milk samples after disinfection to be in the teats. In laboratory cultures act on blood agar and Macconkey agar were done. Catalase, coagulase test was carried out at a next stage in the differentiation mediums of Litmus milk, nitrate, gelatin, mannitol, OF was used and the sensitivity of Staphylococcus with Novobiocin tests was studied. Microscopic slides were prepared. 432 of 1296 cow samples positive for S. aureus and Streptococcus agalactiae 13 cases were positive. Staphylococcus aureus and coagulase-positive, catalase-positive, and a yellow pigment and hemolysis were positive. Streptococcus agalactiae coagulase-negative, catalase-negative, with alpha and beta hemolysis. Milkers hand, the use of common towels, remaining milk in the teats cup, improper milking equipment can be a source of infection is also a factor that we have seen in Visit our of traditional dairy farms using a shared bucket of water to wash the cow's udder all respectively. The milking can be a source of infection. Plays an important role for the joint use of a bucket of water to wash the udder of a cow or cows that pathogen from one cow to the other transmission.

Keywords: Cow, Iran, mastitis, staphylococcus aureus, streptococcus agalactiae

Morphological Investigation of the Buffalo Ovary and Uterus

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Water buffalo breeding is progressively becoming common in our country. While buffalo milk is used for ice cream, various dairy desserts, cheese (mozzarella) and yogurt production; its meat is preferred for the taste and color in sausage manufacturing. Female buffaloes are bred for milk production and prosperity. Buffaloes that are sent to slaughtershouses are usually the ones with decreased productivity (fertility and milk production). In this respect, pathomorphological lesions occurring in the female genital tract are important to investigate in order to achieve more economical efficiency from female buffaloes. So, we thought that histopathological examination of water buffaloes genital organs will be helpful to achieve this goal. For this purpose, totally 198 uteri with ovaries were collected from various cities slaughterhouses, were fixed in 10 % neutral formalin and evaluated macroscopically in Department of Pathology, Faculty of Veterinary Medicine, University of Ankara. Tissues were processed routinly and embedded in parafin, sectioned at 4-6 µ thickness and stained with haematoxylin-eosin (H&E). All microscopical areas were examined under the light microscope. 147 follicular cysts, 22 luteal cysts were noted. In 14 buffaloes, we noticed both follicular and luteal cysts; 12 of them were seen at the same ovary, two of them were located at different ovaries. In one of the follicular cysts, papillary extension that projects into the lumen was seen. In the lumen of other three luteal cysts, hemorrhages were noticed. Also, interstitial edema was noticed in the ovaries of 11 buffaloes. One of these included mononuclear cells consisting of macrophages and plasma cells. In uteri of 15 of the cases interstitial edema was noticed and eight of them were diagnosed as subacute metritis. Desquamation and cystic dilatation also were observed in some uteri. As a result we concluded that the animals sent to slaughterhouses have serious genital problems threatening their fertility. So, in the purpose of productivity improvement and lifespan prolongation, routine clinical examination must be done as an antemortal prevention of disorders and diseases.

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Keywords: Histopathology, ovary, uterus, water buffalo

The Usefulness of Trans-scrotal Ultrasonography at Bull Breeding Soundness Evaluation (BBSE): The Relationship between Testicular Parenchymal Pixel Intensity (PI) and Semen Quality

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Although the use of artificial insemination (AI) is widespread in the UK cattle industry, natural service is still commonly used in suckler cow enterprises and to 'sweep up' following a period of AI in both dairy and beef herds. Bull Breeding Soundness Evaluation (BBSE) is commonly undertaken to identify bulls that are potentially unfit for use as breeding sires, and thus to avoid poor herd reproductive performance and economic losses. Traditionally a BBSE comprises of 5 stages: a general physical examination, manual examination of the external genitalia, manual examination of the internal genitalia, semen evaluation (most commonly obtained via electro-ejaculation (EEJ)) and observation of libido and mating ability. Re-examination of bulls deemed subfertile after an interval of 6-8 weeks to assess whether the subfertility has resolved is often standard practice before making a final judgement. Various large scale studies worldwide have found that approximately 20%of bulls examined during routine screening failed their BBSE, and would be considered subfertile. These failures are for a variety of reasons including physical genital abnormalities and poor semen quality. Multiple papers describe the use of testicular ultrasound as a non-invasive aid in the identification of specific testicular and epididymal lesions; however this is currently not routinely performed at every BBSE. Few field studies have examined the correlation between ultrasonographic testicular parenchymal pixel-intensity (PI) and semen quality. The majority have shown little correlation between the two assessments at the time of testing. Two papers have proposed a link between parenchymal PI and future fertility (Artega 2005; Ahmadi 2013), however, to date, no studies have been published that examine this link. The aim of this study is therefore to assess the relationship between testicular parenchymal PI (measured using trans-scrotal ultrasonography) and semen quality (measured at BBSE) and the usefulness of testicular ultrasonography as an aid in predicting future fertility in bulls, particularly those that are deemed subfertile at first examination. The Farm Animal Practice at the University of Edinburgh performs approximately 150 BBSEs per year; this usually includes around 30 bulls that are deemed subfertile (20% sub fertility rate). These BBSEs consist of part 1 to 4 of the 5 stage method. In this study standardised ultrasonographic assessment of the testicular parenchyma of enrolled animals is undertaken using a 5-8Hz linear ultrasound probe. Two planes of examination of each testicle are stored for computer analysis at a later date. A follow-up examination (BBSE + ultrasonography) is performed 6-8 weeks later on animals deemed subfertile at initial BBSE examination. This time period allows for the completion of the spermatogenic cycle that was in progress at the initial ultrasound examination. Standardized, blinded computer image analysis is undertaken using a free-to-download image analysis package. The testicular parenchymal PI of each image is analysed using a set protocol and produces values on a 8bit 256 greyscale. Mean, mode, maximum, minimum and standard deviation in PI are recorded. This process is repeated 3 times and a mean score for these values produced. The data from all 4 testicular images are then combined to produce overall PI scores for the bull in relation to the examination. At the same time a 5 point scale arbitrary scoring system for gross visual testis fibrosis is carried out for each image and combined to give an overall gross visual assessment of testicular fibrosis on the ultrasound images for each bull. Once image analysis for a BBSE season is completed the databases of image analysis and BBSE results are combined and analysed. To date results from one year (2014) of transscrotal ultrasonography and BBSE are available and further data collection will be undertaken throughout 2015.

Keywords: Bovine testicular ultrasound, bull breeding soundness exam, pixel intensity

Prevalence and Antimicrobial Susceptibility of *Escherichia Coli* Isolated From Uterus of Dairy Cows in the First and Fourteenth Day Postpartum

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Although the contamination of the uterus by bacteria during parturition and postpartum period is almost an unanimous event in cows, defense mechanisms that occur during the physiological postpartum gradually eliminate these contaminants in the first postpartum weeks. The imbalance between contamination and uterine defense mechanisms results in the persistence of pathogenic bacteria and, consequently, the installation of uterine infection, which is responsible for decline in the productive and reproductive performance due to extend postpartum period, delay in the establishment of subsequent pregnancy and even up infertility. *Escherichia coli* (*E. coli*) is one of the more prevalently pathogenic bacteria found in the uterus of cows in the postpartum period and is often associated with increased inflammation, endometrial lesions and severe clinical disease. Furthermore, their presence is considered a predisposing factor for uterine infections by other bacteria. A range of drugs has been used topically or systemically for treatment of uterine infections, but there is no consensus on which is more effectively. In order to assess the sensitivity of *E. coli* present in the postpartum uterus to antibiotics most commonly used in the treatment of uterine infections, this study was conducted in 75 Holstein dairy cows, with a mean age of 2.9 years (± 1.4). All cows had normal calving with complete elimination of fetal membranes within 12 hours after delivery and postpartum free of productive or reproductive complications during the trial period of 14 days. Uterine secretion samples were collected 24 hours (Moment 1) and 14 days (Moment 2) after calving, using sterile disposable pipette for artificial insemination, protected by plastic sanitary shirt to avoid contamination by the vaginal microbiota, coupled to a 20 mL syringe. Uterine secretion samples (Moments 1 and 2) were seeded onto MacConkey agar being held by 48 hours in aerobic conditions at 37°C. For the identification of strains of *E.coli*, colonies ferment lactose or not in MacConkey agar were subjected to biochemical tests for identification of enterobacteria using solid and semi-solids culture media for testing of glucose fermentation, gas production, L-TD (L-tryptophan deaminase), H_2S, urease, Simmons citrate, lysine, motility and indole. *E. coli* isolated from both Moments were subjected to antimicrobial susceptibility testing *in vitro* by disc diffusion method in Mueller Hinton agar and classified into sensitive, partially sensitive and resistant to: Ampicillin (10µg), Ciprofloxacin (5µg), Ceftiofur (30µg), Enrofloxacin (5µg), Florfenicol (2µg), Gentamicin (10µg), Sulfamethoxazole/trimethoprim (Sulphazotrim) (25µg) and Tetracycline (30µg). Of the 75 samples, 46,7% (35/75) resulted in *E. coli* in Moment 1 and 32% (24/75) in Moment 2. Antimicrobial resulting in sensitivity higher than 80% of *E. coli* isolated (Moments 1 and 2, respectively) were Sulphazotrim (33/35 and 23/24), Florfenicol (29/35 and 21/24) and Ciprofloxacin (29/35 and 20/24), and resulting in antibiotic sensitivity lower than 65% were Enrofloxacin (23/35 and 13/24), Tetracycline (22/35 and 13/24) Ceftiofur (19/35 and 9/24), Ampicillin (15/35 and 9/24) and Gentamicin (9/35 and 11/24). *E. coli* isolated (Moments 1 and 2, respectively) showed results of antibacterial resistance lower than 10% to the following agents: Ciprofloxacin (0/35 and 2/24), Sulphazotrim (2/35 and 0/24), Florfenicol (2/35 and 2/24) and Enrofloxacin (3/35 and 2/24). The antimicrobial resulting in bacterial resistance between 10% and 35% were Ceftiofur (4/35 and 5/24), Ampicillin (5/35 and 7/24), Gentamicin (6/35 and 6/24) and Tetracycline (8/35 and 8/24). Thus, it is concluded that the antibacterial sensitivity patterns of *E. coli* isolated from uterus in the first and fourteenth day postpartum are similar, and, additionally that Sulphazotrim, Florfenicol and Ciprofloxacin have best *in vitro* performance for sensitivity and resistance.

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Keywords: Antibiotic sensitivity, bovine, escherichia coli, uterine infection

The Relative Feed Value, In Vitro Digestibility and Quality Characteristics of the Apple Pomace Silage in Ruminants

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This study purposed to determine the effects of the apple pomace (AP) and apple pomace silage (APS) relative feed value (RFV), in vitro digestibility and nutritional quality values for ruminant diets. The apple pomace was chopped into approximately 2.0 cm sizes and ensiled under laboratory conditions in 1.5 L glass jars with clips and rubber gasket. All jars in study were opened on days 60 post-ensiling and were analyzed. The dry matter (DM), crude ash (CA), crude protein (CP), diethyl ether extract (EE) and crude fiber (CF), amylase-treated neutral detergent fiber (aNDF), amylase-treated acid detergent fiber (aADF) and acid detergent lignin (ADL) contents of AP and APS were determined by wet chemical analyzes. The RFV was calculated according to the formulas of Van Dyke and Anderson. Total flavonoid, total anthocyanin, malic and citric acids contents of AP were analyzed spectrometric and HPLC metods. Lactic acid and volatile fatty acids (VFA) of APS were analyzed with HPLC. The pH values in AP and APS were determined using a digital pH meter. The AP and APS added the different levels (10 % or 20 %; AP10 and AP20 or APS10 and APS20, respectively) in a total mixed ration (TMR). In vitro digestibility characteristics of AP, AP10, AP20, APS, APS10 and APS20 were determined using in vitro gas production technique of Menke et al. (1988). Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methane analyzer. The DM, CA, CP, EE, CF, aNDF, aADF, ADL, and pH values were determined as 14,94, 2,65, 4,54, 3,91, 22,10, 38,35, 30,24, 12,97 and 3,83 for AP and 10,44, 3,66, 6,84, 5,96, 32,08, 53,25, 43,05, 14,27 and 3,57 for APS (%). Relative feed values were determined as 158,18 for AP and 96,98 for APS. Citric acid (%), malic acid (%), total anthocyanin (mg Cy-3GE/kg) and total flavonoid (g CatE/kg) levels of AP were analyzed as 0,47, 1,48, 1,34, and 1,59, respectively. Lactic, acetic, propionic, iso-butyric, butyric, and iso-valeric concentrations of APS were 1.49, 1.45, <0.01, 0.02, 0.02, and 0.17, respectively. In the present study, supplementation in 10% level of AP to TMR were determined to have a positive effect compare to supplementation in 20% level of AP to TMR in terms of in vitro digestibility parameters ($P<0.001$). Besides, supplementation in 10 and 20% level of APS to TMR demonstrated similar effects to in vitro digestibility parameters ($P>0.05$). Methane production of AP was lower than APS. As a result, apple pomace silage had a positive value according to apple pomace in terms of the nutrient content and digestibility for ruminant diet. The made of apple pomace silage could be recommended. The digestibility for other animal species of apple pomace and apple pomace silage should investigate.

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Keywords: Apple pomace, in vitro digestibility, relative feed value, silage

Alzheimer Disease and Bovine Spongiform Encephalopathy (BSE) Connections

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Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders affecting animals (BSE, scrapie…) and humans (CJD…). Until recently, TSEs encapsulated a distinct category of neurodegenerative disorder, exclusive in their defining characteristic of infectivity (prion diseases). It now appears that similar mechanisms of self-propagation may underlie other proteinopathies (prion-like diseases) such as Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic lateral sclerosis (ALS). However, only prion disease has been established as the sole "bona fide infectious" disease among these protein misfolding disorders (neurodegenerative diseases). The misfolding and aggregation of endogenous proteins in the central nervous system is a neuropathological hallmark of above mentioned neurodegenerative diseases. Prions („infectious") are produced by recruiting the normal cellular prion protein (PrPc) and stimulating its conversion into the disease causing isoform PrPsc (derived from scrapie). The new prion diseases that have emerged in the last 25 years are BSE and variant Creutzfeldt-Jakob disease (vCJD). The accepted cause of vCJD is that BSE spread from cattle to humans by the consumption of infected beef. However, the evidence that supports this is very thin. Despite probable widespread exposure of the UK population to BSE-contaminated food in the 1980s, there have been only fewer cases of vCJD, than researchers anticipated. The reasons for this are to date uncertain. The temporal relationship between BSE and vCJD (1990s) only coincidentally supported the notion that BSE caused vCJD, and as such is not evidence. The evidence other than this comes from research using mouse models and analysis of subtypes of abnormal prion protein. This supporting evidence was related to four papers published in high-ranking journals (1996- 1999). These experimental mouse models were later supported by the mathematical BSE/vCJD models in about 13 scientific articles (1996- 2006). So these „infectious conclusions "were finished in 2006, when it was found that „Alzheimer's may 'seed' itself like BSE", if proteins taken from the brains of Alzheimer's patients and injected into the brains of genetically engineered mice trigger Alzheimer's-like lesions in the mouse brains, Later, many other similar studies showed that the pathology of AD, PD and ALS can be transmitted to animals in a way similar to that by which a prion disease was transmitted with PrP inoculation. These neurological disorders can be produced by either peripheral (extracerebral) or direct brain (intracerebral) inoculation. Those findings provide evidence of cell-to-cell spread of pathologic proteins of neurological disorders in experimental animals, suggesting those pathological proteins may have seeding abilities, like prion diseases, to transmit pathology. Experimental studies have shown that the aggregation of the AD-associated proteins amyloid-β (Aβ) and tau, and of the PD-associated protein α-synuclein, can be stimulated in laboratory animal models by intracerebral injection of inocula containing aggregated species of the respective proteins. Knowing that amyloid-β and similar proteins act like prions, researchers are left wondering why no one has recorded a case of the proteins passing from person to person, when on the basis of laboratory results, all neurodegenerative diseases should be infectious. However, to date, there is no direct evidence in humans indicating that the diseases caused by misfolded Aβ, tau, α-synuclein are infectious. Again, as in the case of BSE / CJD infection, reasons for this are uncertain. Taken together, these results are consistent with the fact that BSE and scrapie (prion diseases) are not infectious (it has never been scientifically proven), as has been presented at the last World Veterinary Congresses (2008, 2013). Similarly, also other neurodegenerative diseases are not infectious, relevant connections mentioned above will be interpreted at the Congress.

Keywords: Alzheimer disease, BSE, neurodegeneration

Is Intravenous Regional Anaesthesia More Effective than Nerve Blocks to Desensitize Bovine Distal Hind Limb?

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Adequate pain management strategies to prevent serious infectious locomotor system disorders in dairy cows always were interesting for veterinarians to examine another more effective method to have a desensitized distal hind leg prepared for distal hind leg interventions. However, in our knowledge, there is no clinical study to evaluate the effect of intravenous regional anaesthesia as a routine well-known anaesthesia technique compared to another method such as distal limb nerve blocks. Therefore, the aim for this study has been the clinical examination of these two analgesia strategies to recognize the speed of onset of applied anaesthesia method. To identify which anaesthesia method has a rapid onset compared to another technique, 6 non-lactating, non-pregnant HF dairy cows were examined under a cross-over study design. Pain stimulations using Electrical Threshold device as well as Mechanical Pressure and Pin Pricks Threshold means were applied to produce electrical stimuli at latero-dorsal skin of coronary band as well as the skin of soft tissue of heel while lateral and medial Dorsal Fetlock Joint, lateral and medial Lateral Flexor Tendon as well as lateral and medial Bulb of Heel were used for mechanical threshold measurements. The results gained from electrical nociceptive threshold revealed increased mean electrical nociceptive threshold responses after nerve block anaesthesia (NBA) compared to intravenous regional anaesthesia (IVRA) group and demonstrated that the onset of IVRA has about 5-10 minutes delay compared to NBA. In conclusion, although preparing the bovine hind leg for distal limb nerve blocks may take more time than intravenous regional anaesthesia, 4 points nerve block anaesthesia has a faster onset compared to intravenous regional anaesthesia and both methods induce full anaesthesia.

Keywords: Dairy cow, distal hind limb, IVRA, NBA

The Effect of Post-TAI Administration of hCG on Day 4 vs. 5 on Pregnancy Rate in Repeat Breeder Cows

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The Repeat breeder cow is generally defined as any cow that fails to conceive after more than three services with fertile semen despite the normal cyclicity and genital tract. Repeat breeder syndrome causes major economic loss in dairy industry due to higher insemination cost, increased calving interval and increased culling rates. Hormonal factors like delayed progesterone rise after ovulation and decreased total concentrations of progesterone are important etiologic factors of repeat breeders. Also previous studies, reported that progesterone supplementations which have done 5th to 9th day post AI have better results on development of embryos. Since the development of embryo and maintenance of pregnancy critically depends on the levels of progesterone in repeat breeders, this research aimed to increase pregnancy rate via causing post AI ovulation of the first dominant follicle by hCG administration (4 or 5 days after artificial insemination) in repeat breeder cows. Cyclic lactating Holstein dairy cows which had more than three inseminations with no clinical abnormalities of the reproductive tract were enrolled in the present study. All cows (n=214) received the Ovsynch protocol; a GnRH treatment (GnRH1, Buserelin acetate, i.m., 10 µg, Oviren®, Topkim, Turkey) followed by PGF2α (Dinoprost, 25 mg, i.m., Enzaprost®, Ceva, Turkey) 7 d later. Second GnRH treatment (GnRH2) was administered 56 h after PGF2α, and all cows were inseminated at a fixed time (TAI) 16 h to 18 h after GnRH2. Cows were not synchronized (n=22) excluded from the study. After TAI, all cows (n=192) were randomly assigned into three groups; cows in the hCG4 group (n=64) received 1500 IU hCG im on day 4 post TAI; cows in the hCG5 group (n=68) received 1500 IU hCG im on day 5 post TAI; and cows in the control group (n=60) did not receive any treatment post TAI. Ovulations in response to the GnRH and hCG treatments were characterized by disappearance of the responsive follicle and appearance of a new CL via trans-rectal ultrasonography. Pregnancy diagnoses were performed by ultrasonography on 31 and 62 d after TAI. Statistical analyses were conducted using SAS (SAS Institute, 2009) Responses to hCG administrations were found similar between hCG4 (64.1%; 41/64) and hCG5 (64.7%; 44/68). Pregnancy/AI at 31 d was similar among the groups (48.3%, 29/60 in CON, 48.4%, 31/64 in hCG4, 42.7%, 29/68 in hCG5). Pregnancy/AI at 62 d was also similar among the groups (45.0%, 27/60 in CON, 42.2%, 27/64 in hCG4, 39.7%, 27/68 in hCG5). Pregnancy loss between 31 and 62 d was similar among the groups (6.9%, 2/29 in CON, 12.9%, 4/31 in hCG4, 6.9%, 2/29 in hCG5). When hCG treated cows were evaluated, regardless of the day of treatment, the cows that were responsive to hCG (54.1%, 46/85) had a greater (P=0.005) P/AI at 31d than the cows that were not responsive (29.8%, 14/47). In conclusion, hCG administration on day 4 or 5 post-AI in repeat breeders did not increased the pregnancy rate compare to control group. However, pregnancy rate was higher in cows respond to hCG administration compare to non-responsive cows regardless of the day of treatment.

Keywords: hCG, repeat breeder cows, pregnancy

Comparing of CYP19, ERα, PGR Polymorphism between Fertile and Subfertile Holstein-Friesian Heifers

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Dairy heifers are important to dairy farms to improve genetics advance and maximize milk production. Therefore subfertile heifers causes economic lose and obstacle improvement of dairy industry. Many researchers have been focused on etiological factors of subfertility in heifers. Especially hormonal imbalance has been attributed to one of the main factor at subfertility. Polymorphism in genes that regulated reproductive hormones might be causes to hormonal imbalance. The one of the important gene is the aromatase cytochrome P450 enzyme coded by CYP19 gene. The CYP19 realizes conversion of androgens to estrogens and biosynthesis of estrogen by aromatization. The other genes are estrogen receptor α (ERα) and progesterone receptor gene (PGR). It's well known that both hormones are regulating all most reproductive events. So polymorphism in genes managed reproductive hormones causes to the infertility in dairy heifers. The aim of this study was to compare frequency distributions CYP19, ERα, PGR gene mutations between fertile and subfertile Holstein-Friesian heifers. Total 106 Holstein-Friesian heifers were included the study. Heifers (n=51, average age 15.5 ± 0.43 months) got pregnant after first artificial insemination (AI) followed spontaneous estrus were used as a control group. Heifers in study group (n=56) had more than >= 3 AI (average AI number 4.6 ± 0.16 per heifers and average ages 19.9 ± 0.42 months) accepted as subfertile heifers. Blood samples from all heifers were taken for DNA isolation. Total DNA was extracted using commercial genomic DNA purification kit and spectrophotometric methods were used to determine DNA quality and quantity. In order to determinate allele frequencies PCR-RFLP technic was used. To compare genotype frequency distributions between groups two-proportion z-test was used. While two alleles and three genotypes have been found at PGR and ERα loci, two alleles and two genotypes detected at the CYP19 locus. Allele A and AA genotype, G allele and GG genotype, C allele and CT genotype were found to be as predominant at CYP19, ERα and PGR, respectively. According to chi-square test ($\chi 2$) two groups investigated are in Hardy-Weinberg equilibrium. There were no differences detected in allele and genotype frequencies between fertile and subfertile heifers. It's needed to further study to determine relationship between these polymorphisms and fertility in dairy heifers.

Keywords: CYP19, ERα, fertility, holstein, PGR, polymorphism

Comparing the Effects of General Anesthesia Inducted by Mask Induction, Isoflurane and Sevoflurane on Clinical and Physiological Values in Calves

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In this study, calves induced by mask induction, isoflurane and sevoflurane induction of general anesthesia, clinical and physiological findings should be regarded as aimed to compare the effects. Research 0-3 months on the 14 calves were conducted. Such that each group consists of 7 animals isoflurane (Group 1) and sevoflurane (group 2) were divided into 2 groups as. In both groups of calves subcutaneously 15 minutes before anesthesia route 0.02 mg / kg Atropine Sulfate were administered. After 15 minutes application Atropine Sulfate, in the group 1 animals isoflurane, sevoflurane to animals in the group 2 given with mask. 5 minutes of mask induction in all animals endotracheal intubation applied. In group 1, anesthesia was chosen as the initial concentration of 4-5% and 1.5-3% concentrations continued to be maintained. 5-7% of the group 2 and maintenance of anesthesia, the initial concentration was determined as the concentration of 2.5-4%. Calves was monitored throughout the procedure; before anesthesia, premedication and inhalation anesthesia period 5, 15, 30, 45, 60 and 75 minutes in heart rate, heart rate, systolic, diastolic, mean blood pressure, respiratory rate, body temperature and EKG were recorded. Blood samples; before anesthesia, premedication and inhalation anesthesia period 30 and 75 minutes post dose, and red blood cell count (RBC), total leukocyte count (WBC), hemoglobin concentration (Hb), hematocrit (Hct), platelet count (PLT) were evaluated. In the study, isoflurane and sevoflurane anesthesia in calves of the clinical and physiological findings gave similar results of both anesthetic drugs and does not create a significant complication to the conclusion has been reached.

Keywords: Anesthesia, calve, isoflurane, mask induction, sevoflurane

Study of the Relationship between Molecular Markers Associated with Meat Tenderness Presence in Subspecies *Bos Taurus* and *Bos Indicus*

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The softness of meat is considered by consumer as the most important attribute during the palatability. The evaluation the consumer carries out is essential to determine the quality of the meat. Mintert et al. (2000) consider that the softness of the meat is one of the most important characteristics of it. However, this characteristic is difficult to predict and its determination is complex because of it is influenced for some factors such as genetic constitution; feeding; age; exercise; sex; practices during slaughter and subsequent handling meat slaughter among others. In brief, to obtain a meat with an optimal softness it is necessary overlook all the processes and facts mentioned above in order to obtain suitable results in terms of quality of the meat, it is important to watch over from the start of this chain, for this reason it is important to consider the breeding of animals through the characterization of the genes that improve both the quantity and quality of meat. Recently, breeding of cattle has received the attention from researchers and producers. The interest is focused on identify, preserve and potentiate the genetic characteristics that improve the quantity and quality of the meat leading to an increase in the value of it, providing benefits to both producer and consumer. The researches carried out define the genetic causes that affect softness, among them, genes and a specific polimorfism are point out as promoters of this characteristic, among them we can highlight Calpain gene (C-530); Miopaladine (A1795G) and PPARGC1A (A1181G) because of the rol they have in the conversion of the muscle in meat. Therefore the aim of this esudio is to determine the existence of association of genotype with meat tenderness in cattle subspecies, by evaluating these polymorphisms. Materials and methods Biological material: 120 samples from tissue was used (60 samples of each subespecie). The isolation of DNA from tissue will be doing according to protocol proposed by Sambrook. PCR-RFLP for this analysis will be used the primers and restriction enzymes described in the Table 1. The results obtained from PCR and RFLP will be analyzed on a 1.5% agarose gel and on a 8% polyacrylamide gel respectively and followed by staining with ethidium bromide. This study has concluded the whole analyze of the marker C-530 and the results shown there is no statical argument that let the association between this marker and the cattle subspecie so far, however, there is the possibility of that polymorphism affect the gene expression and at the same time, the softness that corresponds to each subspecies, which extends the research landscape, which has been raised further analysis of the levels of genetic expression of the markers studied seeking to establish how these polymorphisms influence on them. The selection using markers has a great potential for improve characteristics of economic interest in cattle. Nowadays, the most important challenge is to find new specific markers for the different cattle species and in that way give a follow to the heritability of interesting characteristics and potentiate them.

Keywords: Bos taurus, bos indicus, calpain gene, polymorphisms, softness of meat

Table 1 - Primers and restriction enzymes of PCR-RFLP

Marker	Primers	Restriction enzymes
Calpain (C-530)	F: 5´- GTGACTTTGTGCTGCGTTTCT -3` R: 5´- CCTTGCTGGCTAGAGACCAA-3 Producto: 261	Ava II
Miopaladine (A1795G)	F: 5´CCTTTGGCTCATTTGGTCTGC-3´ R: 5´-ACTTGCTCATCTGCCACTCACC-3´ Producto: 416pb	Taq I
PPARGC1A (A1181G)	F: 5`- TCAGCAAGACCTCTGTGCTCAGCA-3` R: 5`- TGCTCACCTCCGCGGTCTCT-3` Producto: 255pb	BtsI

Rectal Prolapse in Calves: A Description of 5 Cases

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Rectal prolapse can occur in any breed, age and sex of calves. In rectal prolapse, one or more layers of the rectum protrude beyond the anus due to intestinal, anorectal, or urogenital disease. Prolapse may be classified as incomplete or complete types. Incomplete type is known as mucosal prolapse which only the rectal mucosa is everted. Complete prolapse is the protrusion of all rectal layers through to anus. In this report, operative treatment of rectal prolapse in 5 calves, 2 females (Simmental and Holstein) and 3 males (2 Simmental and 1 Holstein), aged between from 2 monts to 5 monts were carried out under epidural anesthesia. All cases had a complete prolapse and had a 3 to 5 day of history. In clinical examination, rectal prolapse through to anus was noted with edema, congestion and necrosis. The animals could not be defecated. In the first day of the prolapse, the veterinarian tried purse string suture for the treatment but prolapse reoccurred. Therefore, rectal amputation was decided for the all cases. The prolapsed tissue of both cases were cleaned with mild antiseptic solution and hyperosmotic dextrose solution applied topically to reduce edema. The prolapse was stabilized by inserting two needle close to anal opening. A circumferential incision was made just cranial to the necrotic area and removed necrotic part. Serosa, muscle and mucosa layer was closed with simple interrupted suture by monofilament 2 number absorbable material. Needles were removed and allowed to rectum to return to its normal position. In the postoperative period widespread antibiotic was applied. Postoperative controls were carried out 15 days later. There were no significant complication. The result of this study show that complicated type of the complete prolapse can be treated by amputation.

Keywords: Calves, prolapse, rectal

Research on the Immunomodulating Effect of a Phyto-Extract on lymphocyte Population of Cows in Puerperal Period

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The body, as a functional system is balanced as long as antigenic information it receives is identical to their own. Compared to foreign molecules that deviate from the "self" information model, the immune system responds by activating recognition mechanisms to remove "non-self" molecules. Immune system is considered an effective „mobile brain". In terms of structural and functional aspects, defense system of higher organisms has many dualities: -the existence of a nonspecific resistance compartment (innate) and a compartment responsible for specific and adaptive action (immune system); -the presence of two populations of lymphocytes (T and B), which mediate cellular and respectively, humoral immunity -lymphocyte activity is modulated either stimulating or inhibitory, under the action of other cells and humoral factors. In the modern view, the lymphocyte is central cell of the immune system. It is not a "seeded" cell -as it was considered by the old cytologists but has a tremendous reactivity and capacity of differentiation. The T cells (LT) fulfill complex functions of the immune response both effector, cell-mediated and also regulatory, by means of humoral factors, called lymphokines. Lymphocytes B (LB) represent 5-15% of total circulating lymphocytes and is a major functional division of the population. Our immunomodulatory product based on an indigenous plant species extract (under preliminary research for patent application) was administered subcutaneously and it caused a local inflammatory response at the injection place (no clinical significance) that was reabsorbed within 7-10 days. The experimental model was carried out on two cows 14-15 days after calving. From both cows we collected blood before inoculating subcutaneously our product. Hematological and immunological tests were carried out also on blood harvested at 24 hours, 5, 8 and 14 days after administration of the herbal product. Immunological techniques were assayed: lymphocytes separation with Ficoll and Percoll medium; cell separation by adhesion to nylon; E rosetting assay; lymphoblastic transformation test; residual glucose dosing; phagocytosis assay using carbon particles. The experimental results certify that the herbal-based product act as immunomodulator in both cases, with minor differences, a sign that the immune response mechanisms function effectively. At 24 hours after administration of the extract the neutrophil lymphocyte ratio is reversed; correlated to the phagocytic index evaluated by the embedding carbon particles test indicates a non-specific stimulation of the phagocytic major functions performed by the neutrophil population. The administration of immunomodulatory product induced a change in the LT / LB ratio, higher for B lymphocytes in both cases; growth is higher in the case 1 where probably, the activation of the LB clones is faster. A change induced by the administration of the immunomodulatory product is the positive shift of the immune response: LT Helper/ LT Supressor ratio was corrected very quickly in both cases at 24 hours following administration. Experimental results showed a significant leukocytosis at 24 hours after the administration of the extract, which it was maintained even after 14 days. The other elements of white blood cell counts remained normal. Direct parameters of the red blood cells (number of erythrocytes, hematocrit, hemoglobin) and also indirect parameters (MCV, MCH and MCHC) were normal. In correlation with the shift of lymphocytes T / B ratio, it is suggested the priority in the mechanism of blasts activation induced by the administration of the herbal-based product. Further studies are in progress in order to elucidate its specific mechanism of action and the influence on other pathologies.

Keywords: Cows, extract, immunomodulation, puerperal

A Treponema Pedis-like Spirochaete Isolated from a Turkish Dairy Cattle: Preliminary Report

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Dermatitis digitalis (DD), a skin disease, has circumscribed nature (1-4 cm) in the corium coronarium develops after superficial inflammation in the epidermis layer at the aspect of the interdigital space just above the cleft, midway between heel bulbs with the destruction of tissue contiunuation. Lameness due to DD causes important problems in terms of clinic, economic and animal welfare particularly in dairy cattle. Today its prevalance has been reported in various ratios in all continents. Although novel treponema-like anaerobic spirochaetes have been isolated the etiology has not been exclusively defined, yet. In this study isolation of a spirochaete seen in DD diseases carried out in our country's dairy cattle. A punch biopsy in 6 mm diameter from the lesion was taken and divided into two portions. The first portion was placed in tamponated-formalin and the second portion into special transport solution supplemented with rifampisin and enrofloxacin then sent to the laboratory. The first portion was subjected to hematoxilen-eosin and silver nitrate staining (Starry and Whartin method) for histopathological examination and the second portion was used for microorganisms isolation. Oral treponeme enrinchment broth (OTEB) supplemented with rifampisin (5 mg/L) and enrofloxacin (5 mg/L) was used for the isolation of the germs. The biopsy was placed in OTEB medium after chopped into fine particles in an anaerobic chamber and incubated 48h at 36 ºC. At the end of this duration a drop of culture was streaked on fastidious anaerob blood agar supplemented with 5% defibrinated sheep blood, foetal calf serum, rifampisin and enrofloxacin and incubated in the anaerob conditions. Then pure spirochaetes was isolated by tranferring the formed colonies into OTEB. Enzyme profile was determined by API-ZYM and Vitek-II Anaerobe ID enzyme activation kits. 16S rDNA amplification was employed by polymerase chain reaction (PCR). PCR reaction will realized with forward primer (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer (5'-AAG GAG GTG ATC CAG CCG CA-3'). The DNA analysis showed that the spirochaetes genetically shared similar homology with Treponema pedis isolated form a UK cattle. This is the first report that a Treponema pedis-like spirochaete is present in Turkish dairy cattle, indeed.

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Keywords: Cattle, pedis-like, treponema

Research Related to the use of the Rapid Test Method for Early Diagnosis of Pregnancy in Cattle as an Alternative to the Ultrasound Technique

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This study was performed in order to research the possibility of the rapid test technique as an alternative to the ultrasound method for the early diagnosis of pregnancy in cattle. In this study, 150 cattle aged between three and six were used as animal material in the city of Siirt. In the pre-study where the results of 31 animals of the Holstein breed were evaluated, the rapid test technique kit (Idexx Visual pregnancy test), ultrasound device (Hasvet-838) and 7.5 MHz linear probe (SIUI) were used. After synchronization applications in animals artificial insemination was performed. 30 days after insemination applications blood samples were taken through 9mm tubes from vena jugularis of animals. After the samples were brought to lab they were centrifuged and their serums were separated. Pregnancy tests were made compliant with usage procedure of the test kits and data were recorded. In the same animals ultrasound examinations were performed rectally 40 days after insemination. Data obtained from both methods were compared in minitap package program by performing X^2 test. As a result of pregnancy controls made using rapid test technique positive findings were obtained in 18 cattle (58%) while negative findings were obtained in 13 cattle (42%). At the end of study it was determined that the pregnancy results obtained by the ultrasound method were directly parallel with the data obtained by the rapid test method and the difference between methods was not statistically significant (P>0.05). As a result, pregnancy examination results made by the ultrasound technique are directly parallel to rapid test technique findings. The same results were obtained from the same animals and the rapid test technique results in cattle are reliable and can be an alternative method in early diagnosis of pregnancy.

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Keywords: Cattle, early diagnosis of pregnancy, rapid test, ultrasound

Comparative Study of Different Therapeutic Protocols of Bovine Clinical Metritis in the Region of Batna (East of Algeria)

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The objective of this study was to make an inventory of cases of metritis in dairy cows during the post-partum period, to verify with a detailed clinical study on its real incidence in Batna region and to study the treatment of metritis, using different treatment protocols. A total of 432 dairy cows were examined during the post partum period and forty dairy cows having metritis were selected and assigned into four groups of 10 each to be treated as follows: Group I: 10 cows treated with oxytetracyclin intramuscularly according to their bodyweights. Group II: 10 cows treated with oxytetracyclin intramuscularly and a single intramuscular injection of an anti inflammatory drug, the lunixin according to the manufacturer's recommendations.
- Group III: 10 cows treated with prostaglandin F2 alpha at a dosage of 2ml intramuscularly. This group has been subdivided into two groups of 5 cows each as follows:
- Group III(a): PgF2α (2 ml single dose).
- Group III(b): PgF2α (2 ml double dose at 15 day interval).
- Group IV: 10 cows treated with prostaglandin F2 alpha (Estrumate) at a dosage of 3ml by intramuscular route. This group has been subdivided into two groups of 5 cows each as follows:
- Group IV (a): PgF2α (3ml single dose)
- Group IV (b): PgF2α (3ml double dose at 15 day interval).
Visits were programmed to trach the status of uterin involution and different parameters of fecoundity and fertility of cows. The injection of Cloprostenol(a synthetic analogue of PGF2α) has a significant effect particularly on shorter intervals from calving to the first mating, calving to positive fertilization and for the reduction of the number of services of inseminations. Similarly, the association "antibiotic+Flunixin /Meglumine (AvlezanND) has a positive effect on the parameters mentioned above.These results show that the double injection of Cloprostenol in the treatment of metritis improves the performance of fertility of animals and the dose of 0,75 mg of PGF2α had succeeded in giving a positive effect on the different fertility parameters studied, compared to the dose of 0,5 mg.

Keywords: Cow, fertility, metritis, PGF2α, postpartum

Scanning Electron Microscope Study of Cat Enamel after Acid Etching

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The cat enamel was observed by Scanning Electron Microscopy (SEM) after acid etching. In the veterinary literature was used ortophosphoric acid for the time recommended by 30 ", 45" or 60 ", derived from research and experience in human enamel studies. 21 teeth of cat were randomly divided into three groups of 7 (A, B, C): Group 1 was subjected to etching for 30 seconds by means of ortho phosphoric acid to 40% on a circular area with diameter of about 2 mm of the enamel coronal; the Groups 2 and 3 had the same treatment but, respectively, for 45 and 60 seconds. The samples obtained were observed by SEM at 1000 x for each framing. The border area between enamel exposed and not exposed to etching was observed to highlight differences. In the cat enamel, the etching for the times considered is not optimally effective for the adhesives purpose, and the presence of a prismless layer could explain this situation. To improve this condition may clinically in the likeness of what is proposed for the enamel of human deciduous teeth like a bevel or a chamfer of 1 mm on the contour of the cavity to discover the prismatic enamel and increase the bonding surface

Keywords: Cat, enamel, SEM, veterinary dentistry

Low-field Magnetic Resonance Imaging in Diagnosing Tumors of the Brachial Plexus in Dogs

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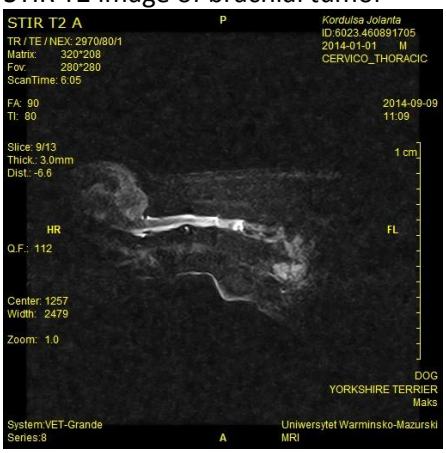
The effectiveness of low-field magnetic resonance imaging (MRI) in diagnosing tumors of the brachial plexus in dogs was evaluated. The aim of the study was to identify MRI sequences that contribute to a reliable diagnosis within a short period of time. In the experiment, tumors of the brachial plexus were diagnosed in seven dogs by MRI with the use of SE, FSE, STIR, Turbo 3 D, 3D HYCE, GE sequences and the gadolinium contrast agent.

Keywords: Dog, magnetic resonance imaging

STIR T2 image of brachial tumor



Homeopathy in Veterinary Medicine

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Homeopathy is a system of medicine based on treating like with like. Homeopathy was developed scientifically by Dr. Samuel Hahnemann. Complementary and alternative therapies, including homeopathy, have a definite place in veterinary medicine today. The public is demanding access to a full range of conventional and complementary therapy. Conventional medicine is based primarily on the concept that diseases are caused by harmful organisms such as bacteria and viruses. Aim of the treatment conventional medicine kills these organisms or to oppose the resulting local symptoms with drugs. Homeopathic treatment strengthens body, acting as catalyst, stimulating and directing the body's ability to fight infection or pathologic diseases. The principle of minimum dose states that the lower the dose of the medication, the greater its effectiveness. Treatments are individualized to each patient—it is not uncommon for different patient with the same condition to receive different treatments. Veterinarians are definitely becoming more aware of the need for and showing more interest in alternative medicine. Homeopathy is more practical and cost effective than other alternative medicine. Nevertheless, their application requires knowledge and experience. Veterinarians teach what the alternative modalities are, whether they are effective, and what it takes to become qualified to practice them. The overriding goal in most veterinarians' minds is to heal animals and provide the best in care, so that animals can live healthy productive lives. Education to keep up with new therapies and medications is paramount to this goal. It follows easily that knowledge of noninvasive treatments with few or no side effects that have the potential to heal animals should be welcomed, and homeopathy, as well as other complementary therapies, fits this description. In this poster presentation, you can find information about homeopathy, the principles of homeopathy, example treatments of animal diseases with using homeopathic methods, the advantages and the disadvantages in veterinary medicine are aimed to be given.

Keywords: Advantages of homeopathy, alternative medicine, disadvantages of homeopathy, homeopathy, theraphy, veterinary medicine

Comparison of Behavioral and Physiological Responses of Kangal Dogs in Different Livestock Flocks

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Kangals are livestock guarding dogs which actively protect different animals against outer threats. In order that those dogs concentrate on threats coming from outside, they should not face any stress arisen from their own flocks. Behavioral and physiological responses of Kangals dogs were examined in the present study. Saliva samples and behavioral responses of 20 Kangal dogs (10 male, 10 female) during the first confrontation with the flocks as well as during active guarding were evaluated in order to assess their stress levels in different livestock flocks. Saliva samples were analyzed using an ELISA method to measure cortisol values. Video records were analyzed in order to evaluate behavioral responses of the dogs. During the confrontation with goats, dogs had significantly higher cortisol values than the values obtained during the confrontation with sheep ($P < 0.05$). However, no significant difference was found when comparing maximum cortisol values during the active guarding in sheep flock and goat flock. No statistical difference was found when comparing direct behavioral reactions of the dogs during the confrontation with the sheep and the goat flocks. Considering behaviours and body language of the dogs during active guarding, only two parameters, “hyperactivity” and “low tail position” showed statistically significant differences ($P < 0.01$). Accordingly, hyperactivity was more frequently observed in sheep flock, whereas low tail position was more frequently displayed in goat flock. This study shows that Kangal dogs, feel less secure and more stressful in the goat flock in comparison to the sheep flock. Dogs should be concentrated come many external threats from the herd while maintaining herd. So those dogs should have positive experiences with the animals that they will guard during the critical period of their lives.

Keywords: Behavior, cortisol, goat, Kangal dog, sheep, stress

Meta-Analysis of Gene Expression in Canine Mammary Tumors Reveals Novel Clinical Biomarkers and Biological Mechanisms

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Mammary cancer is the most common cancer seen in female dogs. It is essential to conduct detailed studies on its molecular background to find out biomarkers for the establishment of an effective clinical diagnosis and treatment (1). Microarray is a high throughput technology used for the determination of differentially expressed genes, which may be used as biomarkers and a meta-analysis refers to methods that focus on combining results from individual microarray studies. Since meta-analysis is not specific to an individual case but to the combination of many studies, it is possible to get more generalized informations for clinical practice (2). The aim of this study is to combine the mRNA microarray studies on canine mammary cancer by a meta-analysis method to identify gene signatures. Finding out cancer specific genes may enable us to understand the pathology and the molecular mechanisms that take part in the progression of the disease. Microarray studies on canine mammary cancer performed so far were searched in microarray databases GEO and ArrayExpress and two studies (GSE14999, GSE22516), which have clinical information, were selected and collected for meta-analysis (3-4). A ranking based meta-analysis approach (5-6), which enables the elimination of discrepancies coming from platform differences was used to combine these two data sets and the top 20% of the genes in result were selected based on their ranking scores and they were further characterized through pathway enrichment analysis and functional annotation. 300 of genes were determined as meta-genes according to their rank scores and differential expression. The selected meta-genes were most significantly found to be as members of protein processing in endoplasmic reticulum (adjP=8.55e-08) and cell cycle (adjP=6.77e-05) pathways, which are known to be effective in the cell life and death decisions. Furthermore 25 of the significant genes were found to be enriched in metabolic pathways (adjP=0.0103) This study indicates the role of metabolic mechanisms and ER stress might be more indispensable in canine mammary tumors and it provides further insight to improve our understanding of development of this disease. Thus, it may contribute to the determination of more effective biomarkers and combinations of anticancer therapies in clinical practice.

Keywords: Canine mammary tumors, meta-analysis, veterinary oncology

Use of a Decreased Dose of Cabergoline to Treat Secondary Anoestrus in Bitches

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Dogs are mono-oestrous and experience an obligate anoestrus of 2 to 10 months following the luteal phase with an average duration of about 75 d. The normal inter-oestrus interval is therefore typically between 5 and 12 months. Abnormalities of the anestrus period are common in bitches. Persistent anestrus is classified as primary or secondary, with primary anestrus defined as lack of estrus by 18 to 24 months of age and secondary anestrus defined as lack of estrus within 12 months after preceding estrus period. Treatment of primary and secondary anestrus should be directed towards identifying and treating the underlying cause. However, estrus induction (EI) may be attempted when an underlying cause is not found. Owners of pure breed bitches with long interval between cycles often request to shorten inter-estrus intervals, so the number of litters per year can be increased. Several protocols with exogenous gonadotropins, synthetic estrogens, GnRH agonists, and dopamine agonists (DA) have been tried for EI in anestrous bitches. These methods differ widely in the efficacy of inducing estrus, as well as in the fertility of the induced estrus. There are limited studies about the use of DA in the treatment of persistent anestrus. Cabergoline has been traditionally used at a dose of 5 µg/kg/d in suppression of lactation, termination of pregnancy or EI. In a previous study of ours, it has been shown that normal and fertile estrus can be induced more economically in bitches at the anestrus period using a quite lower dose (0.6 µg/kg/d) than the prolactin-lowering dose (5 µg/kg/d) of cabergoline. Cabergoline (Galastop, Vetem, Italy), marketed as a veterinary drug in some European countries, successfully induces fertile estrus in most bitches but can be cost prohibitive in the other countries, where this drug is not readily available for veterinary use. Cabergoline (Dostinex, Pharmacia, Italy) is also used in the treatment of hyperprolactinaemia in women and can be purchased easily. The aim of the study was to determine whether a dose (0.6 µg/kg/d) quite lower than the prolactin-lowering dose of cabergoline, prepared for humans, would be a safe and effective method for the stimulation of estrus in bitches at secondary anestrus or late anestrus. Twenty-four pure blood bitches from various breeds were used in the study at their already determined periods of anestrus. The treatment group included bitches at late and prolonged anestrus. Eight bitches that had not shown any signs of estrus for the preceding 370 to 485 d formed the secondary anestrus group. Eight of the 16 bitches at late anestrus (days 165-280) have accomplished the late anestrus group and another 8 have been chosen randomly for the control group (untreated). Cabergoline tablets with 0.5 mg of the active substance were dissolved in distilled water (10 µg/mL) at 37°C and orally administrated until day 2 after the onset of proestrus or for a maximum of 42 d. Blood samples were taken daily from each bitch during the first 5 d of behavioral estrus to measure progesterone concentrations. In the secondary anestrus and late anestrus groups, estrus was induced on days 4-14 and 12-45 at a ratio of 75.0% (6/8) and 87.5% (7/8), respectively. The mean proestrus and behavioral estrus durations, serum progesterone concentrations on day 5 of estrus, ovulation rates, pregnancy rates, and the mean litter sizes in secondary anestrus, late anestrus, and control groups were found to be similar. None of the dogs had any adverse gastrointestinal effects associated with cabergoline administration. The results of the present study suggest that the administration of 0.6 µg/kg/d of cabergoline is a safe and effective treatment for secondary anestrus in bitches.

Keywords: Bitch, cabergoline, estrus induction, late anestrus, secondary anestrus

Comparison of data obtained from dogs in the secondary anestrus, late anestrus, and control groups

Groups	n	Duration of proestrus (d)	Onset of experiment to proestrus (d)	Dogs responding
Secondary anestrus	8	7.0±1.79a	10.3±4.23a	75.0% (6/8)a
Late anestrus	8	8.3±2.14a	30.6±13.02b	87.5% (7/8)a
Control	8	8.1±2.60a	49.5±16.14c	NA

abc: within columns, means with no common letters are statistically different (P<0.0001). NA: does not apply.

Canine Adenovirus Isolation and Identification in Turkey

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The aim of this study was isolation and identification of canine adenovirus from leaving dog shelter in Istanbul, Turkey. This is a first report on isolation and identification of canine adenovirus in Turkey. A total of 15 faecal samples were collected from outpatient ward of clinics, boarding home for dogs, Tuzla, Istanbul. Faecal samples collected from clinical cases were kept at 4ºC until use. MDCK monolayer cell culture was used for virus isolation and the samples were passaged 5 times in cells. The inoculated cells were observed daily for the occurrence of cytopathic effect (CPE) and harvested when a cytopathogenic effect was clearly visible. A CAV-2 vaccine (Vanguard Plus 5L4, Pfizer Animal Health, Lincoln, Nebraska, ABD) strain was used as a positive control in the PCR assay. Total DNA from sample, cell culture supernatant and positive controls were extracted using the DNeasy Blood & Tissue Kit (QIAGEN Group, US) in accordance with supplier's instruction. The specific primers selected from E3 gene of CAV-1 and CAV-2 were used in this study. For sequencing, PCR products were purified using PCR Prufication Kit (Min Elute®, QIAGEN, USA) and the purified products were subjected to automatic sequencer (ABI, USA) in Molecular Genetic Laboratory Department, Pendik Veterinary Control Institute. After 3rd passage in MDCK cells culture, 4 of 5 sample supernatants and positive control were found positive by PCR amplification of E3 gene. The presence of CAV-2 in freeze-dried vaccine was confirmed using the optimized PCR which resulted in amplification of expected 1030 bp fragment. All the four samples gave the amplicon size of 1030 bp in agarose gel electrophoresis. The size of the PCR products clearly indicates that these isolates belong to CAV-2. When the DNA sequences of the PCR products of E3 gene region were compared with the genes and nucleotides of the reference viruses, our CAV isolates (FS1-3) showed an order of nucleotides similar to CAV-2 vaccine strain. Canine Adenoviruses caused serious viral disease in dogs. Although, several study about prevalence of disease in Turkey, there was no genetically identification of CAV until now in Turkey. As in all other disease, identification of genetic differences between vaccine strain and circulating virus is very pivotal in combating the disease. In this study, circulating CAV-2 in Turkey was identified firstly and genetically differences revealed between vaccine strain used in Turkey and circulating virus.

Keywords: Adenovirus, canine, isolation, PCR, MDBK, sequencing

Evaluation of Plasma IGF-2 Concentration and Its Expression in the Uterus and Ovary of the Dogs with Cystic Endometrial Hyperplasia-Pyometra Complex

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The aim of this study was to evaluate the plasma concentrations of insulin-like growth factor-2 (IGF-2) as well as its expression in the uterine and ovarian tissue of healthy dogs and those with cystic endometrial hyperplasia (CEH)-pyometra complex. Material-Methods: The study included seventeen female dogs. Group 1 consisted of 10 bitches with open cervix pyometra and Group 2 consisted of 7 clinically healthy diestrus bitches. The diagnosis of pyometra based on sanguinopurulent vaginal discharge, peripheral leukocytosis as well as vaginoscopic and ultrasonographic findings. Blood samples were taken before the surgery and plasma was separated after centrifugation at 1550 g for 10 minutes. The analysis of plasma IGF-2 was performed using ELISA kit following manufacturers' instructions. Ovarian and uterine tissue samples were collected at the time of surgery from 10 dogs with CEH-pyometra and 7 healthy diestrus dogs presented for routine ovariohysterectomy. Tissues were embedded in paraffin using standart methods and 5 µm sections were prepared. For the expression of IGF-2, a streptavidin-biotin detection system was used. The expression of IGF-2 in corpus luteum, theca cells and interstitial endocrine cells were observed in ovaries of both groups. IGF-2 immunopositive interstitial cells were markedly higher in Group 1. However, in Group 2, a few cells were IGF-2 immunopositive in only 2 cases. IGF-2 immunopositivity was determined in endometrial glandular epithelium from both the healthy dogs and those with pyometra; but epithelial cells of cystic endometrial glands were not stained. Additionally, interstitial fibroblasts and macrophages in the endometrium were also positive in Group 1. The concentration of plasma IGF-2 was higher in Group 1 compared to Group 2 (respectively, 24.76±5.62 ng/ml and 16.90±6.36 ng/ml; $p=0.019$). The concentration was positively correlated with IGF-2 expression in endometrial glands ($r=0.661$, $p<0.05$) of Group 1. However, negative correlation was present between plasma IGF-2 concentration and IGF-2 expression in the interstitial endocrine cells of ovarium in Group 1 ($r=-0.243$, $p>0.05$). The results suggest that IGF-2 plays an important role during inflammatory process occurring in dogs with cystic endometrial hyperplasia/pyometra complex (CEH-pyometra) as well as in the endometrium of healthy diestrus dogs.

Keywords: Canine, insulin-like growth factor-2, pyometra

The Effects of L-Carnitine in Budd-Chiari Syndrome in a Domestic Cat

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Budd-Chiari syndrome (BCS) is a clinical condition resulting from blockage of the main hepatic veins or the inferior vena cava. The blockages in the vena cava can be severe and may cause hepatocellular necrosis, hepatic failure, cirrhosis, and encephalopathy. BCS is very rare in the literature for domestic animals. The aim of this case report was to evaluate Budd-Chiari syndrome in a cat in the light of clinical, ultrasonographic, and laboratory findings, and treatment. Besides, laboratory findings are used to investigate the effects of L-Carnitine. The material of this case report was a 15 year-old female cat, vomiting and having severe abdominal distension. For evaluating Budd-Chiari syndrome in this cat, clinical examinations were performed. Ultrasonographic imaging was made by ESAOTE Aquila Vet (Pie Medical Ultrasound, Czech Republic). Haematological examinations were made by Abacus Junior Vet (Diatron®, Hungary), and biochemical parameters were measured by spectrophotometry according to the standard procedures using commercially available diagnostic kits (DiaSys Diagnontic Systems, Germany). Treatment protocol with antibacterial agent, diurethic drug, and L-carnitine application was combined in the light of clinical, ultrasonographic, and laboratory findings. For this purpose, 160 mg cefuroxime axetyl (Cefaks 250mg/vialTM, IM; Deva®, Turkey), 10 mg furosemide (Lasix 20mg/2mL ampulTM, IM; Sanofi Aventis®, Turkey), and 330 mg L-carnitine (L-Carnitene 1g/scaleTM, PO; Santa Farma®, Turkey) per day were prescribed, and also liver protection and lower salt diet (easy to digest foods that contain high quality protein and highly digestible protein; Prescription Diet® h/d®,l/d® feline-Hill's Pet Nutriton-For Rio, Rio de Janeiro, RJ, Brazil) was administered. There was not any trauma history in the cat, and feline enteric coronavirus could not be detected by Polymerase Chain Reaction (PCR). At intrahepatic region, a blockage of vena cava caudalis was found out by ultrasonographic imaging. The character of abdominal fluid was modified transudate. Liver enzyme levels in serum sample of the cat were increased. The levels of total oxidant status (TOS) and total antioxidant status (TAS) in peritoneal fluid were elevated. Liver protection diet, oral L-carnitine solution, diuretic therapy, and antimicrobial drugs were performed for treatment. Following the treatment, the amount of abdominal fluid decreased, but did not completely dissolve. L-carnitine was administered to cat during three months, and subsequently the levels of liver enzymes decreased. However, the cat died because of recurrent ascites and continuing the blockage of vena cava caudalis. The ultrasonographic examination is a very reliable, non-invasive, and highly useful diagnostic method for BCS, and L-carnitine has crucial effects on the quality of life, energy metabolism, and decreasing liver enzyme levels. However, the blockage of vena cava caudalis could not completely respond to medical treatment, and thrombosis should be eliminated by surgical intervention.

Keywords: Budd-Chiari syndrome, cat, L-carnitine, peritoneal fluid

Clinical Effects of Different Dose of Oral Misoprostol (PGE1) at 30-40 Days of Gestation for Pregnancy Termination in Cats

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The aim of the present study was to determine the clinical effects of different dose of oral misoprostol (MIS) and aglepristone (AGL) for the termination of pregnancy (TP) between 30 to 40 days in pregnant cats. For this purpose 28 healthy pregnant cats of different breeds, ages (18.0±6.2 m) and weight (3.4±0.2 kg) were divided into four groups. In AGL group (n=7) aglepristone (10 mg/kg, sc, on two consecutive days), in AGL+MIS group (n=7) AGL (as in AGL group) and misoprostol (PGE1, 200 µg/cat, oral, 12h interval until TP), in MIS200 group (n=7) MIS (as in MIS200 group) and in MIS400 group (n=7) MIS (400 µg/cat, as in MIS200 group) were used. Clinical and ultrasonographical examinations were performed every 12 hours until the TP. Blood samples were collected to determine whether changes depending of applications immediately before the first applications and four days later. Starting of the treatment, pregnancy days for ALG, AGL+MIS, MIS200 and MIS400 were 34.1±3.5; 35.4±4.2; 34.3±4.5 and 34.3±5.3ds, respectively (P>0.05). As a result of applications, the rate of pregnancy termination were found 71.4%; 100%; 0.0% and 57.1% in groups, respectively. When compared the pregnancy termination rate, it was determined higher in AGL, AGL+MIS and MIS400 groups than MIS200 group (P=0.005; P=0.001 and P=0.02, respectively). Most of the pregnancies were terminated by abortion than resorption in the study. Resorption rate was 28.6% and 14.3% for AGL+MIS and MIS400, respectively. Treatment-start of abortion/resorption interval were found shortest in AGL+MIS (4.7±1.6d) and MIS400 (5.0±0.7d) groups than AGL (6.1±0.6d) group (P=0.04). Similarly, end of abortion were found faster in AGL+MIS and MIS400 groups (6.1±1.6 and 6.0±0.0 ds, respectively) than AGL (7.2±0.4d) group (P=0.05). When evaluate the side effects (SE) of applications, the lowest rate was found in AGL and AGL+MIS groups at 14.3% and highest rate was found in MIS400 at 100% (P=0.002). Although there was no significance SE between MIS200 (71.4%) and MIS400 group (P=0.231), there was a tendency to increase significance between MIS200 and AGL and AGL+MIS groups (P=0.051). The SE that were observed in AGL, AGL+MIS and MIS200 only happened in the aforementioned days and no other time. In contrast, the SE that were seen in MIS400 continued until the TP. The SE were seen 5.3±3.1 applications of MIS on average in MIS200 group. The mean duration for SE initiation for MIS400 was 4.3±0.5 applications. Preliminary findings of the study showed that the oral dose of 200µg/cat MIS when administrated alone does not terminate pregnancy. Beside in higher dose of MIS does not terminate pregnancies in all cats (57.1%). On the other hand, it was determined that MIS support the effect of AGL on TP in cats depending on the effects of smooth muscle of MIS.

Keywords: Abortion, aglepristone, prostaglandin E1, queen

The Production Anti-Albumin Antibodies In Goats For The Accurate Determination Of Albumin In Canine And Feline Plasma/Serum Samples

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Albumin is the most abundant protein of intravascular space, as it constitutes approximately 50-60 % of plasma proteins. Main portion of albumin is found in blood and the remaining is distributed in biological fluids and various tissues. Besides, regulating oncotic pressure albumin plays role in transporting substances in the bloodstream. Thyroid and steroid hormones, fatty acids, bilirubin, vitamin B6, calcium and magnesium are some of the examples of transported elements. Partial compensation of blood pH is also regulated by plasma albumin. Deficiency of albumin may lead disturbances in an organism. In patients with renal failure, serum/plasma albumin levels are used to predict mortality/morbidity. It is recommended that albumin concentrations can be used to monitor patients in dialysis treatment. Dye binding and immunological methods mostly used to determine albumin levels. There are two dye-binding methods, which are Bromocreosol Green (BCG) and Bromocreosol (BCP). The latter is rarely used in veterinary medicine because of demonstrating variability in dye binding affinity among animal species. Moreover, there are several reports in human medicine indicating that dye binding methods giving inaccurate values in patients with renal disease. Immunologic assays are the better choice in such patients. In this study it is aimed to generate anti-albumin antibodies in goats for the possible use in developing immunological assays for cats and dogs, such as ELISA and Radial Immunodiffusion. Canine and Feline albumin were purified by ion-exchange chromatography followed by ethanol precipitation. Product purity was assessed by SDS-PAGE Electrophoresis. Goats were immunized with pure albumin fractions. Hyper immune sera were obtained by booster injections. The immunogenicity was tested by western-blot analysis. In conclusion, low cost polyclonal anti-albumin antibodies were produced for the possible use in the development of immunological assays to accurately estimate albumin levels in dogs and cats with renal failure.

Keywords: Albumin, antibodies, canine, feline, immunological assay

Displacement of Metal Part of Ameroid Ring Constrictor in a Dog with Extrahepatic Portosystemic Shunt

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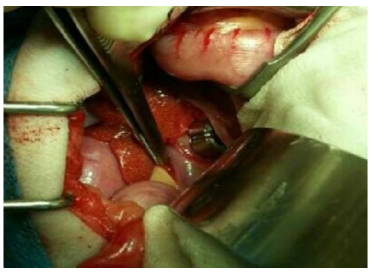
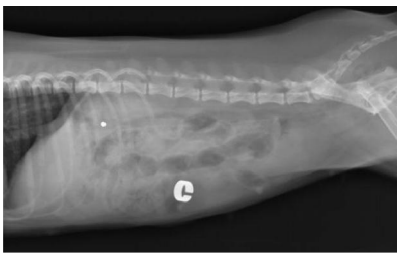
The purpose of this case report is to introduce an unusual case of displacement of ameroid ring constrictor (ARC) and clinical significance in a dog with congenital extrahepatic portosystemic shunts (PSS). PSS are abnormal vascular communications that connect the portal circulation to the systemic circulation, bypassing all or part of the liver in the process. Based on this study, gradual vascular occlusion using the ARC is recommended as a method for treatment of single extrahepatic PSS. Dogs with PSS treated by ARC generally had a good prognosis and prolonged postoperative survival. A 5-year-old intact female Maltese was admitted history about head pressing, dullness, depression. The examinations were conducted through CBC, biochemistry, urinalysis, bile acid and ammonia tolerance test. In general physical examination, CBC and blood electrolyte were unremarkable. But serum biochemical profiles were remarkable results that AST, ALT, GGT, and ALP increased. Ammonia and bile acid were noticeable increased. Abdominal X-ray, ultrasonography and computed tomography (CT) also be used for diagnosis. On abdomen radiograph, small liver, prominent kidneys, and renal calculi were identified. Shunting was observed to the flow of vessel in these images from ultrasonography and CT. Blood vessels in caudal vena cava (CVC) and portal vein were assumed the form of connections among the tortuous down the cranial right kidney. After anesthesia stabilization, the patient was VD recumbent position and laparotomy was performed. Shunt was positioned from left kidney to cranial direction about 2.5 cm. There were confirmed a torsion of right corner of the CVC. The thickness of 3 – 4 mm vessel was coming to the left side and the shunting vessel was clamped temporarily. Contrast media was injected from mesenteric vein and shunting vessel was confirmed by C-arm fluoroscopy. ARC was placed around the vessel and the key was placed and terminated for attaching ring (5 mm diameter ARC, Figure 1). The abdominal was closed routinely. There was no problem in application during surgery. Blood chemistry examination was identified as normal. Head pressing, dullness and depression were disappeared and returned to normal life after surgery. The patient was presented again one year later with other clinical problems unrelated with PSS. Separated ARC was found incidentally in radiography (Figure 2). Then remaining casein and key were maintained their ability to narrow in the shunting vessels and metal ring separate only. There was no failure of the closure of the blood vessels. Until a year after surgery, there was no clinical symptom associated with PSS. Separation of metal ring of ARC is rare but possible, not really affect blood vessels. Separation time after the ARC application is important. The separation of the metal ring at the early postoperative period can cause the failure of the occlusion and improvement of clinical sign may not be considered. The initial separation time of ARC at least 6 weeks after may not cause clinical problem. Continuous monitoring is, therefore, recommended after surgery and further checking of this case is necessary for longer time period.

Keywords: Ameroid ring constrictor, displacement, PSS



Ameroid ring constrictor is placed around the shunting vessel.



Separated ameroid ring constrictor is found incidentally in abdorminal radiography one year later.

Persistent Left Cranial Vena Cava with Congenital Heart Defect in 2 dogs

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This case report introduced the consideration of persistent left cranial vena cava (PLCVC) during thoracic surgery for congenital heart defect. Two dogs with PLCVC were presented with persistent right aortic arch (PRAA) and patent ductus arteriosus (PDA), respectively. First case, a 1-year 6 months old, female, 6.12 kg Cocker Spaniel was admitted with chronic vomiting for 4 months. After thoracic radiography and barium contrast test presented the sign of mega-esophagus caused by obstruction. Clinical signs and radiological findings presented patient was PRAA. Surgery was performed after 20 days after admission hospital with antibiotic therapy and nutrient state correction. During surgery, PLCVC was detected upper side of heart and lifted dorsally since then PRAA was ligated and divided (Figure 1). Patient was managed liquid food with upright posture after surgery. Second case, a 2 months old, female, 1.18 kg Maltese was admitted with chest thrill. Radiological finding showed cardiomegaly, MPA bulging, enlargement of LA and LV. Furthermore echocardiography finding presented abnormal systolic flow in the MPA, LV systolic dysfunction, showed shunting orifice, dilated LA/AO ratio and enlargement of LA and LV. On MRI imaging, abnormal vena cava was detected, it called PLCVC. Due to patient's age, surgery was delayed 3 months. PLCVC was detected and located exactly upper side of the PDA. Thus PLCVC was carefully dissection and lifted ventrally to secure a clear PDA view (Figure 2). Then PDA was ligated with black silk. Both patients were returned to normal life after surgery. PLCVC is formed by the abnormal development of sinus venosus. If the left sided vein does not become block, this vessel persists as a left-sided cranial vena cava, it called PLCVC. PLCVC is relatively rare in dogs and it is however accompanied with congenital heart disease. Therefore, PLCVC could be identified during surgical correction of congenital heart disease. With proper amount of blood flowing to the right atrium, dogs with PLCVC may not have any clinical signs. Nevertheless, due to the fact that PLCVC could bring confusion during congenital heart anomaly surgery, PLCVC should be lifted either dorsally or ventrally in order to secure surgical site and avoid the injury of PLCVC.

Keywords: Patent ductus arteriosus, persistent left cranial vena cava, persistent right aortic arch

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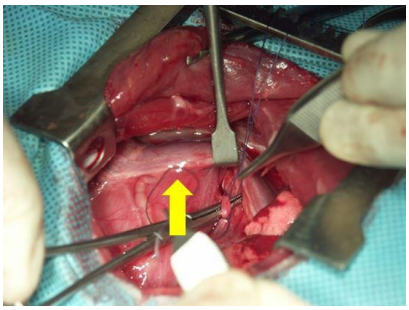
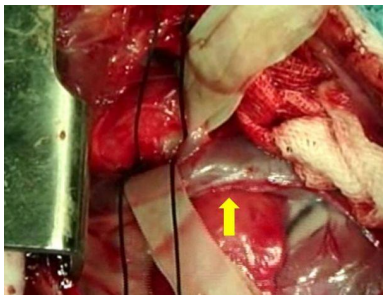


Persistent left cranial vena cava is confirmed upper side of heart and lifted dorsally since then PRAA is ligated.



Persistent left cranial vena cava is lifted ventrally to secure a clear PDA view.

Hematogenous Osteomyelitis Following Open Fracture in a Cat

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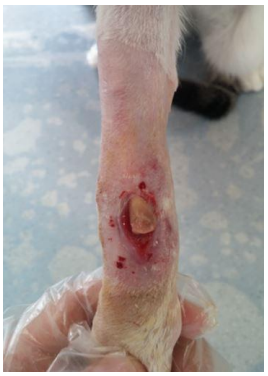
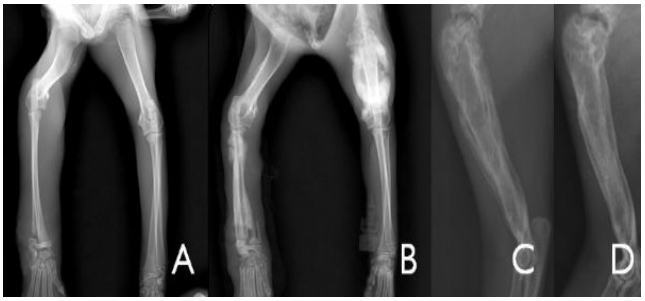
Hematogenous osteomyelitis is a rare form of bone infection in adult cats and dogs. Surgical debridement must be performed within 6 hours in case of open fracture. The purpose of this report is to introduce a hematogenous osteomyelitis spreading from the inappropriate management of open fracture. A 6-month-old, weighing 2.8 kg, female, domestic short-haired cat presented with type 2 open fracture (salter-harris fracture type 1) at right distal radius about a month ago. Exposed right radius bone had avascular bone fragment and fracture instability. Because of excessive inflammatory reaction at fracture site, systemic antimicrobials and corticosteroids had been given for 3 weeks per oral and intravenous at local animal clinics. Physical examination revealed decrease range of motion of the right elbow and pain during flexion. There was no heat, swelling, redness and exudate at fracture site (Figure 1). Blood chemistry and complete blood cell count were normal. Based on radiological findings, hyperplasia at the right radioulnar and left humerus was found. This periosteal reaction was not seen on initial radiograph 2 weeks ago. Results of the cytological examination were inflammatory reaction and reactive osteoblast. Bacteria were not cultured from harvest sample in which cytological findings indicate infection. Debriding necrotic bone was performed by distal radius partial ostectomy with rongeur. K-wire of 1.2 mm diameter was used through cranio-caudal direction to fix radius and ulnar. Post-operative systemic antimicrobial agent had been given for 2 weeks. Inoculation of bacteria can occur from exposure of the bone via an open fracture, or a surgical intervention. Surgical and medical treatment produced good results. Three weeks after operation, the radial bone lysis was seen on radiograph and clinical condition improved. Two months after operation, she can use her both forelimbs despite right elbow have been diagnosed as arthrosclerosis because of periosteal reaction. Six months after operation, clinical symptoms of left forelimb (suspected hematogenous osteomyelitis) and right forelimb (osteomyelitis) was not detected (Figure 2). Between the dog and cat, relative size and dimension of bones may differ, feline ulna is more substantial than canine. Moreover, in comparison with the dog, the distribution of load at a walk is different with the hind limbs of the cat sharing more of the loads. Because of these reasons, she can use her both fore limbs, despite radius was removed. In this case, treatment produced good result without recurrence. Therefore the etiology of hematogenous osteomyelitis is uncertain. The infection can occur to fracture bone (osteomyelitis) and seed from hematogenous spread to another normal bone (hematogenous osteomyelitis). In conclusion, when open fracture occurs, urgent surgical intervention is recommended.

Keywords: Hematogenous osteomyelitis, open fracture, periosteal reaction



Open fractured is shown in digital radius.



Periosteal reaction has improved compared with the inintial radiograph. A, the initail radiograph B, 16 days after wound management C, 3 weeks after surgery D, 8 weeks after surgery

Effect of Some Essential Oils (Allium sativum L., Origanum majorana L.) and Ozonated Olive Oil on the Treatment of Ear Mites (Otodectes cynotis) in Cats

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The objective of this study was to determine the activity of ozonized olive oil (Oo), garlic (Allium sativum L.) oil (As) and marjoram (Origanum majorana L.) oil (Om) against O. cynotis (Oc) infestation in cats and the their activity was compared with permethrin (PE) that is used as a conventional treatment with the intention of identifying an alternative treatment. In the study, 28 cats that performed by the examination of ear secretions under microscope after the physical examination. The level of ulceration, pruritus, pain, amount of secretion and secretory type was scored from 0 to 3 before (day 0) and after treatments (at day 10 and 30). Cats were randomly assigned to treatment groups including; group 1 (n=7): Oo, group 2 (n=7): As, group 3 (n=7): Om and group 4 (n=7): PE. Each treatment was applied to both ears about 5 drops (0.3 ml/daily) for 10 days. All cats (100%) treated with Oo or PE were recovered while 85% and 71% were recovered by Om and As, respectively. At day 10, clinical scores with exception of ulceration in the group 2 and 3 were found to be significantly reduced ($p<0.05$) when compared to other groups. There was no difference in terms of the number of Oc among groups at days 0 and 10. At days 0, 10 and 30 the number of Oc (eggs, adults and young) was counted. The efficacy of the treatments was determined as efficacy percentage (EP) using efficacy formula. Group 4 was found to be the most effective (EP, 100%) followed by Group 1 (99.63%), Group 3 (99.28 %) and Group 2 (98.77%) for egg numbers at day 10. However, Group 1 and 4 had the same efficacy percentage (100%), followed by Group 3 (99.27%) and Group 2 (97.92%) at day 30. Group 2 (99.19%) was found to be the least effective while other treatments had 100% efficacy against young parasites at day 10. At day 30, Group 1 and 4 had the same efficacy percentage (100%) while Group 3 and Group 2 had 99.85 and 98.36%, respectively. For adults, Group 1 showed 100 and 99.77% efficacy at day 10 and 30, respectively. At days 10 and 30, Group 4 and 2 had 100% and 98.74% efficacy, respectively. However, Group 3 had 99.11% and 99.24% efficacy at day 10 and 30, respectively. Hence, the most effective treatments were group 1 and 4 at day 10. For day 30, efficacy level for treatments were in following order Group 4 > Group 1 > Group 3 > Group 2. As a result, it can be said that As, Om and Oo in treatment of Oc infestation in cats would be alternative treatment options as being cheap, easy to apply, no side effects and safe to conventional treatment (PE).

Keywords: Allium sativum, essential oils, origanum majorana, otodoctes cynotis, ozonated olive oil

Factors Related to the Frequency of Cat Ear Mites (Otodectes Cynotis)

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The objective of this study was attempts to identify the factors related to the frequency of Oc infestations in cats in Turkey. A total of 105 cats underwent physical examination and examination of both ear canals using an otoscope. Data on cats sex, age, breed, erythema, ulceration, pruritus, pain, secretion amount- type, and the parasitological examination findings of were recorded. In all, 38 cats were found to be infected with Oc. Waxy material from the ear canals of infested cats was collected by ear cotton swap and examined under microscope by dropping 1–2 ml of mineral oil onto a glass microscope slide to determine the presence or absence of live mites and the total mite count. Mites were identified using the diagnostic key. Statistical analysis was conducted to create a dataset. Cats were categorized by sex (female, male), age (<1 years, 1-4 years, >4 years), lifestyle (indoors alone, indoors with other pets, outdoors), and clinical symptoms (pruritus, eritema, ulceration, ear discharge, pain) to reveal the association of risk factors with Oc using chi-square tests. Associations were considered significant at $p<0.05$. In the study, 28 (27.7%) of 105 cats (male: 49, female: 47 ages: >1–<4) were found to be infected. In the aspect of effective factors of Oc infestation, significant differences were not seen with sex ($p>0.05$) but were with lifestyle of cats ($p<0.05$). Although no statistical differences were found for age, infestation prevalence tended to decrease with age (<1 years: 31.3%; 1–4 years 28.2%; >4 years 11.1%). There was an association between pruritus, ear discharge in clinical symptoms and Oc infestation ($p<0.001$) In conclusion, we found that Oc is a highly common ectoparasite in cats. The potential risk of Oc infestation varies by age and sex. Additionally, lifestyle influenced Oc infestation prevalence. Clinical signs of ear mites were not always apparent, but higher rates of Oc infestation were found among cats with pruritus, eritema, ulceration, ear discharge, and pain.

Keywords: Otodectes cynotis, prevalence, parasites infestation, Turkey

Iodixanol, as a Cryoprotectant, Protects Erythrocyte Membrane

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Iodixanol is a nontoxic nonpenetrating substance used as contrast substance for examination of cardiovascular system in human medicine. In the present study, it was aimed to investigate the effects of nonpenetrating iodixanol usage together with penetrating cryoprotectans, which are effective in protection of erythrocytes from hazardous effects of freezing and thawing. Glycerol was added to control group erythrocytes. Glycerol and 8% iodixanol (60%) were added to the experimental group erythrocytes. Blood bags were kept for 80 minutes in liquid nitrogen at -110°. Blood bags were turned upside down every 20 minutes. Freezing foci were created by seeding procedure when the bags were turning upside down. The frozen experimental and control group bags were stored at -80°C for 12 months. At the end of storage period, frozen samples were thawed and cryoprotectans were removed, and then levels of Thaw hemolysis* (%), osmotic fragility* (%), saline stability (%) changes in erythrocytes were investigated.Osmotic fragility value of experimental group was significantly lower than that of the control group. Thaw hemolysis, which is an another indicator of cell injury, was significantly higher in control group than in the experimental group (Table 1). The accordance observed among thaw hemolysis, saline stability and osmotic fragility results suggest that iodixanol, which does not penetrate into the cell, protects the cell. Accordingly, it can be said that erythrocyte membrane has been damaged less in iodixanol used group. It is concluded that iodixanol possesses cryoprotectant potential that can be used as a protective substance for freezing of canine erythrocytes. However, more researches in which iodixaol used alone and in different doses, are needed.

Keywords: Canine erythrocytes, freezing, iodixanol, glycerol

Table - 1 Thaw hemolysis, osmotic fragility, saline stability changes in frozen erythrocytes

Parameters	Glycerol (Control)			Glycerol +Iodixsanol (Experimental)		
Thaw hemolysis* (%)	4,58	±	0,64	3,00	±	0,38
Osmotic fragility* (%)	83,29	±	0,55	73,63	±	3,19
Saline stability (%)	0,63	±	0,10	0,87	±	0,11

** The difference between groups for he same parameter is significant statistically. (P<0,05)*

Surgical Repair of Primary Spontaneous Pneumothorax

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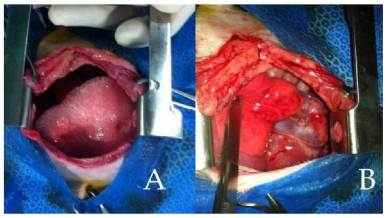
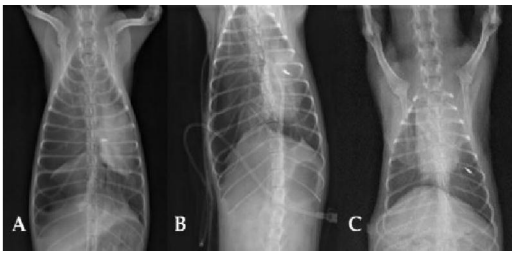
The purpose of this case report is to introduce an unusual case of spontaneous pneumothorax with lesions in the whole lung lobe, which led to a total lobectomy. A 3-year-old female Maltese dog was admitted with chief complaints of cough, dyspnea, and gasping for 3 days. There was no sign of trauma in the history taking, and blood tests (CBC and Chemistry) found no particular abnormality. After thoracic radiography, it was speculated that pneumothorax may have occurred in the right lung lobe, and the patient was stabilized through thoracocentesis. Three hours after thoracocentesis, symptoms of dyspnea and cough have recurred and a chest tube was inserted. For the 5 days of hospitalization, ventilation pattern and air leakage from the chest tube were measured. It was confirmed that air was present in the thoracic cavity even after chest tube insertion, and it lead to the decision for a surgical intervention. After anesthesia stabilization, the patient was placed in left lateral recumbent position and the thoracic cavity was exposed through the right 8th intercostal. The right middle lobe was not composed of normal tissue but was a spongiform, air-containing lobe. Total lobectomy was performed after double ligation of the pulmonary vasculature and bronchus with green polyester suture materials. In addition, atrophy of the right cranial lobe was identified (Figure 1). Through positive pressure ventilation, the right caudal lobe was confirmed to inflate normally. After removing the fluid, chest tube was inserted again and chest was closed routinely. Two days after surgery, it was removed from the chest. The patient showed no abnormal symptoms and the thoracic radiograph showed no signs of recurrence (Figure 2). Histopathological examination of the excised lesion was performed. There were two pathologic changes in the lung sample, both of which were chronic in nature and of minimal/mild severity. A single section of lung from the right middle lobe was examined, revealing two focal, chronic inflammatory changes. First, there was focal accumulation of alveolar macrophages at the periphery of the lung, which is associated with mild fibrosis and presence of a few acicular cholesterol clefts. Second, there was multifocal accumulation of small numbers of lymphocytes and hemosiderin-laden macrophages (chronic hemorrhage) in perivascular locations. Alveolar spaces were otherwise well-inflated. There was no other lesion in the lung sample that could explain non-traumatic pneumothorax. It was diagnosed as spontaneous pneumothorax with the result of the histopathological examination. Infectious secondary pneumothorax was ruled out in the blood test. This case shows that lesions in the whole lung lobe could be seen in primary spontaneous pneumothorax. Unlike the usual primary spontaneous pneumothorax cases that occur in the apex of the lungs, the entire lobe was abnormal in this case. Therefore, total lobectomy was performed in order to reduce the possibility of recurrence.

Keywords: Dog, Non-traumatic pneumothorax, Spontaneous pneumothorax, Total lobectomy



Air filled right middle lung lobe (A) and the cranial lobe of atrophy (B) are identified in operation. The thoracic radiograph shows no signs of recurrence after surgery.



A.the initial radiograph B. at 3 days after appliation of chest tube. C. at 17 days after the surgery.

Clinical Studies on the Treatment of Hip Dysplasia by "Modified DARthroplasty" in Dogs

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Dorsal acetabular rim arthroplasty (DARthroplasty) is a surgical procedure to stabilize the dysplastic hip. A bone graft is planted over the joint capsule at the dorsal acetabular rim. In this study, we aimed to evaluate the effectuality of "Modified Dorsal Acetabular Rim arthroplasty (DARthroplasty)" technique's on treatment of hip dysplasia in dogs. Study was carried out on 20 dogs with hip dysplasia in different breed, sexes (13 Male, 7 Female) and ages (4-24 months). Pelvic radiographs of all dogs, including hip extended views, were taken. DARthroplasty was performed in 16 dogs unilaterally (11 right and 5 left), and in 4 dogs bilaterally. In all dogs, atropine sulfate (0.045 mg/kg), xylazine HCL (1mg/kg) and ketamine HCL (5 mg/kg) were injected intramuscularly for anesthesia induction. Following oro-tracheal intubation procedure, general anesthesia was provided by 2% inhalation of isoflurane. A cranio-dorsal approach to the coxo-femoral joint was used. The exposed bony portion of the dorsal rim of the acetabulum was prepared to accept bone grafts by the drilling of three holes along the dorsal acetabulum, exposing activated cancellous bone. The cortical-cancellous spikes were harvested from the 13rd rib and were penetrated into holes in the acetabular rim, at equal distance to each other. Both surgical wounds were closed with a standard layered protocol. Statistical analysis was performed using Shapiro-Wilk's test, Freidman's test and post hoc Wilcoxon's test. Nominal data (pain tests) were evaluated by using Cochran Q test. $P < 0.05$ was considered significant. There were minimal degenerative changes in the coxo-femoral joint of the dogs. Mean Norberg Angle was measured as 92,32±2,04°, 94,0±1,94° and 95,36±1,95°, preoperative, postoperative 3th and 12th months, respectively. At the postoperative first day, all dogs were ambulant and lively, and started nearly fully weight bearing on the limbs. However, activity of dogs was limited and walking with short leash was permitted for six weeks. The mean results of hip extension tests, abduction external rotation tests, standing and walking tests, gait quality evaluation, lameness test and Norberg angle measurements at 3rd month and 1st year postoperatively were significantly different ($P < 0.05$) than preoperative results. In 3rd and 1st year postoperative examination, better results were obtained from abduction-external rotation test, hip extension test, standing test and walking-running test when compare preoperative findings. As a result, in this technique "Modified DARthroplasty", transplanted cortical cancellous grafts neither extend dorsal acetabular rim nor stopped degenerative changes. But, we observed clinical improvement in all dogs. Also, the owners' satisfaction was very high. Perhaps, cortico-cancellous bone grafts acted as "stem cells".

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Keywords: DARthroplasty, dog, hip joint dysplasia

Cementless Pamuk's Total Hip Replacement (PTKP) Implementation and the Results: The First Evaluation in Dogs

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In this study, application and results of Pamuk's Total Hip Replacement (PTKP) system was evaluated the dogs with hip problems. Twelve dogs with hip problems (dysplasia, luxations and fractures) were used. Equipment, Ortho-Pet, Izmir (Turkey) were manufactured. It was approached with craniolateral incision of craniodorsal sides of the hip trochanter major level. Caput femoris excision was performed by osteotomy. And then, acetabulum was enlarged. After acetabular cup have placed and it was fixed with screws. Then the implants was placed for collum femoris. Implant head of the neck portion was steeled and the hip joint was in place. In controls, inspection, palpation and radiographic examination was performed for post-op 15 days and 1, 3 and 6 months. Seven dogs (58.3%) in the postoperative prognosis is seen good or very good condition. Clinical examination of patients with a good prognosis in 15th and 30th days showed similarities between each other. Comparison of post-operative 90th days with 15th and 30th day prognosis show that the gait problems was eliminated and to considered to be very good condition. It was also observed that the extremities used as intended. The extremities were not used very well and lameness of varying degrees in 5 dogs (41.6%). Radiograhic examination of these dogs were showed loosening of the collum femoris apparatus, subluxation and the acetabular components may not fixed into the acetabulum properly. Although the first impression of cementless PTKP's was shown some technical problems, it has been concluded that it is economic, development devices and encouraging of the first results. In addition, monitoring of longer-term outcomes PTKP's more than 6 months is thought to provide important information for the availability and development.

Keywords: Dog, hip prosthesis, PTKP

Partial Foot Amputatiın in 3 Dogs

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Amputation is a procedure that using for remove the extremity by cutting any bone level. Also disarticulation is a term which explains the separation of two bones at their joint level. Amputation may be perform in cats and dogs with severe irrepable fractures, severe infection unresponsive to treatment, severe distal extremity wounds, excision of tumors, irreversible neurological deficits, osteomyelities, nonunion fractures, severe trauma and severe toe deformities. Medical Records obtained from the University of Selcuk, Faculty of Veterinary Medicine, Department of Surgery. These records are belong to 3 dogs that were treated with foot amputation. The median age of effected dogs was 3 years (range, 2-5 years old) and two of them female, one of them male. Each dog was examined individually via radiogically and rutine clinical. Preoperative radiograph were taken for each effected extremity. For anesthesia Ksilazin HCl (1-3 mg/kg), Ketamin HCL (10 mg/kg) (IM) and isoflurane were used. After being prepared for aseptic surgery an elliptical incision was performed around the affected digits, extending from the distal aspect of palmar or plantar surface of the digits to the dorsal aspect of metacarpus. Branches of the arteries and veins were ligated. Amputation was performed at the level of metacarpophalangeal joint. Wound was cloused with proper sutures material. Penrose drain was placed before suture the skin layer and bandage was applied. After the surgery, postoperative analgesics and antimicrobial theraphy were administered for each dog. Operation wounds healed without any complication. The dogs returned to normal function. Partial foot amputation successfully performed in veterinary medicine to treat commonly untreatble distal extremity problems in pets.

Keywords: Dog, foot amputation

Clinical Experience of Interlocking Nails Stabilization of Dogs with Long Bone Fractures-A Retrospective Study 26 Cases

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The aim of the study, to report clinical and radiographic outcome after use of an interlocking nail (ILN) for stabilization of long bone fractures in dogs. Twenty-six dogs were evaluated. Ages ranged from 1 to 5 years and weighed 15-50 kg. There were 10 femoral fractures, 12 tibial fractures and 4 humeral fractures. Equipment was manufactured by Orthovet (Orthovet, İzmir, Turkey). Three ILN lengths with 3 different diameters (4, 6 and 8 mm) were used. Each ILN had a trocar tip on one end and 4 screw holes (2 distal and 2 proximal). Ten fractures (4 femoral, 5 tibial, 1 humeral) were associated with other orthopedic problems. In 9 (39.1%) patients aseptic nonunion and malunion fractures. Static fixation mode was used in 9 fractures. Dynamic fixation mode was used in 17 fractures (65.3%). Mean surgical time recorded for 45-52 minutes. Three had a major complication requiring a surgical intervention At 6 month, functional outcome was excellent in 15 (57.6%) animals, good in 7 (26.9%), fair in 3 (11.5%), and poor 1(3.8) In conclusion, use of ILNs to repair of diaphyseal fractures of the femur, tibia, and humerus in dogs resulted in a good or excellent functional outcome in most patients.

Keywords: Dogs, interloking nail stabilization, long bone fractures

Morphological Study of Duchenne's Cardiomyopathy in a Canine Model

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Duchenne muscular dystrophy (DMD) is a recessive X-linked disorder characterized for mutation in dystrophin gene and manifested by progressive degeneration and necrosis of skeletal and cardiac muscle with replacement by fat and connective tissue leading to generalized muscular weakness and atrophy. Dystrophin is the largest gene in the human genome and essential member of the dystrophin glycoprotein complex (DGC), which protects the sarcolemma from mechanical stress during repeated cycles of muscle contraction and relaxation. The dog Golden Retriever Muscular Dystrophy (GRMD) is the best experimental model for DMD, with genotypic and phenotypic manifestations closely of human disease. Similar to patients with DMD, heart failure is a major cause of death in GRMD animals. The main of this study was to elucidate the progression of pathological lesions in the heart from GRMD dogs. Methods: Fragments of left ventricle, from 18 GRMD dogs between 6 to 18 months, were collected, fixed by immersion in phosphate-buffered 10% formalin, and after dehydrated, clarified, and embedded in paraffin. Five micrometer thick serial sections were obtained and stained with Hematoxylin-Eosin (HE), Picrosirius red to evaluate cardiac fibrosis and Von Kossa to verify mineralization areas. Results: Myocardial lesions were observed in all GRMD dogs and the sequence of cardiac lesion classified according to the age included: abnormal calcium accumulation verified by Von Kossa staining in GRMD dogs at 6 months of age, myofibrillar necrosis and granulation tissue formation both observed by HE staining in GRMD dogs between 8 to 10 months of age, endomysial and perimysial fibrosis evidenced by picrosirius red staining in cardiac muscles from GRMD dogs at 11 to 15 months of age. Finally, intense fatty infiltration observed by picrosirius red staining was detected in the hearts from dystrophic dogs between 17 to 51 months. Interestingly, several Anitschkow cells were detected in inflammatory infiltrate present at granulation tissue. Our results demonstrate the sequence of cardiac lesions that determine the cardiomyopathy in Golden Retriever dogs affected by DMD. These findings relevant for to clarify the pathogenesis of cardiomyopathy in dogs and humans affected by DMD. (FAPESP 2013/25957-6)

Keywords: Cardiomyopathy, dogs, histopathology, muscular dystrophy

The Importance of Mast Cells in Pathogenesis of Fibrosis in Golden Retriever Muscular Dystrophy

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The Duchenne muscular dystrophy (DMD) is a hereditary myopathy related linked to X chromosome that results in mutation in dystrophin gene. Dystrophin is responsible for connections between the intracellular medium, the actin cytoskeleton and/or the sarcomeric structure and external basement membrane. The Golden Retriever Muscular Dystrophy (GRMD) is the best experimental model for studies of DMD. A histologic hallmark of both human and dog dystrophin-deficiencies is a progressive proliferation of endomysial connective tissue which seems to parallel the clinical course of the disorder in the both species. Ultimately, weakness results in death from respiratory failure before the third decade of patients with DMD. Mast cells are normal inhabitants of the connective tissue known to be involved in wound repair and tissue destruction. In addition, mast cells release specific mediators, among them the tryptase that activate fibroblast growth factor, a potent promoter of fibrosis, or stimulate fibroblasts directly. Based on these correlations, the aim of this study was to evaluate in skeletal muscles from GMRD dogs the morphologic alterations and the role of mast cells in pathogenesis of DMD. Material-Methods: Fragments of skeletal muscles (masseter, diaphragm, triceps brachial and biceps femoris) from GRMD dogs between 2-8 months of age were collected included in paraffin and sectioned in five micrometer thick serial sections for usual histological evaluation. The staining was performed with Hematoxylin-Eosin (HE), toluidine blue, to evaluate the presence of mast cells as a dense granular aggregate in violet staining showing the characteristic metachromasia, and Azan Staining to determine areas of fibrous tissue deposition. The fragments belong to bank of muscles of laboratory Experimental and Comparative Pathology, Department of Veterinary Pathology- FCAV/Unesp. Statistical analysis was performed using GraphPad Prism5. Results: The HE staining evidenced skeletal muscle fibers with different diameters; inflammatory infiltrate and fibrous tissue deposition. The toluidine blue showed increase numbers of mast cells mainly in areas with fibrous tissue deposition in skeletal muscles from GRMD dogs compared with control group. The triceps brachial exhibited increase numbers of mast cells in relation to other muscle groups (±650) as biceps femoris (±590), masseter (±480) and diaphragm (±180). Areas of abundant deposition of collagen were evidenced by Azan Staining in the perimysium of the triceps brachial, biceps femoris and moderate deposition in the masseter and diaphragm of animals affected by DMD. Conclusion: Our results demonstrated the participation of mast cells in the pathogenesis of muscular dystrophy in GRMD dogs. These findings could elucidate the mechanisms that lead to fibrosis in DMD and to contribute to development of new therapeutic approaches to improve life expectancy in humans and dogs affected by muscular dystrophy.

Keywords: Duchenne muscular dystrophy, dogs, inflammatory cells, fibrosis

The Effects of Medetomıdıne-Propofol-Isofluorane Anaesthesia on Blood Gases, Biochemical and Clinical Parameters in Dog

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The aim of this study was to investigate the effects of anaestehesia induced by medetomidine, propofol and isoflorane on blood gases, biochemical and clinical parameters in dogs. The study material consisted of 10 healthy mixed breed dogs. As premedican agent, medetomidine was injected intravenously (iv) at a dose of 20 µg/kg for each dog and after five minutes propofol was administered iv at a dose of 3mg/kg for anaestehesia induction. Following propofol administration, anaesthesia was maintained with 2% isoflorane by inhalation anaesthesia device. Heart and respiratory rate, body temperature was recorded before (0. min) and after 5., 20. minutes of anaesthesia. 10 ml of blood was obtained before and during anaesthesia intervals for blood gases and biochemical analysis. Changes in venous pH, pCO2, pO2, pHCO3 and CtCO2 levels was also determined. Lateral position was observed after 5th minutes of premedication. A significant duction in pedal and palpebral reflex was observed. Anaesthesia induction was achieved after 1-3 (mean 1.5) minutes of propofol injection. All reflexes disappeared at induction. Fairly smooth anaesthesia was achieved by isoflorane administration. Dogs were conscious between 10. and 13. minutes (mean 11 min) after isoflorane application was ceasesed. After awakening from the anesthesia, the dogs were stand up within 25. to 65. min (mean 28 min). The average body temperature during anesthesia was not changed significantly. Heart and respiratory rate decreased during anesthesia. No significant alteratons were detected for the average venous blood HCO3. and CtCO2 values. But, pH, pCO2 and pO2 values changed significantly during 5. and 20. minutes of the anaesthesia. The average serum biochemical parameters ALT, albumin, total protein and glucose values did not show significant changes during anesthesia. However, LDH values changed significantly at the 5 and 20 minutes of anaesthesia. As a result, no significant changes were recorded for the biochemical values for the reported anaesthesia protocol. The presented anaesthesia protocol migth be used confidently if precaution is taken for propofol application since propofol migh cause apnea in some dogs.

Keywords: Dogs, isofluorane, medetomidine, propofol

Interval between Injection of Contrast Material and Positive Contrast Cheliography Affects Accurate Diagnosis of Diaphragmatic Hernia

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The aim of this study was to evaluate the clinical, surgical and diagnostic imaging findings in 11 cats and 3 dogs with suspected acute and chronic traumatic diaphragmatic hernia and to compare the results of positive contrast cheliography (peritoneography) taken immediately and 5 minutes after the injection of contrast material. Thoracic and abdominal radiography, ultrasonography and positive contrast cheliography of all animals were performed. Eight cases were considered as acute and six cases were considered chronic. The contrast images taken immediately after the injection of contrast material revealed the contrast material in the thoracic cavity in 8/8 acute trauma patients, but in none of the chronic cases. In 5/6 of these cases contrast material was seen in the thoracal cavity only in additional images taken after 5 minutes. One patient was diagnosed with FIP and excluded from the study. Twelve cases had complete resolution and one animal died during the early postoperative period. Our results suggest that positive contrast cheliography performed immediately after the injection of contrast material may not reveal chronic cases of diaphragmatic hernia and a second imaging (or imaging after 5 minutes) is indicated in order not to overlook chronic cases.

Keywords: Cat, chronic, contrast medium, diaphragm, dog, peritoneography

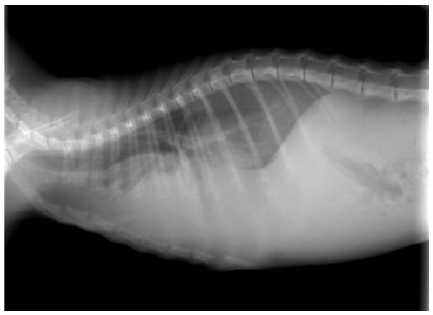

Figure 1
Right lateral thoracic and abdominal radiographic view of a 1-year-old crossbreed cat with diaphragmatic hernia (Case 6). Notice that the diaphragmatic line is lost and a soft tissue opacity is located within the thoracic cavity. The characteristic abdominal gas shadow is also observed. The cardiac shadow is completely obliterated due to pleural effusion and the invasion of abdominal organs.

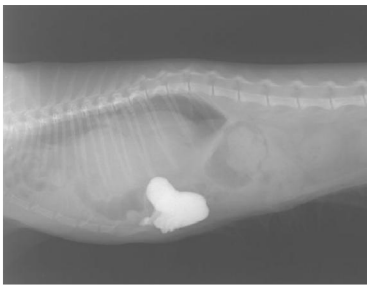

Figure 2
Right lateral thoracic and abdominal cheliographic view of the same case. The radiograph was taken immediately after the injection of the contrast material. The contrast material can not be seen in the thoracal cavity because of adhesions in this chronic case.

Case of Prostate Carcinoma and Cholangiocarcinoma in a Dog

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The aim of the present study is histopathological investigation of prostate carcinoma and cholangiocarcinoma. A fifteen years old terrier male dog was brought from the surgery department of Ankara University and submitted to necropsy in pathology department. The animal came to surgery department with an anamnesis of hematuria, difficulty of defecation and urination as well as lethargy, anorexia, and a progressively growing mass at the perianal region. Pain was revealed during palpation of the abdomen at clinical examination. The animal was submitted to surgery where they figured out that the origin of the tumor was the prostatic gland. During the necropsy macroscopical examination was done then tissues were processed routinely and embedded in paraffin, sectioned at 4-6 µ thickness and stained with haematoxylin-eosin (H&E). All tissues were examined microscopically under the light microscope. At the necropsy; a mass was noticed on the right lateral lobe of the liver. This mass was 4x3x3 cm in size and soft in consistency, had cystic appearance and white-yellowish color on cut surface. At the examination of the uro-genital system of the dog, prostate seemed to be bigger than its normal size, had multilobular, necrotic and dark red appearance on cut surface. Also the wall of urinary bladder was thick and the lumen was stringent. Prostatic urethra was not well distinguished. Histopathological examination of the mass of the liver revealed that the tumor was composed of epithelial neoplastic cells with round to oval vesicular nuclei and narrow eosinophilic cytoplasm. Also, mitotic figures and pleomorphism were seen. Tumor cells were arranged in acinar or tubular pattern and separated by fibrous connective tissue. In some areas, cystic spaces with lobular appearance were noticed. At histopathological examination of prostate; the cells generally had round or cuboidal shape, hyperchromatic nuclei, and clear narrow eosinophilic cytoplasm. These cells were also accompanied by mitotic figures. Neoplastic cells were arranged in nests and variably sized acinar and papillary cystic structures were noticed. While examining the bladder on the light microscope, neoplasic cells were seen in the tunica muscularis. Based on these pathomorphological findings, the tumor in the prostate was diagnosed as prostate carcinoma and the one in the liver as cholangiocarcinoma. Dogs are between mammals the specie that shares with man vulnerability for development of prostate carcinoma; therefore they have been used for years as a suitable model to study mechanisms of human prostate cancer. Prostate carcinoma in dogs shares several common features with man. It occurs more frequently in older dogs. In this case report we accentuate the fact that in old dogs; while hyperplasia has been the first diagnosis to establish in such cases, prostate tumor can be as well a main diagnostic option. And a well developed liver tumor can be diagnosed in a dog not showing any clinical sign related to liver impairment.

Keywords: Cholangiocarcinoma, dog, histopathology, prostate carcinoma

Expression of Sox-2 Embryonic Transcription Factor of Putative Cancer Stem Cells in Canine Cutaneous Mast Cell Tumor

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Cancer Stem Cells hypothesis states that cancer originated from a small subpopulation of cancer cells characterized by have self-renewal and pluripotency (Yunqing and John, 2012) that responsible for treatment and may be rapidly recurrence (Amanda et al., 2014). Canine Cutaneous Mast Cell Tumor (MCT) is the most common skin tumor in dogs. It has various clinical signs. The aimed of this study was to demonstrate that MCT contain the putative CSC which expresses Sox-2. Basically, Sox-2 usually express at the stage of proliferation in embryonic stem cells. A fresh tumor tissue of MCT dog was collected from operating room, Small Animal Hospital Teaching, Faculty of Veterinary Science, Chulalongkorn University. The total RNA was extracted and converted to cDNA for Sox-2 expression analysis using RT-PCR. DNA primers were designed from our laboratory: F: 5'-CGG CAA CCA GAA CAG - 3'and R: 5'-CTG CTG CGA GGA CAT -3'. The study result has preliminarity show the expression of Sox-2 in the MCT specimen. This result implied that the MCT tissue sample might be presented the putative cancer stem cells inside by expressing Sox-2 which controls CSC self –renewal. However, to ensure the role of sox-2 in self-renewal regulation of putative MCT cancer stem cells. The further study in a large MCT population is still required for investigating the expression of Sox-2 in their putative CSCs. This research was supported by the Rachadaphiseksomphot Endowment Fund, Part of the “Strengthen Chulalongkorn University Researcher’s Project.”, 2014. Amanda, K., T., Shinya, M., Anish, B., Anupama,M., Rajagopal, R. (2014).Cancer stem cells: progress and challenges in lung cancer. Stem Cell Investigation, 1(9), 1-18 Yunqing, L. and John, L. (2012).Cancer Stem Cells: Distinct Entities or Dynamically Regulated Phenotypes?. Cancer Res, 72(3), 576-580

Keywords: Cancer stem cell, canine cutaneous mast cell tumor, embryonic transcription factor, RT-PCR, sox-2

Clinical Aspects of Using the Nanofiber Membrane Biodegradable Polymers (PBAT) in the Treatment of Deep Corneal Ulcers in Dogs

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Considering the property of the membrane of biodegradable polymer nanofibers has in provide the support for the cell growth, the aim of this study was to evaluate the feasibility of using membrane poly(butylene adipate-co-terephthale) - PBAT - to treat the deep corneal ulcers in dogs. Six dogs (n=6), male and female, were treated with surgical procedure (keratoplasty) and clinical treatment for corneal deep ulcers under consent of the owner. All patients underwent a general physical and complete ophthalmic examination: Schirmer test, dye fluorescein test, examination by slit lamp biomicroscopy, applanation tonometry and indirect ophthalmoscopy, when possible. The the additional tests to pre-anesthetic and surgical evaluations were performed. All animals received the same clinical treatment protocol from the first day of the treatment: eye drops tobramycin base, EDTA 0.03%, ketorolac tromethamine and atropine 0.5%. Was considered the first moment of evaluation (MB = basal moment), the date of submission to medical consultation. The surgical procedure was performed in the MB or the next day. The postoperative evaluations were performed on days 3, 7, 15 and 30 (M3, M7, M15 and M30). At all time points, the patients underwent complete ophthalmic examination and the specific form filler modified according to Andrade et al. (2010). All patients receiving the sterile membrane PBAT. After routine preparation for ophthalmic surgery, the membrane was cut according to the size of the corneal defect and sutured using simple interrupted pattern non-penetrating, using nylon 8-0. Among the six animals, four were breed Shih Tzu (n=4, 66.67%), Poodle (n=1, 16.67%) and Lhasa Apso (n=1, 16.67%), aged 1 and 5 years (mean: 2.8 years). The causes of the ulcers were attributed to the presence of distichiasis (n=4, 66.67%), keratoconjunctivitis sicca (n=1, 16.67%) and chalazion (n=1, 16.67%). The blepharospasm and intense conjuntical hyperemia were observed until day 15. These events were reducing over time, being absent at 30 days postoperatively. It was observed the presence of mucous ocular discharge until the seventh day after surgery. It was quantitatively reduced in subsequent periods until being absent at 30 days. There was an intensification of corneal vascularization at 15 days after grafting of the membrane near the edges of the ulcer. This gradually decreased over time, but was present in all animals at the late period. At the end of the observation period, we observed the formation of a cicatricial leukoma in all animals, but one can assume that the animals no showed ocular discomfort. It was also observed return to corneal transparency of areas around the ulcer. It was not observed conjunctivalization and anterior synechia in all animal. PBAT membrane seems to be an efficacious alternative for the treatment of corneal deep ulcers in dogs. Further investigations are necessary, especially by adopting longer periods of observation.

Keywords: Dogs, corneal ulcers, membrane, nanofiber membrane, PBAT, polymers

Intermuscular Lipoma in Dog – Case Report

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The lipomas are benign neoplasms, common and mesenchymal origin, have an incidence of approximately 16%. Are classified as subcutaneous and subfasciais with intramuscular variants, intermuscular (rare) and infiltrative. Are more common in large breeds of dogs and old. The lipoma can be invasive, but not aggressive and does not produce metastasis to tissues and / or distant organs. Clinically, most lipomas are asymptomatic. The lesions appear as floating palpable mass consistency. However, in the presence of large tumor masses, the animals may have secondary symptoms such as discomfort or impaired motor function of the affected limb due to compression of adjacent structures to neoplasia. This study aims to report a case of lipoma in intermuscular dog in the forelimb. A dog, male, 8 years of labrador breed, was the Veterinary Hospital at the oncology sector, skin nodule history forelimb, with slow growth, measuring 10 cm. The animal limping on the left forelimb, which was committed by the tumor. Held haematological and biochemical tests, which showed no significative alterations, and chest radiograph, had no pulmonary metastasis. Held cytological examination of the lesion, the report was compatible lipoma, and so walked the patient for surgery. The protocol consisted of pre anesthetic medication intramuscularly (IM) with tramadol hydrochloride (4 mg / kg) associated with chlorpromazine (0.4 mg / kg). Fifteen minutes later, propofol was performed (5 mg / kg) administered intravenously for induction of the patient, and then proceded to tracheal intubation for maintenance of general anesthesia with isoflurane in 100% oxygen. With the anesthetized patient, there was antisepsis with chlorhexidine and 20% alcohol 70%. An incision that extended throughout the tumor extension was made, it was found that the tumor was intermuscular. So we procede to dilatation of the adjacent muscles, and capsule that enveloped. After complete resection of the tumor, the muscles were sutured with Sultan suture pattern with absorbable suture 2.0, dropped dead space with continuous intradermal suture with absorbable suture 3.0, and the dermorrafia with separate simple sutures with nonabsorbable suture 3.0. The material was sent for histopathological examination, the report was lipoma. After surgery was recommended cleaning the wound with saline solution. Ranitidine hydrochloride was prescribed (2mg / kg / BID, 10 days) and gastric protector, cephalexin (30 mg / kg / BID, 10 days) and antibiotic therapy, Tramadol hydrochloride (3 mg / kg / TID / 7 days) and Diripona (25mg / kg / TID, 7 days) as analgesia, Meloxicam (0.1 mg / kg / SID, 3 days) as postoperative antiinflamatória.O therapy progressed well, and ten days after surgery withdrew points. 60 days after surgery the patient was well, and had a medical discharge.

Keywords: Canine, intermuscular, neoplasiaresection

The Treatment of Complete Urethral Obstruction and Bladder Stones with Pneumatic Lithotripsy in a Dog: Preliminary Case Report

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In this preliminary case presentation, use of the minimally invasive cystoscopic pneumatic lithotripsy technique in the treatment of complete urethral obstruction in a dog has been described. The animal material of the case comprised a 5-year old Chihuahua presenting with difficulty during urination (dysuria, stranguria), inappetence, lethargy and inability to urinate for the previous 2 days. Post-renal azotaemia and haematuria was determined in the patient. Physical examination of the dog revealed normal body temperature, heart rate and respiratory rate. Distension and pain in the caudal abdomen together with a full urinary bladder was determined upon abdominal palpation. It was observed that the dog frequently adopted the urinating position but was unable to urinate. A full bladder was seen in direct radiographs with no other abnormalities detected. In ultrasound examination, structures presenting acoustic shadows were identified in the bladder and proximally to the os penis. The urethral catheterization attempt after decompressive cystocentesis was unsuccessful. Therefore, it was decided to perform cystoscopy in the dog. Bladder and urethral stones causing complete urethral obstruction were visualized via cystoscopy, fragmented with a pneumatic lithotripter and the stone fragments were removed using the voiding urohydropulsion method. The pneumatic lithotripsy method was successfully used in the treatment of complete urethral obstruction and fragmentation of bladder stones. The authors recommend the pneumatic lithotripsy method as a useful, low-cost, practical and minimally invasive technique in the fragmenting of urethral and bladder stones.

Keywords: Bladder stone, cystoscopy, dog, pneumatic lithotripsy, urethral obstruction

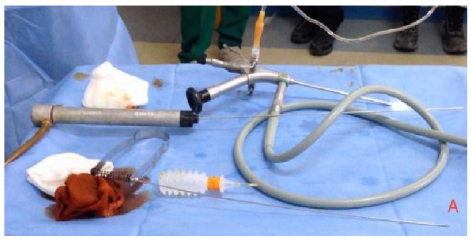

Figure 1. Pneumatic lithotripter and rigid endoscope

Figure 2. Pneumatic lithotripter unit

Be Familiar with Cytopathological Procedures - the Analysis of the Cooperation between Clinician and Cytopathologist

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Cytopathology is a quick, cheap, low-invasive and widely available method of examination of the cells collected from living patients, which allows for obtaining the diagnosis with regards to the main pathological process taking place within the examined tumor. Microscopic examination of the collected smears enables getting correct cytopathological diagnosis even when it is conducted in the ordinary veterinary practices by the non-experienced person. However, in some cases collected samples have to be stained and examined by the specialist. Still many veterinary practices do not have and adequate equipment (e.g. microscope) which is necessary to perform the examination. In many cases clinician collects samples from the patient with detected lesion and sends them to the laboratory. It happens due to the lack of conditions to exam them or because he/she prefers the diagnosis made by the experienced cytopathologist. The aim of the presented study is to show factors (critical points) that can affect the correct cytopathological diagnosis obtained during the microscopic examination of the slides collected by the clinician and examined by the cytopathologist. The study was performed on the basis of the analysis of 100 cases of cytopathological examinations of the smears sent by private practicioners to the Laboratory of Pathomorphology at Warsaw University of Life Sciences and examined by the cytopathologists. Conducted analysis includes the factors depending on the person collecting and sending the sample and that can affect clinical usefulness: the quality of cover letter, the number of smears and macroscopic quality of smears. The results of the microscopic examination of smears analysed in the presented study considered to be nondiagnostic, descriptive and suggestive were obtained in 27%, 14%, and 20% of cases, respectively. In 39% of the cases results of cytopathological examination were considered as cytopathological diagnoses. Dog samples included 80% of the collected material and cats - 20%. Clinical usefulness of the cytoreport was strongly correlated with the macroscopic quality of the smears and the relevance of the cover letter. Close cooperation between clinicians and pathologists is recommended, however the most important factor that allows proper use of cytopathology in veterinary practices is being familiar with biopsy techniques and smear preparation and fixation.

Keywords: Biopsy, cooperation, cover letter, cytopathology, microscopic examination, smear

Urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) Levels of Dogs in Pyoderma

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In this study, it is aimed to investigate correlation between antioxidative metabolism and the significant skin disease pyoderma in dogs and to demonstrate the extent of oxidative damage with 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. Healthy (n=13), females diagnosed as pyoderma (untreated) (n=16), healthy (n=14) males and males diagnosed as pyoderma (untreated) (n=12) consisted the four study groups. In this aspect two control and two diseased groups were formed according to gender. Blood and urine samples were obtained from all the groups and bacterial swabs were collected from the diseased group well. Plasma malondialdehyde (MDA) besides urine 8-hydroxy-2'-deoxyguanosine levels measured with LC/MS/MS. MDA and 8-OHdG levels were increased in diseased groups. Differences among groups were found to be significant ($P \leq 0.05$). While important differences in 8-OHdG levels between the control group and pyoderma group was not observed in female dogs; 8OHdG levels were found significantly higher in pyoderma group than control group in male dogs ($P \leq 0.01$). The difference between the groups in terms of MDA in male dogs were not statistically significant ($P \geq 0.05$). When the results were analyzed, in pyoderma dogs compared to the healthy group, statistically meaningful high 8-OHdG level was detected ($P \leq 0.05$). The increase indicated in 8-OHdG levels which is a product of oxidative damage is considered as an indicator of oxidative skin damage at the organism caused by pyoderma. Again the significant increase ($P \leq 0.05$) in MDA levels at pyoderma dogs support this result. This study was carried out in pyoderma dogs between pyoderma and 8-OHdG levels in urine, which is a product of oxidative DNA damage is seen to be particularly serious correlation. In female dogs because protective factors play a role or several roles an increase is not recorded but in male dogs an increase in 8-OHdG levels is recorded. In conclusion in our opinion untreated pyoderma cases could result in DNA damage.

Keywords: 8-OHdG, dog, MDA, oxidative stress, pyoderma

Chitosan in Minimally Invasive Plate Osteosynthesis Dogs of Tibia

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Minimally invasive plate osteosynthesis (MIPO) has become widely accepted for the treatment of fractures in small animals, especially because it preserves the initial hematoma and minimizes the damage to the soft tissues. Biomaterials using is another way to improve bone healing. The chitosan (CHI) is good example of biomaterial used to that proposal. The lack of research using this treatment encouraged us to develop this project. Ten dogs presenting a tibial fracture referred to Teaching Veterinary Hospital (Unesp – Jaboticabal) were used. They were divided into 2 groups: group 1 (nothing was injected) and group 2 (injection of chitosan). The animals were evaluated for walking, swelling intensity, radiographs changes and bone densitometry at times 0 (M0), 15 (M1), 30 (M2), 60 (M3), 90 (M4) and 120 (M5) days postoperatively. From all animals, 50% were younger than 12 months of age, 55% were mixed breed, 45% of various breeds and 55% males and 45% females. The shaft was the most affected tibial region (85%). There were not statistical differences in swelling, deambulation, location of periosteal callus, presence of bone bridge, restoration of corticals, remodeling and volume of bone callus, and scale of radiographic evaluation between groups. There was a statistical difference to the radiopacity of the fracture line at M1 (15 days), being more radiodense the CHI group. There were not statistical differences in healing time in the group 1 happened with 63 days and group 2 with 66 days. The bone mineral density of the periosteal callus showed no significant statistical difference between the groups in the evaluation in the craniocaudal and mediolateral projections. There was also no difference in density among the moments. CHI provide osteoconductive proteins that serve as a matrix for epithelial migration and subsequent new bone formation and improve the function of polimorfonuclears leukocytes, macrophages and fibroblasts. The chitosan associated with minimally invasive osteosynthesis of the tibia is biocompatible and help in bone regeneration in dogs.

Keywords: Biomaterials, biology of the fracture, bone regeneration

Syringomyelia with Prosencephalic Tumors Concomitance in Three Dogs

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The aim of study is to show the cervical spinal cord syringomyelia with the concomitance of prosencephalic tumors in dogs. The material were three dogs: 16-month-old male French bulldog, 6–years-old male French bulldog and 9-month-old female Yorkshire terrier. They were chosen from the group of 43 dogs, who were University Clinic patients, in which cervical spinal cord was examined using MRI technique. Decision of MRI examination was made on the history: rising difficulties in movement, spastic paresis of hind limbs, cervical spine pain and hyperesthesia. Additional brain diagnosing was performed in these 3 dogs after the results of cervical spine examination. It was performed under general anesthesia using a low-field MRI scanner with magnetic field intensity of 0.25 Tesla (Esaote) in three basic planes: sagittal, transverse and coronal, T1-weighed and T2-weighed sequences and postcontrast T1-weighed (Magnevist - paramagnetic contrast agent). Among all 43 examined dogs there were 6 cases of syringomyelia. Additional brain tests demonstrated Chiari-like malformation in two of them, advanced hydrocephalus in one and prosencephalic tumors in three cases. In two cases tumors coexisted with a slight enlargement of the lateral ventricle of the brain and its displacement. Among these three dogs with concomitance of syringomyelia and prosencephalic tumors one was euthanized. Unofrtunately, contacting the owners of another two was impossible. Euthanized dog, 6-years old French bulldog, exhibited advanced neurological signs, e.g. seizures. In further MRI examination, malignant lymphoma of the central nervous system was diagnosed in this patient. In syringomyelia the oppression of neurons is a result of pathological cavities formation within the spinal cord. This should be distinguished from the expansion of the central channel – hydromyelia. Until recently it was thought, that the collected fluid is a cerebrospinal fluid, but recent studies have shown that its composition is not identical. Most authors agree, that the formation of syringomyelia is associated with the difficulty in cerebrospinal fluid flow – usually at the foramen magnum area. The changes can coexist with the other congenital anomalies, such as: hydrocephalus, Chiari syndrome and syringomyelia (SM). They are most commonly reported in brachycephalic breeds including Cavalier King Charles Spaniel and in this case lesions appear secondary to Chiari-like malformation (CM). This study revealed the presence of syringomyelia accompanied by prosencephalic tumors. Few cases of syringomyelia coexistence with intracranial lesions such as tumors were reported, but only one with prosencephalic one and significant additional hydrocephalus.

Keywords: Dog, prosencephalic tumor, syringomyelia, MRI

Pseudocyst of Hydronephrotic Kidney in a Cat

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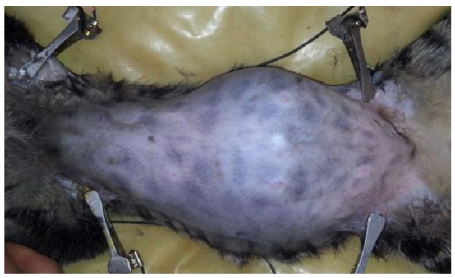
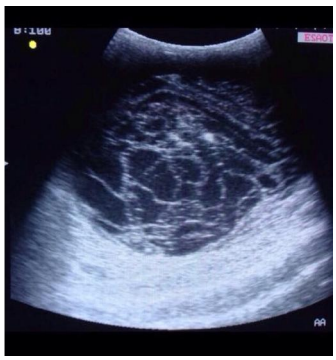
A five and a half months old female cat was referred to Ankara University Faculty of Veterinary Medicine for abdominal distention and constipation. History indicated that cat has always been very active by jumping around in the house and when the abdominal distention formed is unknown. On the physical examination the patient was bright, mucous membranes were pink and TPR, appetite, and urination were normal. Results of CBC and serum urea and creatinine indicated no abnormalities. On abdominal radiographic examination, a mass covering more than half of abdomen with soft tissue opacity was determined. On ultrasonographic examination, a cystic structure of mass was revealed while abdominal organs could not be visualized. The mass was anechogenic and was consisted of cavities. This mass was thought to be an abscess; therefore, an emergency exploratory laparotomy was performed by median incision. The mass was identified in the right side of the abdomen under the liver. Aspiration of mass by a needle attached syringe was performed and 4 ml of fluid was obtained. The mass isolated from the surrounding tissues and vascular structures. Left kidney was not observed while right kidney was present. Moreover, a tubular component from the mass ending into the vesica urinaria was determined; therefore, the mass was decided to be hydronephrotic left kidney. A left nefrectomy was performed. There were no other abnormalities observed in the abdomen. The incision closed routinely with simple interrupted sutures. Analysis of the fluid obtained from mass during surgery indicated that the specific gravity was 1.015, pH was 6.5, and protein and blood were present, but neither parasites nor ova were determined. Post-operative day one urine analysis indicated that specific gravity and pH were 1.030 and 6.5, respectively. Mild inflammatory cell infiltration in the development of cystic fibrous stroma and hemorrhage induced pseudo cyst were determined in the left kidney on histopathologic examination. The cat is healthy after 5 months of the operation.

Keywords: Cat, hydronephrotic kidney, pseudocyst

Abdominal Distention kidney ultrasonography



The mass covers more than half of the abdomen



Anechogenic mass consisting of cavities

Case Report: Ventricular Septal Congenital Defect in a Dog

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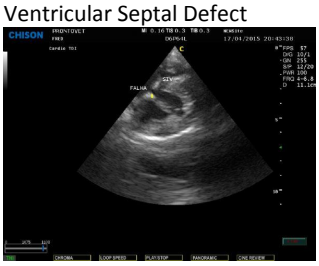
The objective of the study was to describe the clinical, laboratory and echocardiography images of a dog with ventricular septal congenital defect (VSD) and the relationship between clinical signs and laboratory and echocardiographic information as well as the therapeutic procedures. A dog with seven months old, male, Dachshund breed, weighing 6,150 Kg, presented lack of appetite, vomiting, pale oral mucosa, cardiological examination revealed systolic regurgitant murmur at left apex, arterial blood pressure 22/16 mmHg. Diagnostic techniques were performed such as blood and urine exams, ultrasonography and echocardiography. The kidney insufficiency was confirmed by biochemical exams performed in blood, with levels of creatinine of 5.7 mg/dl and urea of 243.8 mg/dl. The urine exams showed the presence of 5 kidney epithelial cells per field and the formation of granular cylinders.The ultrasonography showed kidney alterations and hepatomegaly.The echocardiography exam showed a inlet ventricular septal defect and that there was an important enlargement in left cardiac chambers.The dog received the follow drugs and dosage: aminoacids plus cetoanalogs (Ketosteryl 1 tablet/5kg twice a day), ranitidine (2mg/kg twice a day), ondansetron (0.22mg/kg twice a day); maropitant (1 mg/kg once a day), benazepril (0.5 mg/kg twice a day), carvedilol (0.6mg/kg once a day) and spironolactone (2mg/kg twice a day). The development of the disease in the patient was progressive and acute with the uremic condition worsened, showing signs of blood vomiting and seizures due to blood urea intoxication, resulting on the death of the dog 4 days later of clinical exam.Considering the age, and the clinical and exams results, the kidney insufficiency was due to the early low cardiac debit, creating low blood pressures. The response of the kidney was to liberate renin that promotes the release of angiotensin 1 and angiotensin 2, leading to vessel constriction and the release of aldosterone improving cardiac debit. The aldosteron retains sodium and water creating systemic hypertension, but the debit continues to decreases, for it, turns itself in a vicious cycle. The high level of blood pressure promoted kidney failure and was responsible by presence of kidney epithelial cells in urine samples and high levels of creatinine and urea. In the echocardiography exam was observed a inlet VSD type because they could be located anywhere in the ventricular septum under the mitral and tricuspid valve leaflets. This is a type of endocardial cushion defect and usually this kind of defect solves itself. However, in this case the VSD proved to be causing enlargement of cardiac chambers, leading to a low cardiac ejection, decreasing the debit. Even with all the drugs administrations it was not possible to decrease the blood pressure, that was harmful to the glomerulus, generating irreversible tissue damage, creating a situation where urea became circulant in blood vessels, hurting tissues all over the organism, such as mucous and serous tissues of the stomach and intestines leading to blood vomiting and also seizures caused by urea intoxication and probably brain edema. Despite clinical and laboratory information have directed to VSD, the definitive diagnosis could be possible only because the echocardiogram exam was realized. Subclinical cases of VSD could cause confusion regarding only the clinical and laboratory information. In this situation the definitive diagnostic using echocardiogram exam was essential for an accurate diagnosis.

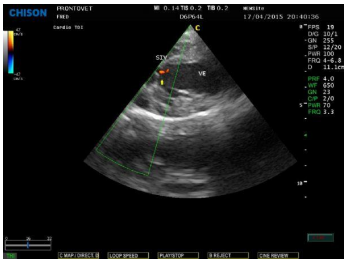
Keywords: Echocardiography, ventricular septal defect

Ventricular Septal Defect



Inlet ventricular Septal defect, in diastole.

Ventricular septal defect with collordopper



Collor doppler in diastole showing the blood flux from left to right, trought the inlet septal defect.

Medical and Surgical Approach to Cutaneous Histiocytoma in a Dog

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The aim of this case report was to evaluate aggresive growth of cutaneous histiocytoma, its response to surgical excision and medical therapy with systemic corticosteroid administration in a dog. An 11 year-old, male Boxer dog was brought to the clinics with 1 month history of a solid subcutaneous mass at dorsal adjunction of the left ear to the head. Initial therapy was surgery because of the rapid growth of tumor. Histopathological examination revealed cutaneous histiocytoma. Three months later, ulcerative mass reoccurred close to the same region and on dorsal surface of the left auricula. At the second surgery, histopathological examination revealed the same tumor type. Six months after first surgery, tumor reoccurred on the left dorsal auricular surface aggresively. There were two more masses, one on the right medial femoral region and the other near parotis region. At this time, medical therapy with metilprednisolone (1 mg/kg, BID, PO) was preferred for 2 weeks. At the end of 15th day, the tumor on the leg was observed to disappear completely. The other tumors on auricula and head were smaller. Therefore, dosage of metilprednisolone was reduced, but tumor tissues had grown aggresively one week later, and corticosteroid administration was begun with the initial dose again. One week after this administration, the dog was brought to clinic with weakness, dispnea and severe anemia. Despite all interventions, dog has died and necropsy was done. This case showed that cutaneous histiocytoma could grow aggresively especially after surgical removal. Corticosteroid treatment was more effective in inducing remission of tumor tissues. However, side effects of large dose corticosteroids might be lethal in geriatric dogs.

Keywords: Cutaneous histiocytoma, dog

Addison Disease Accompanied by Severe Skin Crusted Wounds in a Cat

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A ten years old, 4 kg, female Ankara cat was referred to Ankara University, Faculty of Veterinary Medicine, Department of Internal Medicine with sever skin wounds that crusty and unresponsive to treatment whole its body. Other historical signs included Hyperkeratosis, lethargy, weight loss, muscle weakness and itchiness. TSH and free T4 was normal and Cortizol (7.5 ug/dl; reference interval: 1.5-6.5 ug/dl) and T-3 (77 ng/dl; reference interval 15-60 ng/dl) was high. skin scrapings were taken and at microscopic examination no evidence of any skin parasite revealed. 0.4 cm diameter punch biopsy was taken from the skin from head and hind leg. In the biyopsy was taken from hind leg degenerative hair and sebaceous glands with large areas of bleeding and inflammatory changes were observed in the dermis (nonspecific dermatitis and bleeding) and In the biyopsy was taken from the head hyperkeratosis was detected. The results of a complete blood count including white blood cell count 6.3_ 109/l (reference interval:5.5-19.5 _ 109/l), red blood cell count 7.65 _ 1012/l (RI 5.00-11 _ 1012/l), haemoglobin 11.9 g/dl (RI 8.0-15.0 g/dl), haematocrit 29.2% (RI 25-45%) and platelet count 32_ 109/ml (RI 200-500_ 109/l), and The results of serum biochemical analysis including blood glucose 127.2 mg/dl (RI 70-110 mg/dl), blood urea 41.3 mg/dl (RI 15-64.2 mg/dl), creatinine 0.59 mg/dl (RI 0.8-1.8mg/dl), aspartate aminotransferase 16.1 U/l (AST; RI 26.0-43.0 U/l), alanine aminotransferase 26 U/l (ALT; RI 6.0-83 U/l), alkaline phosphatase 43.3 U/l (RI 25.0-93 U/l), total bilirubin 0.05 mg/dl (RI 0.1-0.2 mg/dl), total cholesterol 200 mg/dl (RI 95-130 mg/dl), total protein 7.4 g/dl (RI 5.4-7.8 g/dl), sodium 148.8 mmol/l (RI 146-159 mmol/l), potassium 3.7 mEq/l (RI 3.8-5.3 mEq/l), Creatine kinase 135.0 IU/l (RI <=130 IU/l), calcium 10.8 mg/dl (RI 6.2-10.2 mg/dl). sodium-to-potassium ratio was 40.2. Hypercalcaemia, hypokalaemia and hypophosphataemia was detected. Beacuse of many antibiotics, antihistamines application and one year treatment probably the results of the blood analysis did not match with the classic symptoms of the disease. Beacuse of high cortizol, We performed low dose dexamethasone test but befor and after dexamethasone cortizol was low (<1.0 ug/dl). Then we apply the ACTH stimulation test and as a result befor and after ACTH cortizol was low (<1.0 ug/dl). Addison (hypoadrenocorticism) was diagnosed as a result of this assay. As therapy oral Prednisolone and Famotidine was applied. After one week itching has stopped and wounds begans to heal. After one month gait and behavior of cat was recovered, returned to normal and gained weight.

Keywords: Addison, cat, skin, wound

Survey on External Ear Canal Fungal Flora in Clinically Healthy Dogs, in Urmia, Northwest Iran

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External otitis exists in 5-20 percent of normal dogs. External ear canal fungal flora can change because of numerous anatomical, senile, behavioral, environmental, sanitary condition and sex parameters. The change of fungal flora of external ear canal, from saprophyte to disease causing form, has considered as a causative agent of dogs otitis. The present study has designed and conducted to evaluate the fungal flora of the external ear canal of clinically healthy dogs referred to Urmia branch, Islamic Azad University's small animal clinic. The study was conducted from April 6 up to the late July 2010. The samples of 96 dogs were gathered through sterilized swabs from the junction point of vertical and horizontal canals of the external ear. The subjects included 59 male and 37 female dogs which ranged from 0-6 years and have no signs of the external ear involvement. The samples were examined by direct smear microscopy, plate culture, slide culture and germ tube test examinations. Results: Seventy one out of 96 (74%) samples were infected to different gena and species consisting of Aspergillus spp (45%), Candida albicans(8%), Alternaria spp(7%), Geotrichum spp(6%), Scopulariopsis spp(3%), Pseudoallescheria boydi (3%)and Penicillium spp(2%). Statistical analysis showed no significant difference about isolated species of the left and right ears but there were significant differences about all of other studied parameters ($P<0.05$). Conclusion: According to the results of this study, it can be concluded that the isolated fungi in 1-3 year age group, male dogs, pendulous form ears, bad sanitary condition, and outdoor housing type were more prevalent compared to other age groups, female ones, erect form ears, good sanitary condition and indoor housing type, respectively.

Keywords: Dog, external ear canal, fungal flora, Iran, Urmia

Changes in the Hip Joints in Cats up to 36 Months of Age

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The aim of this study was to show the frequency and variety of changes in hip joints in young cats. In the present report the material consisted of 46 cats whose hip joints were examined with X-ray technique. Various types of anomalies were found on the radiographs. Cats presented in the study ranged from 6 to 36 months of age, both males and females, belonging to the different breeds. The largest group was Domestic Shorthair – 19 individuals, British Shorthair - 11, Maine Coon - 10, and others - Siamese, Persian, Norwegian Forest. The reason for further diagnosing in these cats were different levels of walking difficulties. In 42 cats X-ray image was performed under general anesthesia. Cats were only subjected to a single study. Ventro-dorsal view of the extended hip and knee joints (OFA view) was performed in 38 cats. These studies were performed by direct digital radiography (Medical ECO NET, Germany). Test parameters were 55-68kV and 12mAs. During femoral head amputation surgery, samples were taken for histopathological examination. It helped to differentiate slipped capital femoral epiphysis (SCFE) from post-traumatic exfoliation of femoral epiphysis. Among the cats diagnosed with fractures, Salter-Harris fracture type I of the femoral head was found in 15 individuals; bilateral hip dysplasia in 13; lateral dysplasia in 9; SCFE - confirmed by histopathological examination of the growth plate in 8 (in 3 changes were bilateral); hip luxation in 6; osteoarthritis in 3; neoplastic changes in 1. Legg-Perthes disease was not diagnosed in the studied cats. The earlier the disease is diagnosed, the better the prognosis for the affected joint is. Proper care of cats is associated with taking care of joints development. In young animals common cause of movement disorders are injuries. The most common changes in the tested cats were fractures and luxations which have occurred as a result of trauma. The changes were most frequently diagnosed post-traumatically in Domestic Shorthair breed. Almost as often, however, developmental disorders such as hip dysplasia or SCFE were found. Hip dysplasia occurred the most often in the Maine Coons and SCFE in the British Shorthairs. No cases of Legg-Perthes disease may result from a double femoral head vascularity in cats. In such young animals only single cases of joint degenerative changes were diagnosed. Radiographs obtained from one cat showed the changes in one hip joint suggesting neoplastic process.

Keywords: Cats, dysplasia, fracture, hip joint, luxation, X-ray

Acoustic Radiation Force Impulse (ARFI) Elastography in Healthy Canine Pancreas - Results Preliminary

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To describe the technique of the ARFI elastography the evaluation of the pancreatic tissue of healthy dogs, in order to establish the qualitative and quantitative patterns have not yet described in veterinary medicine. This preliminary study used three healthy dogs, Beagles, with a mean age of 4 years of life, belonging of the Animal Nutrition Laboratory of the Faculty of Agricultural and Veterinary Sciences - UNESP / Jaboticabal, Brazil. The selected animals were considered healthy after clinical evaluation and the results of laboratory tests (blood count and biochemical profile). The animals were submitted to conventional ultrasonography and elastography of the pancreas using ACUSON S2000 system / SIEMENS and with linear transducer (matricial and multifrequency) of 9.0 MHz. By B-mode ultrasonography were evaluated in transverse and longitudinal sections the echotexture (homogeneous or heterogeneous) echogenicity (anechoic, hypoechoic, hyperechoic or mixed) and the size of the board in its different portions (right and left lobe and body). Using specific software for ARFI elastography, the pancreatic tissue was evaluated by qualitative and quantitative method, determining the elastogram (images formed to grayscale of different pancreatic portions, assessing the relative tissue stiffness) of the tissue evaluated, the presence or absence of tissue deformity and the values of shear velocity (m /s) (were obtained at least five sampling of the quantitative technique) of the various portions of the pancreas. In all patients evaluated at B-mode ultrasound examination, the pancreatic parenchyma showed homogeneous echotexture, preserved echogenicity and dimensions. By qualitative ARFI elastography it was verified that pancreatic tissue of animals presented itself homogeneous, non-deformable and with greater stiffness than the tissues adjacent (mesentery or omentum). At the quantitative ARFI method were obtained mean values for the shear velocity of 0.95 m / s in all evaluated portions. Quantitative and qualitative ARFI elastography of the pancreas in healthy dogs was easily implemented in preliminary study, demonstrating that this technique can be used to conduct further research about the pancreatic stiffness in animals and evaluating its applicability in diseased animals.

Keywords: Dogs, elastogram, ultrasonography

Risk Factors of Canine Dementia

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The increasing prevalence of cognitive impairment in an aging canine population poses a serious health problem. Identifying modifiable risk factors which may influence the onset of cognitive decline is becoming increasingly important. Here we investigated whether age, sex, weight, nutrition, dogs' keeping and reproductive state are associated with increased risk of canine dementia. The study was designed as a cross-sectional study. Two-sample Z-test of log odds was applied for evaluation of risk factors. We found that sex, weight, reproductive state and dogs' keeping were not significantly correlated with cognitive decline. Nutrition emerged as a significant predictor variable. We demonstrate that dogs fed balanced diets had 2.8 times lower chance of developing dementia when compared with dogs fed home-made or other uncontrolled diets. Finally, we found that prevalence of canine dementia was similar in both small and medium/large dogs in the age period 8–11years (13 % vs 16 %), however it differed in older dogs, 11–13 years old (41 % vs 55 %). Clinical Significance Nutrition and age were found to be the most prominent risk factor of canine dementia. These findings may provide new insights into nutritional interventions that can modify canine brain ageing processes.

Keywords: Canine dementia, cognitive decline, epidemiology, risk factor, prevalence

Treatment of Caudal Coxofemoral Luxation with Toggle Pin Technique in an English Pointer

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Coxofemoralluxation is a common traumatic disorder that accounts for 90% of all joint luxations in dogs. The most common cause of hipluxation is trauma, with vehicle accidents accounting for approximately 60%. The majority of hipluxationsarecraniodorsal, probably cause of the type of injury and contraction of the gluteal muscles, which are strong extensors and abductors of the hip joint. Ventrocaudal displacements, where the femoral head may lodge within the obturator foramen, occurs less frequently. This type of luxation is often a result of a fall. The hipmayalsoluxatemedially, in associated with an acetabular fracture. The patient, six months aged English pointer, was admitted to the clinic of Surgery Department, Ankara University Faculty of Veterinary Medicine with history of lameness caused by a traffic accident. After clinical and radiographic examinations, caudal coxofemoralluxation was diagnosed. Patient was operated with toggle pin technique. The toggle pin itself serves as a bone anchor by locking against the medial wall of the acetabulum. Post-operative radiographs were taken at 10th and 45th days after the operation to control the status of the hip joint. After the follow up process, treatment was successful.

Keywords: Luxation, pin, toggle

Immunohistochemical Consideration of Lymphocyte Types Infiltrate into the Canine Seminomas

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Seminoma is frequently observed in human and canine testes especially in cryptorchids. Canine and human seminomas are typically associated with leukocytic infiltration. We aimed to identify the type of lymphocytes that infiltrate into seminomas. The materials consisted of formalin-fixed paraffin embedded tissue blocks of five formalin fixed, testicular canine tumors, all of which were diagnosed as diffuse seminomas. Tumor infiltrating lymphocytes were evaluated by immunohistological techniques in 5 dogs with diffuse seminoma. Routine pathological examination of these specimens showed diffuse seminoma with tumor infiltrating lymphocytes in all cases. Lymphocytes in seminomas were predominantly T cytotoxic (CD3+, CD8+) cells. These cells were distributed diffusely, but patchy around the vessels. B-cells were also identified as some rare single cells diffusely scattered in the tumoral parenchyma. The results of this study showed that the lymphocyte infiltrating cells are mainly T lymphocytes particularly CD8 cytotoxic type. These findings, associated with the prevalence of cytotoxic CD8+ lymphocytes, suggest that in canine seminomas inflammatory cells play an active anti tumoral role.

Keywords: Immunohistochemistry, T lymphocyte, tumor

A Coinfection Model of Classcical Swine Fever Virus and Porcine Circovirus Type 2 *In Vitro*

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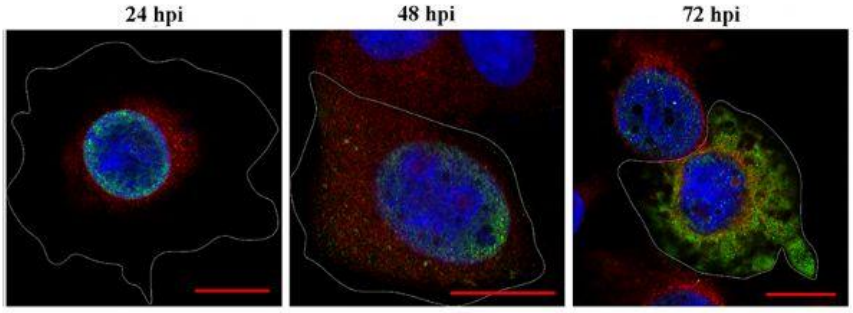
Increasing clinical lines of evidence have shown the coinfection/superinfection of PCV2 and CSFV. Here, we aimed to develop an in vitro coinfection model system in which PCV2 and CSFV could infect the same cell productively. PK15 cells were inoculated with CSFV or PCV2 for 72h for virus titration. Cells inoculated with the CSFV C-strain were subcultured continuously and random passages were selected to detect CSFV-positive rate (flow cytometry) and virus titration (TCID50 and absolute real-time PCR). Proliferation and apoptosis of cells harboring CSFV were tested using CCK-8 assay and TUNEL assay, respectively. Virus subcellular localization were observed using confocal laser scanning microscopy (CLSM). Then virus coinfection rate in cells were measured by counting the numbers of positive cells under microscopy. PK15 cells could be infected with PCV2 and CSFV, and had productive progeny viruses. So, PK15 cells were selected to establish cell lines harboring replicating CSFV named PK15-CSFV. After serial subcultured, passages of PK15-CSFV remained relatively stable in virology (by flow cytometry and virus titration) and in cytobiology (by CCK-8 assay and TUNEL assay). Using CLSM, CSFV and PCV2 signals could be detected simultaneously in PK15 cells (FIG. 1). Dynamic analysis in PK15 showed PCV2 could fulfill an entire life in CSFV-infected cells, but the localization of CSFV E2 were abnormal, probably as the result of PCV2 infection. In both PK15 and PK15-CSFV, the data of coinfection efficiency of PCV2 showed that the percentage of cells infected with PCV2 was dose-dependen, and there was no difference in percentage of PCV2-positive cells in PK15 cells and PCV2-CSFV dual-positive cells in PK15-CSFV cells ($P > 0.05$), indicating that the infection efficiency of PCV2 in PK15-CSFV was similar to that of PCV2 in PK15 cells. Our results demonstrated the coinfection/superinfection of PCV2 and CSFV within the same cell, and provided an in vitro model to facilitate further investigation of the underlying mechanism of CSFV and PCV2 coinfection.

Keywords: Coinfection model, classical swine fever virus, porcine circovirus type 2

FIG. 1 Subcellular localization of viral proteins in PK15.



Cells coinfected with PCV2 and CSFV. Cells were inoculated with PCV2, fixed, immunostained for PCV2 Cap (green) and CSFV E2 protein (red). The red bar represents 10 μm.

Prevalence of *Salmonella* spp. in exotic small mammals imported as pets to Japan

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Every year, a lot of exotic small mammals of various species are being imported as pet to Japan. In order to prevent the introduction of pathogens carried by these mammals, to Japan, the Infectious Disease Control Law in Japan was revised in 2005, and a health certificate issued by the exporting country became mandatory when importing those animals. However, only seven infectious diseases are regulated by the law and other infectious diseases such as salmonellosis are excluded, and prevalence of various pathogens in imported exotic small mammals is unknown. This study was carried out to investigate the prevalence of *Salmonella* in such mammals, immediately after arrival in Japan, and in those already kept as pets for a certain period of time in Japan. During the period from 2003 to 2012, a total of 701 fecal samples of imported exotic small mammals, just arrived in Japan, consisting of 30 species of 3 orders, and 65 fecal samples of sugar glider (*Petaurus breviceps*), which have been kept as pets in Japan, were collected. The fecal samples were suspended in 2 ml of sterile saline, and 1 ml of the suspension was inoculated into 10 ml of buffered peptone water (BPW). After incubation at 37°C for 24 hr, 1 ml of cultured BPW was transferred to 10 ml tetrathionate broth. The broth was incubated at 37°C for 24 hr. Desoxycholate hydrogen sulfide lactose agar (DHL), Mannitol lysine crystal violet brilliant green agar (MLCB), ES *Salmonella* agar II (ES II) were used as isolation medium. Isolates were identified by biochemical tests and serotyped, and antibiotic susceptibility were determined by the disc diffusion method. *Salmonella* was isolated from 77 (11.0%) of 701 fecal samples of the imported exotic small mammals, just arrived in Japan, comprising 11 of the 30 species analyzed. *Salmonella* were isolated from 38 (6.0%) of 631 Rodentia, 32 (53.3%) of 60 Marsupialia (sugar glider), 7 (70.0%) of 10 Insectivora (long-eared hedgehog; *Hemiechinus auritus*). Isolation rates of *Salmonella* in imported Rodentia before and after the review of the law were 5.3% (27/508) and 8.9% (11/123) respectively. The isolates were identified into 22 serovars, and included *S.* Enteritidis, *S.* Typhimurium, *S.* Litchfield, *S.* Newport, that are the serovars usually associated with human gastroenteritis in Japan. Most isolates were susceptible to the 11 antibiotics used in this study. Fourteen isolates from 7 sugar gliders and 7 long-eared hedgehogs were resistant to 2 or 5 antibiotics. On the other hand, *Salmonella* were isolated from only 3 (4.6%) of 65 fecal samples of sugar glider kept in Japan. Interestingly, this isolation rate was significantly lower than of those ones that have just arrived in Japan (53.3%). Prevalence of *Salmonella* in imported exotic small mammals was 11.0%. The isolation rates differed according to the species and origin, and isolation rates of Rodentia did not decreased after the changes in the law. Some strains were multidrug resistant, and imported exotic small mammals might play an important role as a carrier of *Salmonella*. This result suggest that they might to be a potential source of infection to humans and the importance of continuous monitoring of the prevalence of *Salmonella* of imported exotic small mammals. Isolation rate of *Salmonella* in sugar gliders kept as pets in Japan were significantly lower than that in imported ones. This result indicates that prevalence of *Salmonella* might decrease according to the period living in Japan.

Keywords: Exotic small mammal, salmonella

Leptospirosis in Humans and Animals in Turkey

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In this study, it is aimed to determine the dissemination of Leptospirosis and the Leptospira serotypes that causes disease in humans and animals. As a material, 3,521 human and animal blood serum samples that sent to our laboratory in the year of 2014 were tested with Microscopic Agglutination Test (MAT, OIE 2008). Animal serum samples were tested with L. grippotyphosa Moskva V, L. bratislava Jez Bratislava, L. canicola Hond Utrech IV, L. hardjo Hardjoprajitno, L. pomona Pomona, L. icterohaemorrhagiae Ictero RGA serotypes and human serum samples with the abovementioned serotypes as well as with L. hebdomadis Hebdomadis ve L. patoc Patoc I. Of all 56 human patients' serum samples sent to our laboratory for the diagnosis of Leptospirosis, 34 were found negative and 22 positive. The positivity detected provinces are as follows: Rize (9), Zonguldak (4), Ankara (3), Bursa (3), Adana (2) ve Istanbul (1). In total 286 horse serum samples sent to our laboratory and from these samples 215 were found negative and 71 positive. The positivity detected provinces are as follows: Malatya (29), Eskisehir (14), Istanbul (8), Sanliurfa (8), Sakarya (5), Bursa (4), Adana (1), Izmir (1), Kocaeli (1). Of all 2,556 cattle serum samples 1,590 were found negative and 966 positive. The positivity detected provinces are as follows: Adana (813), Kırklareli (112), Gaziantep (19), Konya (7), Edirne (4), Samsun (3), Bursa (2), Afyon (1), Ankara (1), Eskisehir (1), Istanbul (1), Sanliurfa (1), Yozgat (1). Of all 623 sheep and goat serum samples 597 were found negative and 23 positive. The positivity detected provinces are as follows: Bursa (9), Balikesir (7), Konya (7), Izmir (2) ve Kirklareli (1). When the results of the study are evaluated, it can be seen that Leptospirosis is widespread in Turkey.

Keywords: Leptospirosis, leptospira, livestock, MAT

Molecular Epidemiology of Canine Coronavirus in Ankara Dog Population

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Since the first identification of the virus in 1971, the disease caused by canine coronavirus (CCoV) has not been adequately investigated, and the role that the virus plays in canine enteric illness has not been well established. CCoV is an etiologic agent of diarrhea in dogs and is known to have spread world wide. Infection is usually characterized by high morbidity and low mortality, predominantly affecting dogs in kennels and rescue shelters. CCoV replication is mainly limited to the gastrointestinal tract and can eventually lead to death, particularly when co-infection with canine parvovirus (CPV-2), canine adenovirus type 1 (CAV-1) or canine distemper virus (CDV) are present. To date, two different genotypes of CCoV are known, CCoV type 1 and CCoV type 2. CCoV type 2 is divided in two subtypes, CCoV-2a (classical strains) and CCoV-2b, with CCoV-2b emerging as a result of a putative recombination between CCoV-IIa and transmissible gastroenteritis virus (TGEV). In this study, enlucidating not only the prevalance of CCoV, important disease of dogs, but also molecular characterization of the viruses with M and S genes based RT-PCR and sequencing were aimed. During this study 100 dogs were sampled. Samples were collected from dogs with diarrhea from kennels, pet shops and veterinary clinics of Ankara/TURKEY region between 2012 and 2013. Dogs were grouped according to the information collected from the questionnaires filled by the owners on age, breed, gender, housing, environment and vaccination status. These questionnaires revealed that CCoV infection was seen widespread in dog population (%40), diseases could be caused by virus regardless of clinical symptome, vaccination status, gender and housing (indoor /outdoor). Diarrhoea is the common clinical symptom of CCoV. The PCR results indicated that not only the M primers but also the S gene primers should be used in order to determine and genotyping of the CCoV. The sequence analysis pointed out the CCoV's (CCoV type 1 and 2) in Turkey, were genetically related to the other CCoV's worldwide. As a result we concluded that studies for determining molecular epidemiology of canine coronaviruses must be continuous; and these studies will contribute to the choice of commercial vaccines and help new studies for developing vaccines and contribute to healthy animal in several ways.

Keywords: Epidemiology, canine coronavirus, molecular, molecular phylogeny, RT-PCR

Isolation and Biotyping of Brucella Species In Abortions From Middle And Eastern Blacksea Region

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Brucellosis is a zoonotic infection showing chronic, contagious, necrotic and inflammatory reactions, which causes abortions and infertility with genital organs involvement including testes, mammary gland, uterus in cattle, sheep, goat, pig, dog and ram. Currently there are 10 Brucella species known in Brucella genus. Classification based on the host specifity include Brucella melitensis in sheep and goats, B. abortus in cattle, B. canis in dog, B. ovis in sheep and ram, B. neotomae in desert rat. Out of 6 species, there are further isolations including B. ceti in dolphins, pig, fish and whales, B. pinnipedialis in generally seals, B. microti in many rodents and foxes, which are unknown if they are pathogen for humans. Recently, B. inopinata has been isolated from human mammary implants. In the Brucella genus, there are 3 biotypes of B. melitensis and 7 of B. abortus and 5 of B. suis. In the present study, biotyping of brucella spp. strains from 9 different provinces (Samsun, Amasya, Tokat, Sivas, Ordu, Sinop, Giresun, Trabzon and Rize) have been carried out by Samsun Veterinary Control Institute Directorate in Middle-eastern Blacksea Region from year 2013 to 2014. Brucella spp were isolated from 275 of 2547 abortion materials. These isolates were sent to Pendik Veterinary Control Institute Directorate for biotyping. In conclusion, 76 B. abortus bt-3 (73 cattle, 2 sheep and 1 water buffalo), B. abortus bt-1 from 8 cattle, S-19 from 4 cattle, B.melitensis bt-1 from 1 sheep, B.melitensis bt-3 from 4 cattle and 150 sheep and goats, Rough B.abortus mutant strain from 2 cattle, B.abortus and B.melitensis mixed strain from 2 cattle, Rev-1 from 3 sheep and 24 contaminated strain from 24 specimens were detected. One of these strains could not re-isolated.

Keywords: Abortus, biotyping, brucella, isolation

A Pilot Study for Vaccinate to Free from FMD (In Regarding Regional and Territorial of FMD Control and Eradication Programme) in Ankara Province

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In many countries with endemic foot and mouth disease (FMD) or with frequent introductions of FMD virus, the control of the disease mainly relies on vaccination of cattle and other susceptible species. Control of FMD depends on the disease control policies and epidemiological status of the country. In countries where the disease is endemic, protective vaccinations are performed with inactive vaccines of suitable serotype and measures are taken in order to reduce the prevalence of the disease. In all over the Anatolia, serotypes A, O and Asia1 are endemic. In this study, all cattle, in 11 districts of Ankara province selected according to several epidemiological surveillance and statistical criteria, vaccinated with 6 PD50 oil adjuanted Foot and Mouth Disease vaccine in February, March (booster vaccination) and September, a total of 3 times, during and after the vaccination programme. Blood sera samples were collected from the NSP(-) cattle that selected by conveniente sampling method at 0, 30, 60, 180, 210 and 240 days and tested by LPB-ELISA, NSP ELISA. Obtained results evaluated together with field observations and number of the infected premises by apropriate statistical methods. This is the first study in Turkey using a high potency vaccine in various vaccination coverage of population. It was targeted that, results are going to lead control and eradication programmes of country and regionwide. It was evaluated that effect of two different potency levels of vaccines on population immunity. This is the first study on this topic, so it is going to contribute to the literature. The average annual number of FMD outbreaks (last five years) of the study carried out in 11 districts was thirty. Only 2 FMD outbreaks in Sincan and Gölbaşı were detected during the study. According to the test results; there was high and stabil vaccine potency against the three of FMD serotype (O, A, ASİA-1). Finally; FMD outbreaks were taken under the control as a result of scientific and administrative measures and animal movement restriction.

Keywords: 6PD50, Ankara, FMD vaccine, FMD vaccine's effectiveness, mass vaccination

The Distribution of Anthraks Cases Detected in Middle and Eastern Blacksea Region (2010-2014)

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Anthrax is one of important zoonotic diseases in Turkey. It is an infectious disease with septicemic and toxemic features caused by Bacillus anthracis. Anthrax, worldwide zoonotic disease, occurs mainly in sheep, goats, cattle and water buffalo as well as horse, donkey, camel, elephant, pig, dog, cat and guinea pig. In general, it develops sporadically, but sometimes enzootically. The infection is characterized by splenomegaly, dark and unclotted blood, sero-hemorrhagic infiltration in skin and subcutaneous tissues and high body temperature. The causative agent of the disease, Bacillus anthracis, is an aerobic, gram positive, non-motile, sporulated and capsulated microorganism. In this study, anthrax cases from 9 different provinces (Samsun, Amasya, Tokat, Sivas, Ordu, Sinop, Giresun, Trabzon and Rize) were investigated by Samsun Veterinary Control Institute Directorate in Middle Eastern Blacksea Region. All materials submitted with Anthrax suspicion (blood soaked cotton, smears, spleen specimen if necropsy performed) were stained with Giemsa and polychrome methylene blue and then examined under microscopy. Examination of the 63/375 anthrax suspected cases were positive between 2010-2014 periods. In these periods, 7 of 24 cases were from Amasya (6 cattle, 1 sheep), 1 of 33 from Ordu (cattle), 6 of 22 from Rize (cattle), 22 of 141 from Samsun (19 cattle, 3 sheep), 2 of 18 from Sinop (cattle), 19 of 83 from Sivas (cattle) and 6 of 26 from Tokat (cattle) were positive for anthrax. Five cases from Giresun and 23 cases from Trabzon were negative for any kind of infectious agent.

Keywords: Bacillus anthracis, giemsa, microscopy, polychrome methylene blue

Investigation of Coxiella burnetii Prevelance in Cattle, Sheep and Goat Abortus

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Q fever is a zoonosis infection that cause to abortion in cattle, sheep, goats and cattle threaten to healty of human in all world. Coxiella burnetii is a gram negative, tiny (0.3-1.5 µm X 0.2-0.4 µm) and an obligat intracelluler bacterium. Due to its resistance to physical agents, probably related to its sporulation process, C. burnetii survives for long periods in the environment. Moleculer techniques are use to detection of DNA of C.burnetii from clinical samples and cell cultures successfully. Experimental studies by Trans-PCR are shown that can detection even 0.007 ng/µl DNA of C. burnetii and only single cell of C. burnetii from milk samples. The aim of this study was to investigate the presence of C. burnetii in sheep, goat and cattle by Polymerase Chain Reaction (PCR) in Samsun and neighboring provinces. Aborted fetus which were delivered to Samsun Veterinary Control Institute in 2012 and 2014 were used as meterials. Totally, C. burnetii investigated in 227 abortion materials IS1111a gene with Trans-1 and Trans-2 spesific primers. Two (%0.8) sheep aborted fetus were detected as positive from all 227 materials including 55 sheep, 14 goat and 158 cattle aborted fetus.

Keywords: Abortus, coxiella burnetii, PCR

Evaluation Cases of Young Ruminants with Diarrhea Symptoms in Marmara Region of Turkey

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The aim of this study was to determine bacterial, parasitological and viral agents of diarrhea in young animals in Marmara Region of Turkey between 2013-2014. The material of this study was consisted of 15 calves, 88 lambs, and 26 kids which were younger than 6 months old. For bacterial, clostridial and viral agents, Conventional Cultural Methods, Toxin Neutralization Test (TNT), Real-time PCR and ELISA and for parasitological agents, Flotation and Carbol-fuchsin staining methods were used. A total of 31 Escherchia coli (24.03%), 29 Eimeria spp. (22.48%), 12 Cryptosporidium spp. (9.3%) and 6 Clostridium perfringens Toxin (4.6%) were identified from samples. In addition Rotavirus, Coronavirus and Pestivirus were detected in 4, 1 and 5 of 129 of samples respectively. In conclusion, Identification of the agents that cause diarrhea in young animals, will give an idea to veterinarians on which agents are prevalent in the region. This may increase the chances of treatment and as a result of this reduction of mortality and prevention of large economic loses from occuring

Keywords: Diarrhea, young ruminants, marmara region, Turkey

Retrospective Evaluation of Anatolian Serosurveillances for Foot and Mouth Disease

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Serosurveillance activities have been initiated in 2008 and regularly conducted in order to lead control of Foot and Mouth Disease (FMD) in Anatolia. Main goal to conduct serosurveillance is to provide an estimate of the proportion of seropositivity to non-structural proteins of FMDV (NSPs), and to assess vaccine efficacy in selected provinces by measuring antibody levels to structural FMD proteins (SPs) 30 days post-vaccination in cattle. In this study, evaluation of the results of Anatolian serosurveillances between 2008-2012 was aimed. Two-stage sampling strategy was preferred. Primary sampling units were the villages and secondary sampling units “animals” in selected villages. Age of sampled animals was clustered between 4-24 months for cattle and 4-18 months for sheep-goat. 565 villages were selected by % 10 expected prevalence with a % 95 confidence interval. Animal number was calculated by test criteria of % 88 sensitivity and % 99 specificity within the selected villages. And % 80 expecting antibody level with % 95 confidence interval were preferred for vaccine efficacy survey calculations. Only cattle were sampled in 2008-2009, sheep and goats were sampled as well as cattle in 2010 and 2012. WinEpiscope 2.0 and Survey Toolbox 1.0 Softwares were used for sample size calculations. Samples were analysised in laboratory by NSP ELISA mainly using the PrioCHECK® FMDV NS Kit for detection of antibodies directed against 3ABC NSP. NSP and LPB ELISAs (for detection of antibodies directed against FMDV SP) were used for vaccine efficacy survey. Comparing the results of each year, gradual increase has been detected on the cumulative prevalence. Prevelances of % 8.27 (2008), %10.03 (2009), %14.72 (2010) and %21.0 (2012) were calculated, respectively. Congruently, prevelance of young age (4-12 months) group were detected as % 4 (2008), %8 (2009), %12 (2010) and %16 (2012). Significant prevelance differences have been determined between sheep-goat and cattle in 2010. Prevelance in sheep (%18.59) was clearly higher than the other species %12.3 (cattle) and %7.9 (goat). Sheep and cattle prevalances were increased progressively comparing the 2010 and 2012 results; Sheep %18,5 (2010); %24.1 (2012) and cattle %12.3 (2010); %17,4 (2012). Progressive prevelance increases in years 2008-2010 were confirmed with the disease dynamics detected through these years. Persisting increase of prevelance in 2012 might be possible cause of Asia-1 epidemic after 9 years. Although sheep prevelance was higher than the cattle, transmission between species was not determined since cattle were NSP negative despite sheep has high NSP positivity in the same epi-unit in 2012. Sustained increase of sheep prevalence in 2012 can be explained by two possibilities; subclinical infection might be spread in small ruminant population or bias on the sampling. This study is important for further serosurveillance plan towards developing effective FMD control strategies in Turkey.

Keywords: Anatolia, FMD, serosurveillance, retrospective

Laboratory Result of Animal Movements From Anatolia to Thrace Prior to Eid- Ul Adha for Foot and Mouth Disease

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Turkish Thrace region has been recognized by the World Organisation of Animal Health (OIE) as Foot and Mouth Disease (FMD) free –with- vaccination since 2010. To conduct this status, meat producing animals transported from Anatolia to Thrace prior to Eid- Ul- Adha have been controlled in FMD Serology Laboratory with regard to FMD virus circulation since 2011. In this paper, it was aimed to evaluate laboratory studies between 2011-2014. This process has been realized by the help of two legislations (2010/7 and 2010/13) of General Directorate of Food and Control, Turkey regulated by OIE. According to legislations, there should not be symptom of FMD on the transport day of animals, their premises should not be changed since their birth and kept at least 3 months in the same premise, FMD should not be detected 10 kilometer around the premise at least 3 months, animals should be quarantined in a premise 30 days prior to the transport and end of this period blood sera should be tested and gave negative antibody result against FMDV 3ABC Non Structural Protein (NSP). Lastly, animals should not be exposed FMD during transport. Laboratory analysis of the sera was performed by NSP ELISA mainly using the PrioCHECK® FMDV NS Kit for detection of antibodies directed against 3ABC NSP of FMDV. Sera/premises numbers tested in laboratory were calculated 9805/325 (2011), 23232/685 (2012), 22665/1143 (2013) and 32237/1289 (2014), respectively. Percentage of seropositive premises was detected as %73 (2011), %59 (2012), % 24 (2013) and % 25 (2014) respectively. When the results evaluated regarding to province, the most intense animal movement to Thrace is from Kastamonu province. Seroposivity rates in every year were determined to be %11 (2012), %13 (2013), %11 (2014) for sheep, %20 (2013), %50 (2014) for goat, % 64 (2012), %24 (2013), % 26 (2014) for cattle, %45 (2012), %33 (2013), %26 (2014) for mixed premises (there was no data entry in 2011 with regard to species basis and goat blood sample was not sent to laboratory in 2012). Generally, first two years seropositive premises-unaccepted premises were higher than the last two years. Actually, the real situation of seronegative premises or really negative (risk-free) in terms of virus circulation should be an important question to answer in this evaluation. Animal movement is a significant epidemiologic risk factor for highly contagious FMDV circulation. In conclusion, it is for the first time that a FMD Serology Laboratory to evaluate intensive animal movements from Anatolia to Thrace prior to Eid ul Adha.

Keywords: Eid-Ul-Adha, FMD, NSP, Thrace

Current Situation of Brucellosis Outbreaks in Turkey after Mass Vaccination Started in 2012

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Mass vaccination is generally accepted as one of the most efficient and economical tool to control and eradicate the disease in countries where brucellosis is endemic. In this report it was aimed to evaluate general situation of the brucellosis outbreaks in Turkey during mass vaccination campaing between the years 2012 and 2014 in cattles, sheep and goats. Human brucellosis cases were also evaluated in respective years. A total of 4420 new outbreaks have been reported from livestock since mass vaccination campaing started at the beginning of 2012 in Turkey. New outbreaks of the disease during the following years were analyzed for cattles, sheep and goats. According to the results of the year 2012, number of outbreaks were 1696 in cattles, 189 in sheep and 33 in goats. In 2013, number of outbreaks were 1319 in cattles, 217 in sheep and 295 in goats. In 2014, number of outbreaks were 596 in cattles, 51 in sheep and 24 in goats. According to the data by Ministry of Health, the number of human cases were 6759, 7225 and 4403 in 2012, 2013 and 2014, respectively. When the number of outbreaks were evaluated annually, brucellosis incidence were decreased sharply in livestock except the figures obtained from goats in year 2013. The reason of this high outbreak number in goats was the result of vaccine induced abortion in this species which are known to be more susceptible to Rev.1 infection than sheep. Consequently, mass vaccination campaign caused the decrease of the disease incidence. A sharp decline in the number of disease outbreaks in humans were also quite remarkable. According to the data obtained from the both ministries clearly demonstrate that mass vaccination should be continued meticulously along with other sanitary and control measurements in order to control the disease in both animal and human efficiently and come to the point of eradication.

Keywords: Brucellosis, eradication, mass vaccination, outbreaks

Biovar Distribution of Brucella Isolates from Livestock in Turkey between 2010-2014

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The main aim of this study is to evaluate the biovar distribution of Brucella(B) isolates gathered from livestock between 2010 and 2014. The second goal is to determine the most prevalent biovars circulating in sheep, goat and cattle in Turkey. 4673 Brucella isolates from livestock sent to Pendik Veterinary Control Institute between 2010 and 2014 were examined through conventional biotyping method. Biovar identification of isolates was carried out according to CO2 requirement, H2S production, Thionin, Basicfuchcin, Safranine, Penicilin, Streptomycin, and I-erythritol sensitivity, lysis with Tibilisi and R/C phages and agglutination with monospecific A and M antisera. When the general results obtained between 2010 and 2014 are taken into consideration, 85 isolates were identified as B.melitensis biovar-1, 655 isolates as B. melitensis biovar-3, 1 isolate as B.melitensis biovar-2, and 1329 isolates as Rev1 vaccine strain. Moreover, 116 isolates were identified as B.abortus biovar-1, 2466 isolates as B.abortus biovar-3 and 21 isolates as S-19 vaccine strain. After the results are classified in accordance with sheep, goat and cattle separately; out of 2587 isolates from cattle, the majority of the isolates were identified as B.abortus biovar-3 and B.abortus biovar-1, respectively.Out of 892 isolates from sheep, the majority of the isolates were identified as B.melitensis biovar-3 and Rev1 vaccine strain, respectively. Out of 1194 isolates from goat, the majority of the isolates were identified as Rev1 vaccine strain, B.melitensis biovar-3, and B.melitensis biovar-1, respectively. Even though the number of biovar identification was changeable annually, B.melitensis biovar-3 for sheep and goat, B.melitensis biovar-1 for goat and B. abortus biovar-3 for cattle have high identification percentages as field strains. S-19 and Rev1 identification stem from vaccine induced abortions as an expected result during mass vaccination program when pregnancy status of animals is ignored. The rest of the biovars with low identification percentages are not responsible for outbreaks as much as B.melitensis biovar-3, B.melitensis biovar-1, and B.abortus biovar-3. Focusing on the trace back of field strains by biovar identification and following the sharp rises in less common biovars are beneficial for both epidemiological studies and control-eradication program.

Keywords: B.abortus, biotyping, B.melitensis, brucella biovars

Bacterial and Viral Agents Which Were Isolated from Material of Ruminant Abortions That Brought to Pendik Veterinary Control Institute, Between 2010 and 2014

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The aim of this poster session is evaluation of infectious agent that cause abortion in ruminants using aborted material brought to Pendik Veterinary Control Institute between 2010 and 2014 from Marmara region. In this study, totally 1156 material including aborted fetus and waginal swaps were examined with clasiccal bacteriological isolation and identification method using blood agar, farrel medium, skirrow medium and McConkey agar. Of examined 1156 aborted material, etiological agent were dedected in 606. Most frequently isolated abortion agent was Brusella sp., followed by Leptospira sp. and Pestivirus. No isolation done in 550. One of the most important reason that effect of profitability of stock farming is abortions. There are many things than can disrupt a healthy pregnancy in ruminats: Genetic abnormalities, feeding deficiency, heat stres, mycotoxins, toxic agents. Although the cause of many abortions is never determined, infectious agents represent the most commonly diagnosed cause of abortions in many laboratories. They reduced fertility and some may also infect humans (zoonotic diseases). It has been reported that aproximately 30 % of ruminants abort infectious agents are Virus, bacteria and parasitic agents. We could dedected aproximately 50% etiological of agents of ruminant abortions. Being as a highly contagious zoonosis Brusellosis is one the major reason of ruminat abortions in most countries in the world. It is also a notifable disease in Turkey and mass vaccination has been done with konjunctival S19 and Rew 1 vaccine for sheep/goat and cattle for controlling the disease. In our study the dominant pathogen was also Brucella sp. This results is fitting the result of research. It is concluded that the major etiological agent responsible from ruminant abortion in Turkey is Brucella sp.

Keywords: Abort, bacteria, ruminant, virus

Listeriosis Cases Diagnosed in Pendik Veterinary Control Instute, According to the Months and Animal Species, between 2010-2014

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In this study we aimed to point to the seasonal impact which is an important part of environmental factors for the epidemiology of this infection, with determining the range of listeriosis cases in Marmara region by months. This study will give an opinion for future studies to determine the risk factors and fight strategies against listeriosis in this region. The genus Listeria contains 6 species: L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L.ivanovii, and L. grayi. L. ivanovii and L. monocytogenes are pathogenic for animals. L. monocytogenes is the causal organism of listeriosis. The main clinical manifestations of animal listeriosis are encephalitis, septicaemia, abortion, and mastitis. The disease is often associated with cold weather and stored forages, usually silage. Poor quality (pH >5.5) maize and silage wherein the organism can multiply, is the most importent source of infection. In our study, the suspects animal tissues aspecieally central nerveus system tissues like cerebrum,cerebellum,cerebrospinal fluid sent to Listeriosis Reference Laboratory in Pendik Veterinary Control Institute between 2010-2014 from animals with nerveus system sypmtoms, tested with cultural methods and Vitek II system. In cultural method; Enrichment medium with supplements and listeria sellective agar base (Oxford formulation) used for isolation. Gram stain, biochemical tests are applied using FDA-BAM 'Detection and enumaration of L.monocytogenes' method, and Vitek II system was used too for identification of the agent. 26 of 88 sheep sample was found positive; 1 on January, 9 on february,5 on March, 8 on April, 1 on May, 1 on June, 1 on December. 4 of 24 goat sample was positive; 1 on January, 1 on April and 2 on December. 4 of 24 cattle sample was found positive; 1 on January, 2 on April and 1 on May.At total 3 positives on January, 9 on February, 5 on March, 11 on April, 2 on May and 1 on June was found. Listeriosis is an important flock disease in Turkey. This study, shows the instance of Listeriosis for last 5 years in Marmara region, can be a guide about fight strategies of the disease. We can say that Listeriosis is efficent between the months December to June for Marmara region.

Keywords: Encephalitis, listeriosis, listeria monocytogenes, marmara region, silage

Epidemiological Assessment of Rabies Cases in Marmara Region in Last Five Years

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The aim of this study is the assessment of five years analysis of possible rabies cases coming from district towns and received from Pendik Veterinary Control Institute's Rabies Diagnosis Laboratory between 2010-2014. Our laboratory has a total of 1320 suspected cases of rabies in last five years. For rabies diagnosis; Seller's painting and Fluorescent Antibody Technique (FAT) has been applied, if all materials are FAT negative, Mouse Inoculation Test (MIT) is examined. In the case of testing autolysis, RT-PCR is performed. In our laboratory 236 cases out of 1320 are detected positive cases of rabies. The distribution of these positive cases by province is as follows; Balıkesir 90, Çanakale 81, Bursa 29, İstanbul 27, Bilecik 6, Düzce 2 and Kocaeli 1. 199 from 236 positive cases of rabies were detected from domestic animals, while 37 positive cases were detected from wild animals. In the last 5 years, examination of the rabies cases shows that rabies cases for domestic animals majorly for cats and dogs is seen in İstanbul. Whereas,its remarkable that according to the analysis of last 5 years' cases, most of the rabies cases are concentrated in Balıkesir and Çanakkale with also domestic animals like Cow, Sheep and Goat. Mass rabies cases in Aegean Region occurring in 2007 on farm animals coincide with these regions and years. In 2007, the first rabies case in Balıkesir was detected at a fox in İvrindi, a neighboor district to İzmir. Following this, at the same year, two dog rabies cases were also detected in İvrindi. Furthermore, during 2008, 2 out of 6 rabies cases in Balıkesir were detected in district of İvrindi. In 2009, the rabies cases pervaded in Balıkesir where 14 cases were detected in 6 different districts. Rabies cases which started in wild animals then has observed in stray dogs or farm dogs and consequently spreaded to farm animals. In 2010, 17 out of 25 rabies cases observed in Balıkesir are detected in farm animals like sheep, goat and cow. Although there were no rabies cases detected in Çanakkale between years 2007 and 2010, in 2011, 17 cases are detected. Between 2010 and 2014, there is a decreasing trend in rabies cases in Balıkesir while, on the contrary there is an increasing one in Çanakkale.

Keywords: Epidemiology, marmara region, rabies

Nosemosis in Queen Bee Enterprises in Turkey

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Queen bee is the mother of all the bees in her hive and she is the determinant of their morphological features as well as their diligence, calmness, resistance to illnesses, their good and bad tempers. Conducting commercial beekeeping depends on working with healthy, efficient and young queen bees. Nosemosis is one of the most common diseases of adult honeybees. The agent of the disease is the protozoons named N. apis and N. ceranae. The disease has fecal-oral transmission. Nosema reproduces in the gastro-intestinal epithelial cells of adult honeybees, disrupts the digestive system and causes a decrease in efficiency of infected colonies. The purpose of this study was to compare nosemosis disease in the honeybees and queen bees of 30 major queen bee enterprises in different parts of Turkey. The cities chosen were in different geographical regions; queen bee and bee samples were taken from a total of 30 major queen bee enterprises in 11 cities: 5 from Ankara, 2 from Kırklareli, 5 from Mersin, 1 from Adana, 5 from Artvin, 1 from Ordu, 1 from Samsun, 5 from Ardahan, 1 from Elazığ, 2 from Aydın and 2 from İzmir. The samples were taken with the cooperation of Provincial Directorates and Beekeeping Unions of these enterprises. As a result of the queen bee and worker bee examinations of the samples taken from 30 major queen bee enterprises, nosemosis was found in 21 (70%) of these enterprises. N. apis was not found in any of these enterprises. N. ceranae was found with a rate of 100%. 10 (33.33%) nosemosis was found in the queen bees of the enterprises. 3 queen bees and 300 bees were taken from each colony of each queen bee enterprise. In the queen bees and adult bees taken from these enterprises, N. apis and N. ceranae spores were searched by using polarization microscope with an enlargement of 40X following native examination and dying. For the examination of N. apis and N. ceranae by using multiplex PCR, the queen bee taken from each enterprise was considered as one sample while 20 bees taken from the bees of the same colony were considered as one sample. Consequently, a total of 180 homogenization of 90 queen bee homogenizations and 90 bee homogenizations were examined against the two types of nosema by using multiplex PCR method. All the queen bee and bee samples taken gave N. ceranae DNA band at 219 bp at agarose-gel electrophoresis. N. apis was not found at 319 bp at agarose-gel electrophoresis in any of the queen bee or bee samples. This study proves that nosemosis is an important problem in queen bee enterprises. It was concluded that queen bee enterprises should be examined regularly in terms of nosemosis, they should be treated and the sale of queen bees with diseases should be prevented.

Keywords: Nosema, queen bee

Epidemiological Monitoring of BHV-1 Infection on Dairy Cattle Herds

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In this study, we aimed to show possibility of use of present modeling's that might follow BoHV-1 status in 10 dairy herds located in Corlu County, Tekirdag province, by detection of correlation between BoHV-1 occurrence frequency and herds' general conditions, by detecting factors triggering occurrence of the infection and finally assessment of all these data for the herds under similar circumstances. For this purpose, a total of 1006 serum samples from 383 different animals were collected by samplings in 4 month intervals during the 1.5 years from 10 dairy cattle herds. During the study, herds in the animal's movements (entry and exit of animals) and any changes that may affect virus infection (vaccination, birth, etc.) were recorded for the monitoring. Avidity test was performed as described before (Ozkul et al, 2008) using a commercial BHV-1 ELISA kit (Herdchek* IBRgB). Two of the herds were found to be seroconverted for BHV-1. The avidity indexes were evaluated at these two positive herds. The avidity scores of the herds revealed possible recurrences of the infection. Epidemiological assessments explaining status of BHV-1 infection on the herds were done based on their individual management features (including milk production level, vaccines administered, animal trafficking, owner educational level, etc). Evaluation of the data obtained revealed that purchased animals from the other herds is the biggest risk factor at the acquiring BoHV-1 by the herd. On the other hand, risk of the infection occurrence was found lower via other ways of contact and/or virus reactivation from latently infected animals within herds. The herd's administrative and physical characteristics besides of BoHV-1 infection previously reported data were questioned on the availability for application of mathematical modeling techniques. Although R value, expressed as a "rate of occurrence of the disease", of the assessments has been calculated using data received from another study, assessments for detection of transmissions at the earliest time within the herd and/or spreading of the disease between herds having similar structure in many herds within the scope of potential or existing control program can not be made due to lack of data. On the other hand, the unforeseen parameters that would allow determining to take the disease under control and the use of mathematical modeling have been identified.

Keywords: BHV-1, cattle, dairy herd, epidemiology

Application of Molecular Techniques in Routine Laboratory Diagnosis of Classical Rabies Virus in Post-Mortem Brain Samples from Naturally Infected Different Species of Animals in Turkey

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Rabies is one of the most significant zoonotic diseases. Rabies is an enzootic disease in Turkey. Rapid and accurate diagnosis of rabies in animals is important for prevention and control. The aim of this study is to emphasize the importance of the applications of molecular techniques in the rutin laboratory diagnosis of rabies. The material consisted of 139 suspected brain samples brought from 11 different provinces, belonging Eastern and Southeastern Anatolia region, including Elazığ, Malatya, Diyarbakır, Van, Mardin, Tunceli, Muş, Bingöl, Siirt, Şırnak and Bitlis in year of 2013. The real-time RT- PCR assay was applied for routine laboratory diagnosis of classical rabies with fluorescent antibody technique (FAT) and animal inoculation test (MIT).These samples were from 43 dogs (39 normal, 4 autolytic), 63 cattle(61 normal, 2 autolytic), 6 sheep, 1 donkey,5 foxes, 5 wolves, 10 cats,1bedger, 1 marten and 4 goats(3 normal, 1 autolytic), The samples were autolytic in 7 of 139 (5.03%), whereas 72 (94.96%) of 132 samples were freshly arrived to the laboratory. Due to badly autolysis of 7 samples, they could not be examined by fluorescent antibody test (FAT) and mouse inoculation test method (MIT). Seventy two of 132 samples were tested with both fluorescent antibody test (FAT) and mouse inoculation test method (MIT), whereas all the samples (139 of 139) were examined by optimizing real-time RT- PCR assay in which the primary for classical rabies virus and TaqMan prob set were used. Fresh brain homogenates from Cornu ammonis in carnivores and Cerebellum in herbivores were used for FAT and MIT. As a result, 112 of 132 samples were positive with FAT, 115 of 132 samples with MIT, whereas 115 of 132 were positive in real-time RT-PCR. In fresh brain samples, with MIT results in mouse brain, real time RT- PCR test results showed complete relation each other. As both fluorescent antibody test (FAT) and mouse inoculation test method (MIT) could not be applied to the autolytic samples, the real time RT-PCR assay was the only choice. Consequently, real-time RT-PCR assay could be utilized in autolytic samples conveniently as well as confirmatory to FAT in in vitro conditions. Partial nucleotid sequences of the positive primary PCR amplifications were identified from the selected 7 bovines and 2 foxes brain samples and published in GenBank. Sequencing results of the positive primary PCR amplifications confirmed that all eight isolates are rabies virüs (RABV) by NCBI Programme. Phylogenetic analysis of RABV identified according to the partial nucleoprotein N gene 0-321 sequences. As a result, nucleotid sequences of isolate were found as identical with the isolate obtained from these provinces, as well as related with isolates sequences from neighboring countries of Turkey in the Middle East.

Keywords: Classical rabies virus, molecular techniques, routine laboratory diagnosis

Table 1 -Results of diagnostic methods according to the materials sampled.

Table 1 -Results of diagnostic methods according to the materials sampled

ANİMAL SPECİES		NUMBER OF MATERİALS	DİAGNOSTİC METHODS		
			FAT	MIT	REAL TİME RT-PCR
CARNİVORES	DOG	39+4*	29	29	29+4*
	CAT	10	6	6	6
	FOX	5	5	5	5
	WOLF	5	5	5	5
	BEDGER	1	1	1	1
	MARTEN	1	1	1	1
HERBİVORES	CATTLE	61+2*	55	58	58+2*
	SHEEP	6	6	6	6
	GOAT	3+1*	3	3	3+1*
	DONKEY	1	1	1	1
TOTALLY		132+7*	112	115	115+7*

A Serological Investigation for Some Viral Diseases on Cattle, Sheep and Goat in Private Enterprises in East and Southeast Anatolia Regions

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East and Southeast Atolian Regions have been prominent areas in farm animal breeding in Turkey. Geographic and climatic conditions are very suitable especially for small ruminant breeding. Family type enterprises are quite widespread. However, data on contaigious diseases is ratherly limited comparing other regions in Turkey. Aim of the study is to reveal healthy prophile for manly latent-persistent infections in cattle, shep and goat that have breeding in small scale enterprises. Samples were collected from 13 provinces (Elazığ, Malatya, Bingöl, Tunceli, Hakkari, Bitlis, Van, Muş, Siirt, Diyarbakır, Şanlıurfa, Mardin and Şırnak) in East and Southeast Anatolian provinces. Cattle samples (n=2.118) were obtained from 12 provinces, sheep 12 (n=2.418) and goat 7 (n=1.175). Total of 5.711 samples were collected. Ages of the animals were varied between 6 month old and 12 years old according to the species. The big majority of the sampled animals were female related to the breeding aims and clinically healthy during sampling. The samples were controlled for Leukosis Virus (BLV), Visna-Maedi Virus (VMV), Caprine Arthritis-Encephalitis Virus (CAEV) and Bovine Herpesvirus Type1 (BHV1) using indirect ELISA and microneutralisation test were utilised for Bovine Viral Diarrhea Virus (BVDV), Parainfluenza Type 3 (PI3) and Bovine Enterovirus Type 1 (BEV1). According to the test results; BLV was detected in 3 provinces; Elazığ 3.1% (8/255), Hakkari 0.9% (2/204) and Şanlıurfa 2.2% (4/181). Out of 2.118 cattle, 14 (0.6%) was found tobe BLV positive. VMV is more prevalent, positivity was detected in all studied provinces between 3.2% (3/92) Diyarbakır and 27.9% (80/286) Bitlis, in total 14.7% (356/2.418) was detemined as positive. CAEV was also detected in whole 7 provinces between 0.6% (1/46, Elazığ) and (21.2, Bitlis). Out of 1.175 goat, 71 (6%) detected as CAEV positive). BHV1 was detected as 42.8% (908/2.118), 2.7% (66/2.418), 29.1% (342/1.175) in cattle, sheep and goat, respectively. Pestiviruses antibody presence was 77.7% (1.646/2.118), 50.5% (1.122/2.418) and 44.6% (525/1.175) in cattle, sheep and goat, respectively. PI3 proportion in cattle was found to be 69.9% (1.481/2.118), while 36.6% (887/2.418) and 43.2% (508/1.175) in sheep and goat, respectively. In total, 1.525 cattle was serpositive for BEV1 (72%), proportion was 43.7% (1.057/2.418) and 33.1% (389/1.175) in sheep and goat, respectively. As a result, Small Ruminant Lentiviruses was detected as higher comparing other regions of Turkey. BLV, BVDV, BHV1, PI3 and BEV1 proportions were seem slightly higher then previous data. However, obtained data showed that, proportions are high than expectations for family type enterprises and control-eradication studies are a necessity especially for Retroviral infections.

Keywords: East and Southeast Anatolia Regions, seroprevalence, some viral diseases,

A Serological and Molecular Investigatıon of Pestivirus Infections and Coxiella Burnetii from Abortion Materials of Sheep in Eastern Turkey

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Whether the purpose of rearing cattle, sheep and goats is production of milk, wool or meat, these processes are dependent on the ability of the animals to reproduce successfully. Any decrease in the total number of reproductive animals in a flock will cause important economic losses that could close the operation. Infertilization results from two main factors: non-infectious and infectious factors. Noninfectious factors include genetic and nutritionalor toxic causes. Infectious ones are the mostimportant factors causing abortions in the small ruminants. Pestiviruses are among viral infections causing abortion in sheep. In the study 89 fötüs samples from sheep that cause of abort cases 89 flocks obtained from Elazığ, Malatya, Tunceli and Bitlis. The presence of pestivirus was detected in. 22/89 (% 24.71) fötüs samples collected from aborted sheep. 546 serum samples obtained from aborted flocs belonging to them, aborted sheep. Antibodies against pestivirus detected in 58.05 % of 546 sera samples using ELISA. The presence of pestivirus and Coxiella burnetii reported as an important cause of abort cases in sheep was investigated serologically, bacteriologically and virologically in aborted sheep. Q fever is a zoonotic disease that occurs worldwide and is caused by the obligate intracellular bacterium Coxiella burnetii. Coxiella burnetii can infect people and many animal species including mammals, birds, and ticks. Infected animals are usually asymptomatic, but infection can cause abortion and stillbirth in ruminants. C. burnetii doesn't grow on standard laboratory bacteriological media. Isolation of C. burnetii is difficult and time-consuming. The aim of this study was to investigate the presence of C. burnetii infection in aborted sheep in Elazığ and neighboring provinces by serological and molecular methods. A total of 550 samples (350 serum samples and 200 fetuses) were collected from aborted sheep. Serum samples were examined to detect antibodies specific to C. burnetii by ELISA, whereas foetal organ samples (lung, spleen, kidney and liver) were also analyzed by PCR test. C. burnetii specific antibodies were detected in 56 (16%) of 350 serum samples. In PCR from tissue samples directly, 4 samples (2%) were positive. In PCR from yolk sacs of embryonated chicken eggs, C.burnetii DNA was detected in 5 samples (2.5%). This study shows that Pestiviruses and C. burnetii infections has an important role in sheep abortions in Elazığ, Malatya, Tunceli and Bitlis province.

Keywords: Abortion, C. burnetii, isolation, pestivirus, sheep, PCR

An investigation for Equine Viral Arteritis Virus Infection in Donkeys in East and Southeast Anatolia regions, Turkey

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The Equine viral arteritis (EVA) is a prevalent infection of horses described as “abortion storm”. The equine arteritis virus (EAV) is an enveloped, single stranded, positive RNA virus classified in the Arteriviridae, in the order Nidovirales. Beside main host, donkeys, mules and alpacas were detected as susceptible to the natural infection as well. Equine viral arteritis (EVA) Virus infection is cause to reproductive and respiratory tract disorders in horse, donkey and mule. In this study, the infection was investigated serologically in donkeys to obtain first data from in 6 provinces in East and Southeast Anatolia regions of Turkey. Donkeys have been using as a draft animals and they are breeding mostly with ruminant species in rural areas in Turkey. Average number of breeding donkeys is 1 to 3 in every farm. Clinically normal adult (1>= years old) donkeys have been randomly selected for sampling. Sex of the animals was ignored. Out of 1,492 serum samples, 53 (3.5%) were positive by Enzyme Linked Immunosorbent Assay (ELISA) test. Seropositivity was detected in all provinces; Elazığ (7%, 17/241), Tunceli (2.4%, 3/122), Van (2.9%, 10/342), Bitlis (7.4%, 5/67), Şırnak (2.7%, 12/440) and Siirt (2.1%, 6/280). Out of 28 locations in 6 provinces, seropositivity was detected in 22 locations. This is the first data for EVA infection for the studied regions. Even though detected proportions were not high, results revealed the presence of the infection or antibodies the most of the studied locations. Health monitoring of donkey seems to need more attention to take control and preventive precautions in the aspect of infections.

Keywords: Donkey, ELISA, equine viral arteritis, seroprevalence, Turkey

Epidemiology of Parasitic and Viral Bee Diseases in the Bee Enterprises of Central and East Black Sea

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Apiculture is one of the most important agricultural activities in Turkey in terms of both its own geographical location and its flora. Parasitic and viral bee diseases have an important place among the primary problems of technical beekeeping sector. Varroa destructor living on the larvas, pupas and matured ones of the honeybees, increasing in the eye of combs ina short time, considerably showing no clinical signs for a long time is a harmful mite which gives harm to the bees. Nosemosis is one of the most common diseases of the matured honeybees. The factors of the disease are the protozoons named N.apis and N. ceranae. The recent researches have shown that bee viruses also have active roles in colony deaths. This study examined 67 hives from 24 bee enterprises in the cities of Amasya, Giresun, Ordu, Rize, Samsun, Sinop, Tokat and Trabzon, all of which are within the service area of Samsun Veterinary Control Institute. The presence of Varroa destructor and Nosema spp. were examined in terms of parasites while the presence of Acute Bee Paralysis virus, Black Queen Cell Virus, Deformed Wing Virus, Kashmir Bee Virus, Chronic Bee Paralysis Virus and Sacbrood Virus was examined in terms of viral diseases. V. destructor was found in 47 samples(%70.14) and Nosema spp. was found in 15 samples (%22.8) in our study As a conclusion, it is very important to have a combined fight with parasites and to ensure that beekeepers participate regularly in regional disinfestation altogether. Although the chemical fight for varroosis was quite successful at first, a resistance for chemicals developed in varroa in time. In addition, these chemicals began to be harmful for human health through food chain and they even polluted the environment. Organic acid and volatile oils which began to be used recently have not produced the desired success, either. For nosemosis, in addition to similar fight, the hives should be kept away from damp environment, strong hives should be formed and disinfestation should be made twice in spring and autumn. Fight with parasites becomes more important in beekeeping since varroa and nosema can transmit viral diseases.

Keywords: Honeybee, honeybee viruses, nosema, varroa

Investigation of *Coxiella Burnetii* in Ticks by Molecular Methods in Middle and East Black Sea Region

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Coxiella burnetii, an obligat intracellular bacterium is the causative agent of sistemic Q Fever in humans and animals. Ticks may play significant role in the transmission of agent. There is little study on investigation of C. burnetii on ticks in Turkey. In this study, the presence of *C. burnetii* were investigated by Trans-PCR method in collected ticks from some of provinces in Middle and East Black Sea Region. For this purpose; 591 (333 female, 258 male) ticks were collected on totaly 160 farm animals (140 cattle, 16 sheep and 4 goat) from Samsun, Sinop, Ordu, Giresun, Amasya, Tokat and Sivas provinces on April to September 2014. Species and gender of collected ticks were identified and 221 groups were constituted. Following homogenisation and DNA extraction, the samples were examinated for the presences of *C. burnetii* DNA by using the primers Trans-1 and Trans-2 by Trans-PCR method. *C. burnetii* DNA couldn't detected from samples at the end of the study.

Keywords: Coxiella burnetii, ticks, trans-PCR

A Serological Survey for Bovine Enterovirus Type 1 in Human and Farm Animals in Elazığ Province, East Anatolia

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Contrary to bovine enterovirus type 2, host spectrum is quite wide of type 1 infection. In this study, BEV1 was serologically investigated in human and 6 domestic animal species in Elazığ province, East Anatolia, Turkey. Blood samples were collected from 751 human, 191 cattle, 200 sheep, 160 goat, 87 donkey, 91 horse and 46 dog. Serum samples were controlled using standard Virus Neutralisation Test. Cattle have the highest proportion as expected (85.3%). Values were found to be high in donkey (73.5%) and goats (71.8%). Close values were detected in sheep, horse and dog as 46.5%, 43.9% and 41.3%, respectively. In total, out of 1.526 samples, 742 (48.6%) has BEV1 specific antibodies. The lowest seropositivity was detected in human as 33% (248/751). Human samples were classified acoording to the settlement as city center and rural locations for evaluation of viral expose. However, no difference was detected, positivity was 32.6% (154/472) in the center settlements while 33.6% (94/279) in the persons from rural areas. Ages of the samples were varied between 2 and 94. Serum neutralisation50 test (SN50) showed that the highest antibody titter rates were among 40 and 70 years old. According to the gender 60.9% of the positivity was found to be in females (151/248). Despite BEV1 is cause to subclinic-mild disease, propotion is not low in many species. Considering stability in field conditions, the virus can be accepted among environmental pollutants.

Keywords: Bovine Enterovirus type 1, Elazığ, serology, Turkey

The Sero-Epidemiologic Analysıs of Cattle Hypodermosis in the Central Black Sea Region

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Cattle hypodermosis in Turkey is one of the most important parasitic diseases causing economic losses in meat, milk and leather industry by reducing meat and milk yield as well as due to the damage to hide. Hypodermosis is still considered as a threat for the herd health. The microscopic diagnosis of Hypoderma is mainly carried out on third-stage larvae (L3). The morphological differentiation of L3 is not difficult, but can sometimes be troublesome because Hypoderma spp. have similar biological and morphological features. The techniques for the molecular identification of larvae have been developed day by day. The present study aimed to determine epidemiologic characteristics of hypoderma infestations by serological methods in the Central Black Sea Region to help creating strategies against the spreading of the disease. A total of 1560 blood serum cattle were collected in Central Black Sea Region (Samsun, Amasya, Tokat, Sinop and Ordu). Serum samples were tested for anti-hypoderma antibodies by ELISA. The analysis of 1560 serum samples by ELISA revealed 35.2% seropositivity in the region. The seroprevalence was recorded as 56.4% in Samsun, 50.6% in Sinop, 36.8% in Amasya, 23.1% in Ordu and 9.2% in Tokat. The regional spread of hypodermosis among the cities and the counties was found statistically significant ($P < 0.01$). In accordance with the life cycle of the larva, it was concluded that October-November for the fall term and March-April for the spring term are the best times for conducting fumigation before the larvae complete their migration to the spinal canal. The L3 larva stage was determined as the stage of the larva that falls to the earth during the May-June period in Samsun, Sinop, Amasya, Tokat, and Ordu. Nevertheless, for the early intervention against hypodermosis, an insecticide application preceded by the detection of L1 stage antibodies by ELISA would be more economical.

Keywords: Cattle, hypoderma

Seroprevalance of Equine Viral Arteritis in Horse and Donkeys in East Anatolia Region, Turkey

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Equine arteritis virus (EAV) is a member of the genus Arterivirus of the family Arteriviridae; its host range is limited to equids. Equine viral arteritis (EVA) Virus infection is cause to reproductive and respiratory tract disorders in horse, donkey and mule. In this study, Equine viral arteritis (EVA) infection was investigated serologically in adult and clinically normal horses and donkeys in 5 different locations in two adjacent provinces, Elazığ (38o41´N-39o14´E) and Tunceli (39o07´N-39o32´E) in the East-Anatolia region of Turkey. The blood samples from donkeys and horses were collected arbitrarily from small private enterprises in rural areas in these provinces between March 2009 and August 2010.According to ELISA result, seroprevalence varied between 6.4 and 24.3% in horses, in total out of 193 samples, 29 (15%) was found to be positive. The obtained values were lower in donkeys as 8.3% (19/227), ranging between 2.4 and 14.2%. Gender of the sampled horses were almost equal (99f-94m), 13 stallion and 16 mares was found to be seropositive. Age distribution was 1-16 and average age of the seropositives was 7.4. Majority of the donkeys were female due to breeding styles (220/227). Out of 19 positive donkeys, only 1 was stallion. Age distribution of sampled donkeys were 4 to 30, average of the positive donkeys 11.8. Health monitoring of donkey seems to need more attention to take control and preventive precautions in the aspect of infections.

Keywords: Donkey, East Anatolia, equine viral arteritis, horse, Turkey

Seroprevalence of T*oxoplasma Gondii* Infection Among Horses And Donkeys in Tiaret Province, Northwestern Algeria

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Toxoplasma gondii is an important zoonotic pathogen infecting humans and almost all warm-blooded animals. The most common sources of human infection are ingestion of tissue cysts in raw or undercooked meat. However, limited information is available about *T. gondii* infection in horses and donkeys in Algeria. In the present study, we report the seroprevalence of *T. gondii* infection in horses and donkeys in Tiaret province, northwestern Algeria. A total of 293 and 30 serum samples were obtained from clinically healthy horses and Donkeys respectively, in three regions of Tiaret Province. The modified agglutination test (MAT) was used to test the specific antibodies to *T. gondii.* In this study, 75 of 293 (25.59%) horses were seropositive for *T. gondii* with titers of 1:25 in 43, 1:50 in 19, 1:100 in 11, and 1:200 in 2. Antibodies to *T. gondii* were found in 9 of 30 (30%) donkeys with titers of 1:25 in 3, 1:50 in 3, 1:100 in 3. Seroprevalence varied in 3 different regions, ranging from 17.9% to 33.5%. The results of the present study indicated that the rate of infection with *T. gondii*in horses and donkeys is a little high in Tiaret province, northwestern Algeria in comparison to other surveys in Algeria, which suggests that consumption of horse meat in this area may represent a potential source for human infection with *T. gondii*.

Keywords: Algeria, donkey, horse, MAT, seroprevalence, toxoplasma gondii

Fetal Age Estimation in Bedouin Goat: Comparison between Three Methods

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In small ruminant's research, the veterinarians and researchers are often faced with difficulties in determining fetal age which is one of the main diagnostic tools of abortions etiologies. In this study three methods of fetal age estimation are applied on Bedouin goat runts and fetuses, in order to precise the most reliable method which must be applied in this breed. The study was carried out at Beni-Abbes region in the southwest of Algeria. Three fetuses were collected at the slaughterhouse and thirteen runts were collected from different nomadic herds of the region. The first method used to estimate fetal age is that of Keller (cited by Pavaux, 1979) based on taking the crown-rump length, the second method is that of Huggett and Widdas (1951) based on taking the body weight and the last method is that described by Sivachelvan and al. (1996) based on the appreciation of the degree of development of morphological characters. Weighing and morphometric results were correlated with the nonparametric Spearman test. The results obtained by the method of Keller and development horizons method are in agreement and are more reliable for the young fetuses of Bedouin breed. Beyond 119 days, the method of Huggett and Widdas seems to give more reliable results in this breed. Indeed, during the last month of gestation, differentiation of fetal morphological characters is completed; it is only its size and weight which will increase. Morphological characters become no longer an estimation age criterion. Results of weight and measurements depend on breed, litter size and sexual dimorphism, limiting the application of the methods of Keller and Huggett and Widdas. A very highly significant correlation was found between the crown-rump length and body weight ($r = 0.85$; $p <= 0.0001$). In perspective, it would be interesting to extend this study with a larger number of fetuses of the same breed. Moreover, it would be interesting to establish a formula relating the body weight and the crown-rump length for more reliability. Finally, it would be sensible to carry out a study on a control group of pregnant females.

Keywords: Age, bedouin goat, foetus, runt

Genes Associated with Pathogenicity of Avian Escherichia Coli (APEC) Isolated from Feces and Oropharynx of Backyard Chickens

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The creations of backyard chickens is the main activity performed by small producers in the world. Sanitary problems are the main limiting factor of these creations, which may contribute to the dissemination of diseases to both poultry and the consumer. Among the bacterial agents cause of diseases in birds there is the Escherichia coli pathogenic for poultry (APEC). The mechanisms of virulence have been studied continuously, and are considered to be multifactorial. Thus, understanding the genetic factors that are able to generate the mechanisms may be helpful for detecting pathogenic isolates. The present work aimed to characterize virulence related genes in E. coli isolated from backyard chickens. For that, 250 fecal and 250 oropharyngeal samples were collected. DNA template was prepared from inoculated BHI broth, using a thermal lyses approach. Thereafter, a multiplex-PCR screening was performed for detecting cvaC, hlyF, ompT, iroN, iss and iutA. Those cultures that were positive for any of the genes were employed for the detection of APEC isolates. For obtaining isolates, the positive broths were streaked onto MacConkey plates. After incubation, ten colonies of each plate were inoculated in BHI broth. These isolated were also submitted to a themal lyses and testing my multiplex-PCR for the same genes analysed previosly. From the 500 samples, 139 (27.8%) presented at leat five of the virulence genes. The frequency of genes observed in the E. coli isolates were 66.7% for cvaC, 100.0% for hlyF, 100.0% for iss, 87.5% for iroN, 100.0% for ompT and 88.0% for iutA. The data obtained in this work provide an overall panorama regarding the virulence characteristics of the APEC pathotype isolated from backyard chickens of the region of Ribeirão Preto-SP, Brazil. Therewith, future works, based on these initial characteristics, may be developed. Other tecniques, that allow the comparison between APEC samples, and that may be helpful in the elucidation of the APEC way to develop ilness in distinct hosts, may also be developed.

Keywords: APEC, backyard chickens, escherichia coli, virulence genes

Molecular Detection and Genetic Analysis of *Giardia Duodenalis* in Diarrheal Feces of Calves in Korea

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To identify the infection status of *Giardia duodenalis* in diarrheal feces of calves using ELISA and PCR and to characterize the genetic characteristics of *G. duodenalis*. A total of 671 diarrheal feces of claves were collected throughout the nation during November 2013 to March 2015. For statistical analysis, type of feces, location and season of sampling collected were recorded. To detect *G. duodenalis*, two assays were applied, including PCR and ELISA. DNA was extracted from diarrheal feces using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The ß-giardin gene fragments of *G. duodenalis* were amplified using a nested PCR with two primer sets, G7/G759 and G7n/G759n, as previously described. In addition, ELISA was performed using the Giardia Antigen Test Kit (IDEXX Laboratories Inc., USA) according to the manufacturer's instructions. A $P<0.05$ was considered as significant difference. Two PCR positive samples were sequenced and genetic analysis was performed. Overall, 70 (10.4%) and 75 (11.2%) samples out of 671 samples were found to be positive against *G. duodenalis* using ELISA and PCR assay, respectively (Table 1). The twenty-nine samples showed positivity in both ELISA and PCR. Of the 70 ELISA positive samples, 41 were negative in PCR, and of the 75 PCR positive samples, 46 were negative in ELISA. Significant differences were observed when the results were analyzed according to the types of feces using ELISA, and season of sampling collected using PCR. Of the 75 PCR positive samples, two samples were sequenced. A total of 511 bp from *G. duodenalis* ß-giardin gene was sequenced from the two samples with 100% identity to each other. By genetic analysis, it is revealed that the sequences in this study belong to Assemblage E which is specific to cattle. The results in this study suggest that *G. duodenalis* was associated to calves with diarrhea. Therefore, continuous monitoring to calves with diarrhea is required. Since *G. duodenalis* is prevalent in feces of calves, further study in the aspect of zoonosis is required in animal feces.

Keywords: Cattle, diarrhea, giardia duodenalis

Molecular detection of Giardia duodenalis in 671 diarrheal feces of calves in Korea, according to type of feces, collected location, and season.

			No. positive (%)	
Variable		No. tested	ELISA	PCR
Type of feces	Bloody	67	9 (13.4)*	12 (17.9)
	Watery	258	36 (14.0)*	27 (10.5)
	Pasty	346	25 (7.2)*	36 (10.4)
Collected location	Northern	134	16 (11.9)	12 (9.0)
	Central	157	10 (6.4)	12 (7.6)
	Southern	380	44 (11.6)	51 (13.4)
Season	Spring	126	9 (7.1)	10 (7.9)*
	Summer	240	29 (12.1)	41 (17.1)*
	Fall	117	15 (12.8)	11 (9.4)*
	Winter	188	17 (9.0)	13 (6.9)*
Total		671	70 (10.4)	75 (11.2)

** P < 0.05 is considered statistically significant difference.*

Occurrence of Co-Infection between Avian Pathogenic *Escherichia Coli* and Infectious Bronchitis Virus in Broilers

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The infectious diseases remain the leading causes of the most severe economic impacts on poultry production, especially infections caused by avian pathogenic Escherichia coli (APEC) and infectious bronchitis virus (IBV). These diseases result in significant losses annually for the poultry industry due to growth retardation, and condemnation of broiler carcasses in slaughterhouses. Indeed, most of APEC strains are opportunistic pathogens that usually invade birds following primary infection with other respiratory pathogens such as IBV. Thus, the current study was proposed, aiming to investigate the occurrence of co-infection of APEC and IBV and to characterize the virulence profiles of APEC strains in broilers reared in Brazilian poultry flocks. A set of 105 broilers were sampled at two slaughterhouses for the collection of tracheal, air sac and cloacal swabs as well as heart and liver samples. The air sac, part of cloacal swabs and liver and heart samples were grown in selective media and processed by PCR for the presence of six virulence factor (VFs) genes (*cvaC, hlyF iss, ironN, ompT and iutA*), while the tracheal swabs and the other part of cloacal swabs were examined by RT-Nested-PCR for the detection of IBV. The presence of another set of APEC VF genes (*tratT, iucC; fim H, iuc D; irp2, fyuA, tsh, sitA, papGI, papGII, papGIII, papC, cnf1, hlyA, astA and vat*) were also investigated by PCR. Overall, the results showed that a total of 83 APEC isolates carrying at least five out of six VFs were recovered from all the samples collected from these broilers. IBV was detected in 42 tracheal swabs and in 48 cloacal swabs, and the co-infection between IBV (tracheal and cloacal swabs) and APEC was found in 70.37%, 72.22%, 83.33% and 75% of air sac, cloacal, heart and liver samples, respectively. PCR results showed also a high frequency (>=60%) of APEC virulence genes such as, ironN, iss, iucD, traT, fimH, tsh, and astA in APEC isolates, regardless to the anatomical site in which the initial samples were collected for these isolates (air sac swabs, cloacal swabs, heart or liver). Additionally, some virulence genes exhibited different percentages of occurrence for a specific anatomical site, so that the frequency of *tsh* gene was higher in air sac samples (92.6%), compared to the occurrences of this gene in samples derived from cloacal swabs (16.7%), heart (50.0%), or liver (60.0%). Our findings indicated that the broilers from these investigated Brazilian poultry flocks were co-infected by APEC and IBV in a high frequency and the APEC isolates were carrying relevant virulence genes that can increase the occurrence and severity of colibacillosis in these birds. Therefore it is important that additional studies are conducted to better characterize these pathogens and the co-infection process.

Keywords: APEC, avian coronavirus, chickens, epidemiology, virulence factors

Development and Evaluation of a Inactivated Vaccine against Brazilian Variant Strain of Avian Infectious Bronchitis Virus

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The infectious bronchitis (BI) is an infectious disease caused by avian coronavirus (infectious bronchitis virus - IBV) that is widespread among commercial poultry flocks in the world. There is currently in Brazil, a predominance of infections caused by strains of IBV classified in variant genotype (BR-I), which reveal striking differences in antigenicity with regard to the Massachusetts vaccine strain, routinely used in this country. The consequence is a low cross-immunity and lower level of protection against Brazilian field isolates of genotype BR-I. Thus, the main objectives of this study were to formulate an experimental inactivated vaccine with a variant strain of IBV previously characterized as genotype BR-I and added of a new oil adjuvant and test it two weeks after administration, at one day old, of a commercial attenuated Massachusetts vaccine, followed by the evaluation of humoral and cell-mediated-immune (CMI) responses, as well as the protection status upon challenge with this variant strain of IBV by histopathological examination and quantification of viral load present in the trachea, and kidneys of the vaccinated and challenged birds with this variant strain. The results of this study showed that this immunoprophylactic approach was able to elicit significant increases in the serum and tear levels of anti-IBV antibodies of IgG isotype, and also in the expression of CMI genes, such as CD8β chain and Granzyme A in the vaccinated birds. All these immunological parameters were associated with decreased histological lesions and reduced viral load in the trachea and kidney of vaccinated and challenged birds. It was concluded that humoral and cellular memory immune responses conferred by vaccination with this Brazilian variant strain of IBV combined to a previous vaccination with Massachusetts strain, induced an effective protection against infection with a homologous strain of IBV, and has the potential to provide protection against other strains from the variant genotype BR-I of IBV.

Keywords: Avian coronavirus, cell-mediated immunity, humoral immunity, pathotype, protectotype, variant strain

Prolactin Like-Receptors in *Toxocara Canis* Larvae-2 cells

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Toxocariosis is a zoonotic disease in which the definitive hosts are dogs, mainly puppies. In adult dogs, the larvae of *Toxocara canis* is enquisted in different tissues, during pregnancy, these larvae are activated and transmitted by transplacental and lactogenic way to the breed. Physiologically, one of the most important events in this stage is the hormonal variation, so it has been proposed tat these variations are related to the reactivation of the larvae. This would imply that the larvae are able to recognize this hormonal variations and use them for its development. Because of this, the objective of this study was to identify the presence of prolactin binding proteins resembling receptors in larvae of *T. canis.*. The larvae of *T. canis* were cultured in RPMI-1640 in the presence of 40 ng/mL of prolactin during 20 days. Cells of larvae were disagregated before being marked with the antibody which recognizes the prolactin receptor. As secondary antobody was used anti-rabbit Ig-G coupled with APC and they were analyzed by flow citometry. A group of larvae were incubated with the same antibodies (staining *in toto*) and flourescence was detected by confocal microscopy. The percentage of positive cells to prolactin-like receptor by flow citometry was 13%. Confocal microscopy showed the presence of prolactin-like receptors in the intestine of the larvae. These results suggest that cells of larvae of *T. canis* have prolactin-like receptors.

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Keywords: Larvae, prolactin like-receptor, toxocara canis

Spatial Analysis and Risk Factor to Presence of Streptococcus Agalactiae in Holstein Dairy Herds under Tropical Conditions

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This study evaluated the prevalence of S. agalactiae in areas previously classified according spatial analysis of bulk tank somatic cell counts (BTSCC) and identified risk factors to new and chronic infections based on individual somatic cell counts. The spatial dependence for BTSCC was evaluated using semivariogram. In case of spatial dependence, the values of BTSCC at non-sampled locations were estimated with minimum bias and variance by Kriging method for interpolation data. Determination coefficient (R2) and spatial dependence degree (SDD) were calculated in function of exponential model parameters. The prevalence of S. agalactiae was estimated according to areas previously classified according BTSCC. Logistic regression was performed to identify the risk factors to new and chronic infections based on individual somatic cell counts. Moderate spatial dependence to BTSCC was observed (SDD=37.6 and R2=0.31) with reach until 50 Km.The areas was classified as low (BTSCC<=250,000), medium (250,000<BTSCC<=600,000) and high somatic cell counts (BTSCC>600,000). The general real prevalence was 0.41. The real prevalence in low plus medium BTSCC area and high BTSCC area were 0.16 and 0.93($p<0.05$), respectively. Risk factors to new infections were no chronic infection cow culling (OR=2.39) and no bacteriological exam support (OR=1.92). Risk factors to chronic infection were lactation period (OR=3.85), calving number (OR=1.95), no chronic infection cow culling (OR=2.11) and to buy a new animal and introduce into herd (OR=3.93). Streptococcus agalactiae is an obligate intramammary pathogen, which usually causes subclinical mastitis in dairy cows. This pathogen responds very well to intramammary antibiotic treatment. Blitz therapy involves antibiotic intramammary treatment into all four quarters during lactation to eradicate S. agalactiae infections. These results allow the farmers' association making decision at region and herd level in prevention, control and eradication of S. agalactiae. Additionally, the information generated in these epidemiological studies could be the first step to introduce the surveillance system to S. agalactiae in these dairy herds.

Keywords: Epidemiology, mastitis, streptococcus agalactiae

Correlation Study of Incidence Rate of Human Brucellosis and Bovine Brucellosis Rate during 11 years (2003-2014) in Iran

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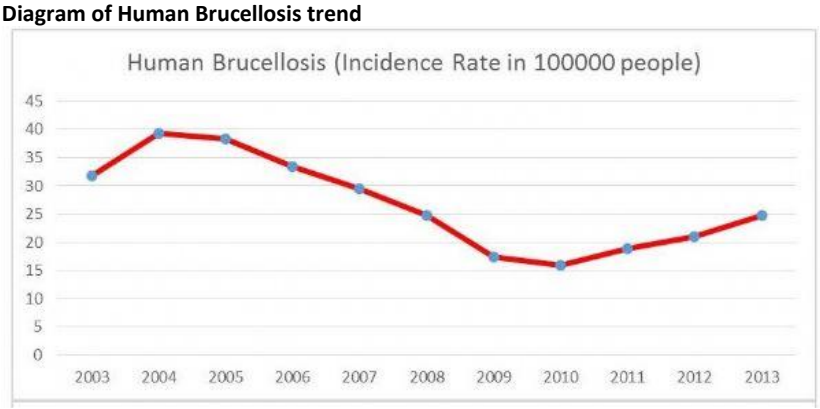
Brucellosis is one of the most important zoonoses and has always received a great deal of attention for its quite possible transmission from animals to human. Several types of brucella bacteria cause lots of economic lost in animal productions and induce Malta fever in human population. During last years, Brucellosis has been controlled in both human and animal populations via some strategies including pasteurization of the dairy products, test and slaughter program in cattle population, and vaccination in sheep, cattle and goat populations. Since the most dairy products are produced from the industrial cattle farms in Iran, the correlation between human brucellosis and cattle brucellosis is a vital aspect of the transmission of this disease in Iran. This investigation is an effort for evaluation of mentioned correlation by an ecological survey in 11 years (21 March 2003 to 20 March 2014) in Iran. Human brucellosis data were provided from the Ministry of Health and Medical Education and the bovine brucellosis data were received from Veterinary Organization of the Islamic Republic of Iran. Analysis by SPSS (version 16) showed a mean of 26.815 (SD=8.24) for incidence rate of human brucellosis and a mean of 0.199 (SD=0.087) for the rate of reactor to test cattle. Positive correlation (ρ=0.816, p=0.002) between human and cattle brucellosis at the confidence level of 95% in the time of study. Based on the other studies, Human and cattle brucellosis are dependent to each other. Similarly, this study had an affirmative concept of controlling cattle brucellosis in industrial farms and continuation of programs in which the reduction and prevention of this disease have been targeted.

Keywords: cattle brucellosis, correlation, human brucellosis, Iran

Diagram of Human Brucellosis trend



Differentiation and Comparison of *Erysipelothrix* spp. Strains based on the *gyr*B Gene Sequencing

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The genus *Erysipelothrix* consists mainly of two named species;
the *Erysipelothrix rhusiopathiae* and *E. tonsillarum*, and, as *E. rhusiopathiae* is a zoonotic bacterium, causing the swine erysipelas and human erysipeloid, differentiating these two species is of a great concern in animal and public health. Usually, those two species has been differentiated based on the serovars. However, molecular genetic techniques demonstrated that some isolates of same serovar might belong to different species, and also that the serovars 13 and 18 might be classified into two new and separate species. In this study, we analyzed the possibility of differentiating the species, serovars and isolates based on the sequence and restriction fragment analysis of *gyr*B gene of strains of this genus and also its diversity and usefulness as a component for molecular phylogenetic analysis. Twenty-seven strains representative of the serovars of each *Erysipelothrix* spp. strains, and 65 strains of serovar 2 isolated from meat samples were used. DNA of the strains was extracted using the Wizard Genomic DNA Purification Kit (Promega). The *gyr*B genes were first amplified using the universal primers described by Yamamoto et al.. Those PCR products were sequenced and specific primers for amplify the *gyr*B gene of *Erysipelothrix*spp. were designed and used in the subsequent sequencing analysis. The *gyr*B gene amplicons were digested with *Afa* Ⅰ and *Nal*Ⅲ and the obtained fragment patterns were used for cluster analysis of the strains. PFGE using*Sma* Ⅰ was also carried out Cluster analysis, based on the *gyr*B gene sequence, divided the 27 serovar representative strains into 4 clusters, each one comprised by strains of each species and serovars 13 and 18. By the *Afa* Ⅰ and *Nal*Ⅲ fragment pattern analysis, the *E. rhusiopathiae* and *E. tonsillarum* strains were clustered into 3 clusters. The 65 meat isolates could not be differentiated by those two restriction enzymes, but by PFGE, they were divided into 9 clusters, and the strains were clustered according to the meat species from which the strain was isolated, and the chicken meat isolates were clustered according to the region where it was produced or according to the retail shop where it was bought. This study shown that *Afa* Ⅰ and *Nal*Ⅲ restriction fragment analysis of *gyr*B gene is able to differentiate the species of this genus, while differences among the meat isolates could not be found. On the other hand, PFGE identified the isolates according to the meat species and according to the origin of the meat, and demonstrated that it might be a useful tool for epidemiological analysis of the *E. rhusiopathiae* isolates of specific animal species meat. The *gyr*B gene sequence variation was also enough to differentiate between the species and also some strains of *E. rhusiopathiae* were grouped into a different sub-cluster. Thus, the *gyr*B gene sequence, in combination of other house-keeping genes sequences, might be useful for detailed molecular phylogenetic analysis of the strains of this genus.

Keywords: Erysipelothrix, erysipelas, swine

Search of Vaccinia Virus in Wild Mammals

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The emergence or re-emergence diseases process are mostly related to wildlife. In this sense, "cowpox" zoonosis caused by Vaccinia virus (VACV), is presented as an important re-emerging disease in Brazil. Since the end of global vaccination program against smallpox in 1980 VACV outbreaks are described in various regions of Brazil. There is little information about their reservoirs, however in 1963, the VACV was isolated in one rodent Oryzomys gender, suggesting that wild rodents act as reservoirs. Although several species of rodents, carnivores and marsupials are seen in areas adjacent to farms, there is a lack of studies on the prevalence of Orthopoxvirus (OPV) in Brazilian mammals. According to this possibility this work had as objective to evaluate the prevalence of VACV in wild rodents and other wild mammals. Forty seven dairy farms located in Midwest region of the State of São Paulo-Brazil, with and without historical of VACV outbreaks were studied. All of these farms had native forest areas. The capture was realized in these native areas for 5 consecutive days. For this three traps of Tomahawk, containing thigh or drumstick chicken as bait, 20 traps Sherman type, baited with a mixture of canned sardines, peanut butter, corn and oat flakes, and 6 pitfall traps type were placed. The captured mice were anesthetized using diethyl ether and other mammals with Tiletamine and Zolazepan for blood samples collection by cardiac or jugular puncture for serologic (serum) and molecular (whole blood) survey of VACV. From 103 serum samples from small rodents, nine (9%) were positive [4 (6%) were from Oligoryzomys nigripes 3 (18%) from Oligoryzomys flavenscens and 2 (15%) from Sooretamys angouya]. Of the 57 sera from Opossums white ear (Didelphis albiventris), 4 (7%) were positive. Of the 16 sera of black ear opossums (Didelphis aurita) 2 (12%) were positive. From 4 Quatis (Nasua nasua) evaluated, 1 (25%) showed seropositivity. Seropositivity was not observed in samples of 4 dogs of the woods (Cerdocyon thous), 1 Ocelot (Leopardus pardalis) and 8 Cuicas (Gracilinomus microtarsus). Blood PCR positivity was obtained in 4 (5%) rodents, 22 (39%) D. albiventris, 6 (37%) D. aurita, 1 (25%) C. thous 1 (25%) N. nasua, and 1 (17%) G. microtarsus. Since the only isolation of VACV in a rodent Oryzomys gender, rodents are suggested as VACV reservoirs but our results show a low percentage of positivity in both the neutralization and in PCR test evaluation. On the other hand although there was a low prevalence of antibodies (Ab) in wild mammals (7% of D. albiventris and 12% of D. aurita,) these species showed higher positivity in the PCR (39% and 37% respectively.) The presence of viral DNA in the absence of antibody titles may suggest an acute infection in which has not yet occurred seroconversion or just a virus contact without the development of an virus infection. At the time of capture no suggestive lesions of infection with OPV were observed. Despite the lack of studies of the prevalence of OPV in wild mammals, few reports refer to zoo animals in Europe or African free life animals, but all describe characteristics injury and often death. Our results do not allow us to state that the Olygoryzomys nigripes, Olygoryzomys flavenscens, Akodon montensis, Sooretamys angouya, Nectomys squamipes, and Calomys tener species, act as a reservoir for the VACV and do not exclude the possibility of other species of wild rodents or marsupials act as reservoirs.

Keywords: Epidemiology, serology, vaccinia virus, wild animals

The Effect of Long-Term Exposure of Mice to 900 MHz GSM Radiation on Healing the Experimental Cutaneous Candidiasis

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Mobile phones are communicating with base stations using 900 MHz microwaves. The current study aimed at evaluating the effects of long term 900 MHz microwave exposure of mice on experimentally induced cutaneous candidiasis. Forty inbred, 5-7 weeks old male BALB/c mice were randomly divided into 4 groups. Candida infected skin lesions were experimentally produced in the lateral-back skin of the 20 mice through repeated inoculation of the yeast. Half of the mice (LCW) were exposed for about 6 hours per day, 7 days per week, to 900 MHz microwave radiation, while the other unexposed half was considered as the control (LC). Two unexposed control groups were also included (N, KC groups). The skin lesions were monitored and the live Candida density of wounds was enumerated. The process was repeated again after 1 week resting interval. One week later, all mice were challenged through intra tail veins using LD90 dose of Candida albicans. Mortality of the mice were recorded and the candida load of the kidney homogenates from died animals were counted. 900 MHz microwave exposed mice had 1.5 days and 3.7 days delay on wound healing in stage 1 and 2, respectively. LCW mice also showed higher yeast loads in skin lesions at days 5, 7 and 9 post inoculations. Survival analysis of live Candida challenged mice showed radiation exposed group is prone to death induced by systemic infection and candida enumeration from the kidney homogenates showed radiation exposed animals have had significantly higher yeast load in the tissue. Overall, long term 900 MHz radiation exposure of mice led to longevity of skin wounds and susceptibility of the animals to systemic challenge and higher incidences of microorganisms in internal tissues.

Keywords: Cutaneous candidiasis, microwave candida albicans, survival analysis

Seroprevalance Oaaf Dourine and Equine Piroplasmosis in Breeding Racehorses between the Years of 2010 and 2014

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Dourine is an venereal disease of horses, donkeys and mules placed in Office International des Epizooties (OIE) notifiable disease list. The aethiologic agent of the disease is a protozoan tissue parasite named Trypanasoma equiperdum. The diagnosis of the disease is carried out by CFT that is OIE gold standard test. No vaccine available and treatment the disease with drugs may result in inapparent carriers. In Turkey, it is compulsory to check the breeding horses if they have dourine. Second disease we have studied on is Equine Proplasmosis that is responsible for important economic losses in the equine industry. Diagnosis of this disease is based on clinical signs, identification of the parasite and serological tests. For this disease, IFAT and c-ELISA are the OIE prescribed tests. Our study was carried out in Veterinary Control and Research Institute, Parasitology Laboratory between the years of 2010 and 2014 with the aim of determining the status of dourine and equine piroplasmosis in thoroughbred breeding racehorses in Turkey. A total of 33475 serum samples were tested with CFT for dourine and 164 serum samples were tested with IFAT and 121 were tested with c-ELISA for equine pirolasmosis. As a result, dourine was tested with CFT and seropositivity was not detected. Equine pirolasmosis was tested with IFAT and 36 of 164 (21%) serum samples were found positive for Theileria equi and 5 (3.048 %) were found positive for Babesi caballi. Out of all 121 serum samples were tested with c-ELISA, 44 (36.36) were found positive for T. equi and 1 (0.082 %) was found positive for B. caballi. In conclusion, we need to be careful about dourine and continue further studies on mules, donkeys, and horses. This study also showed that to control equine piroplasmosis we have to continue effective tick control, seromonitoring of animals and treatment of sick animals in Turkey.

Keywords: Dourine, equine piroplasmosis, horse

Laminitis Observed Following Colic Case and Hoof Disorders in a Horse

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Laminitis resulting in disruption of integrity between dermal laminae of the distal phalanx and epidermal laminae of the hoof wall and causing severe pain and a lameness of characteristics is a complex metabolic disease. Acute gastrointestinal disease (colic), the excessive consumption of feed grains and other diseases that cause endotoxemia occur as sequelae. This study discussed an acute laminitis case begining after severe colic and medical therapy and corrective rasp. The horse turned into chronic stage returned to its athletic performance by appropriate treatment and patient work.

Keywords: Corrective shoeing, horse, laminitis, medical treatment

The Changes in Blood Haematological and Biochemical Parameters after Multiple-Dose Administration of Cefquinome in Horse

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Cefquinome is a member of the fourth-generation of cephalosporins that has been developed for use in animals only. It is active against the *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Moraxella* spp.,*Haemophilus* spp., *oryne bacterium*, *Enterococcus*, *Escherichia coli* and Gram (-) (+) anaerobs. The objective of this study was to evaluate the effect on blood hematological (WBC, LYPMPH, MONO, GRAN, RBC, Haemoglobin, HCT, MCV, MCHC, MCH, PLT) and biochemical parameters (AST, ALT, ALP, Total Bilirubin, Total Protein, Albumin, Creatinine, Urea) after the administration at multiple ascending doses of cefquinome (CEQ) in the horse. The study was performed on the sixteen healthy unmedicated mature horses with age and body weight of 4.6 ± 2.1 years and 302 ± 38 kg (mean±SD), respectively. Animals were randomly divided to four dose groups. CFQ was administered to each horse at dose levels of 1, 2, 4, and 6 mg/kg. Each animal received a total of 13 injections every 12 h at a given dose level. Blood samples for hematological and biochemical analyses were obtained the jugular vein before dosing (0 day) and 1, 3, 7 and 14 days after the administration of first CEQ. There were no significant differences in serum biochemical parameters among dose groups ($p>0.05$). Although significantly differences in some haematological parameters (MONO, GRAN, RBC, Haemoglobin, HCT, MCH, PCT) among dose groups ($p<0.05$), all of the values were within normal limits. These results indicate that CEQ could be safe and well tolerated in horse following the administration of multiple doses of up to 6 mg/kg dose.

Keywords: Blood haematological parameters, biochemical parameters, cefquinome, horse, multiple dose

Age Related Histopathological and Immunohistochemical Changes in Horse Brains

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With the study consists of aging-related pathological findings in horses aimed to evaluate in detail using routine staining and immunohistochemical methods. Firstly the pathological findings were especially compared with previous studies in horses with different and similar aspects. Furthermore the second aim of the study was to compare our findings both human beings and as well as with animal models. In the study 23 old (10>= years old), 7 young (between 3-6 years old) horse brains were examined for macroscopically, histopathologically and immunohistohemically. After death brains were removed and they were fixed in 10% buffered formalin and routinely processed for histological examination by embedding in paraffin wax. Brain tissue samples were collected from telencephalon, mesencephalon, rhombencephalon. Sections were cut 5-8 µm in thickness. Sections were stained by the haematoxylin and eosin (HE). Additional sections were dewaxed and rehydrated by routine methods for immunohistochemical staining method. Glial Fibrillary Acidic Protein (GFAP), ubiquitin, 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase (CNPase), neuron-specific enolase (NSE), β-amyloid protein (Aβ), tyrosine hydroxylase antibodies were used in immunohistochemical staining. Colour labelling was developed by a final incubation step using 3-amino-9-ethyl-carbazole (AEC). Sections were evaulated under light microscope and microphotography was taken. When compared with young brains at aged brains macroscopically only ventricular dilatation was observed, microscopically satellitozis, neuronal vacuolization, status spongiosis, ventricular dilatation and ependymal undulation, calsium deposits and axonal swellings were seen. At old brains immunohistochemically positive labellings were observed with Glial Fibrillary Acidic Protein (GFAP), ubiquitin, 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase (CNPase), neuron-specific enolase (NSE), β-amyloid protein (Aβ), tyrosine hydroxylase antibodies. As a result we observed that aging had a significant effects on brain tissue. The histopathological and immunohistochemical findings encountered in the brain related to aging in horses are similar with earlier studies, other animal species and humans. With further molecular studies pathogenesis of these lesions will be explained in detail.

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Keywords: Aging, brain, histopathology, horse, immunohistochemistry

A Case of Abortion by Equine Herpesvirus-1 in Horse, Korea

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Equine herpesvirus 1 is in the family Herpesviridae, subfamily Alphaherpesvirinae, genus Variellovirus. It is widespread throughout the world and causes respiratory, neurologic and generalized neonatal diseases as well as abortion in horses. We describe a case of abortion by equine herpesvirus-1 (EHV-1) in korea. A aborted foal was submitted to Animal and Plant Quarantine Agency for laboratory diagnostic tests. After necropsy, tissue samples were collected for PCR test. Also the samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, stained with hematoxylin and eosin and immunohistochemistry for histopathology. Grossly, lung was failed to collapse, heavy and rubbery, showed the impressions of the ribs and exhibit a pitting response to pressure. Pus was present in the bronchi. The spleen was enlarged twice than normal. There were 1∼2mm gray-to-white foci in capsule of liver. Histopathologically, the pulmonary interlobular septa are edematous and infiltrated with monocytes and macrophages in lumen. There were acidophilic inclusion bodies in the nuclei of bronchial epithelium and leukocytic infiltration in the necrotic foci in liver. Immunohistochemically, EHV-1 was positive in lung and liver. PCR was positive at EHV-1. This is the first case with antigen positive and pathogenic lesion in Korea, because there were few examinations about abortion cases of horses. Despite of equine herpesvirus vaccination, still we have abortion case of EHV-1.

Keywords: Abortion, equine herpesvirus-1, histopathology, Korea, PCR

Questionnaire Study on Feeding and Managements of Rahvan Horses in Aegean and Marmara Regions of Turkey

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Horse necessity decreases day to day in the modern world but still race horse industries develop. Some countries have traditional horse races like Rahvan. Yorga horses walk in Rahvan style in Kirghiz region. Rahvan horses were breeding in Turkey from the Ottoman times. Nowadays, Rahvan breeding is common in Marmara and Aegean regions as a traditional hobby. Local races are arranged during the year by Non Governmental Organizations, municipality and federation supports. When Rahvan horse is moving, only sound of two steps is heard. The front and back feet on the right of the body move forward together, then the front and back feet on the left move forward together. It is like pacing but the steps are more rhythmic and quick. Riders and horses tire less than the other walking types. When we compare to the race horse industry, Rahvan breeding is very amateur and unique. The aim of this study was to learn about feeding and management practices of the Rahvan horses in Aegean and Marmara regions of Turkey. Target population of this study was Rahvan horses in the Turkey. To have the information about Rahvan horse feeding and management practices in the Turkey, a questionnaire which has 69 questions was designed. The questionnaire was divided into four sections (General information of breeders, farm conditions, feeding habits, nutritional diseases). 40 of the 69 questions were consisted of multiple choices and yes/no questions, 29 were open ended questions (feeding materials, number of the animals etc.). Web based questionnaire software (Google® Docs) was created for the online surveys. With the print outs of the survey, personal interviews were done on 31 August 2014 Golcuk Rahvan Races. Questionnaire was shared with e-mails and social platforms about Rahvan Horses. Questionnaire was online for 10 months. Rahvan horses' breeder and horse population records are not clear. Number of the breeders and horses were estimated by personal communications. Data were downloaded as Microsoft Excel© and evaluated in SPSS 22©. Evaluation was done according to frequencies and percentages. Questionnaire was completed by 50 horse owner from Aegean (62.0%), Marmara (38.0%) regions. 60.0% of the respondents were experienced more than 10 years in this industry. 72.0% of the horse barns contained 1-5 horses. Major source of energy met from oats. Main diets contained alfalfa (60.0%), pasture (56.0%) and oat hay (48.0%). Respondents generally (86.0%) separate foal and mother after 6 months. According to owners average feed consumption of an adult horse is 50 kg/month. They feed horses commonly (56.0%) two times a day. 52.0 % of the owners disburse 0-5.000 TL for feed materials in a year. 10.0% of respondents use corn silage. 26.0% reported that they turn out in all seasons, while the others turn out horses except winter. They usually (76.0 %) prepare concentrated feeds and soak oats and barley (60.0%). Owners reported that, usually they do not use oil (78.0%) and feed additives (84.0%), but they give fruits regularly (68.0%). Respondents never medicate (30.0%) or medicate once in 3-6 months (58.0%) with antiparasitic drugs. 46.0% of the respondents do not rasp their horses' teeth. There are few studies on Rahvan horses in Turkey. This survey presents primarily information about feeding and management practices of Rahvan horses which are one of the heritages from Ottoman to us. To understand these horses, we need more scientific studies and clear data. Owners should be informed about animal welfare and nutrition. Good genetic resources should be protected and reclamation studies performed for this heritage's future.

Keywords: Equine nutrition, rahvan horse, nutritional diseases, pace horse, Turkey, yorga horse

Figure-1 Preferred nutrative condition of Rahvan horses.

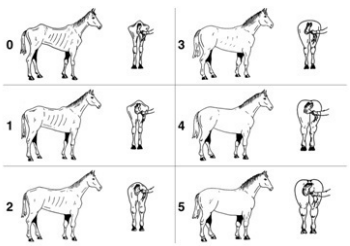

Figure-1 50.0% of the respondents preferred nutrative condition score (NCS) 3, 26.0% preferred NCS 2, 24.0% preferred NCS 4.

Table-1 Feeding meterials' usage (percent).

Animal	Feeding Material	Frequency	Percent
Foal	Fabric feed	5	11.9
	Barley	9	21.4
	Oat	19	45.2
	Maize	1	2.3
	Bitter Vetch	1	2.3
	Tare	1	2.3
	Bran	3	7.14
	Hay-Turn out	42	100
	Bagasse	1	2.3
	Vitamin Mineral Premix	4	9.5
Mare	Fabric feed	2	8.3
	Barley	3	12.5
	Oat	15	62.5
	Hay-Turn out	24	100
Pregnant Mare	Fabric feed	6	18.7
	Barley	10	31.2
	Oat	21	65.6
	Bran	1	3.1
	Corn	1	3.1
	Vitamin Mineral Premix	1	3.1
	Hay-Turn out	32	100
Stallion	Fabric feed	5	17.8
	Barley	2	7.14
	Oat	7	25
	Hay-Turn out	28	100

Table-2 Colic reosans according to respondents.

Cause	Frequency	Frequency
No comment	1	2.0
Drinking water after work or journey	37	74.0
Over feeding	32	64.0
Gastro interstinal parasites	16	32.0
Contaminated feed with other animal's feces	1	2.0
Rotten, mouldinees and bacterial contaminated feed	36	72.0

Malignant Melanoma in a Grey Pony, Korea

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It is proposed that, in grey horses, a disturbance in melanin metabolism and transfer associated with progressive greying of the hair, due to increasing age, results in intra-cellular accumulation of pigment. Excess pigment deposition then stimulates formation of new melanoblasts or increased melanoblast activity resulting in focal areas of overproduction and neoplastic transformation of melanocytes. Melanocytic neoplasms have been reported to represent up to 18.7% of all equine cutaneous neoplasm. These are very rarely seen, but the tumors are malignant and frequently metastasize. We describe here a case of malignant melanoma in a grey pony, Korea. A 16 year-old female grey pony was submitted to Animal and Plant Quarantine agency for diagnosis in Feb 2, 2015. The horse had the masses under the tail and around the anus. After necropsy, parenchymal organs were fixed in 10% neutral buffered formalin, embedded in paraffin wax and stained with hematoxylin and eosin for histopathology. And immunohistochemistry was performed to investigate the expression of PNL2, Melan A, S100 and PGP 9.5 in the mass. On gross examination, multiple pigmented masses, likely melanomas, were detected peri-anally and under the tail. Further metastatic spread to the spleen, liver, lung and lymph nodes was also identified. Histopathologically, anaplastic and pleomorphic melanocytes which may be fusiform or epithelioid in shape and contain much or little intracytoplasmic melanin and melanophages were found in the mass. The tumor may display an interwoven or whorled pattern of fusiform cells, or nests of epithelioid cells with an interstitial, fine, fibrovascular stroma. Sometimes neoplastic cells were observed in the vessels. PNL2, S100 and PGP 9.5 protein were detected, but Melan A was not expressed in the neoplastic melanocytes. In conclusion, veterinarians should recognize that cutaneous melanomas can indeed have life-threatening consequences due to the potential malignant behavior of them. It would be important for veterinarians to find benign cutaneous melanomas in the early stage and treat or excise them to prevent metastasis to other organs.

Keywords: Grey pony, malignant melanoma, metastasis

The First Virus Isolation and Partial Characterization of Equine Herpesvirus-4 in a Horse, Korea

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Equine herpesvirus-4 (EHV-4) along with equine herpesvirus-1 (EHV-1) is alpha herpesvirus with the clinical symptoms of acute respiratory diseases known as equine rhinopneumonitis (ER) in horse. The EHVs have a major economic and welfare impact on all sectors of the horse industry worldwide. In Korea, EHV-4 along with EHV-1 was detected in nasal swabs collected from horses suffering from respiratory diseases by PCR methods in Korea, 2008. But, the isolation or characterization of EHV-4 in Korea to our knowledge has been not reported so far. This study was performed to isolate and characterize circulating EHV-4 in Korean equine populations so that in future they will be used as a material for sero-surveillance and development of vaccine or diagnostics against ER. Nasal swab was collected from in a 2-year-old foal with the acute phase of the clinical course with respiratory diseases in Korea, 2010. E. Derm (ED) cells (ATCC CCL-57) were used for virus isolation. The cells were grown in Dulbelcco's modified Eagle's medium supplemented with 10% fetal bovine serum, 300mg/L L-glutamine and 1X antibiotics-antimycotics solution. The cells were inoculated with the prepared sample when monolayers achieved 70-80% confluence. The cultures were observed daily cytopathic effect (CPE) and were frozen at -70°C when 70% CPE was reached. Further passages were done with prepared material by freezing-thawing methods. The indirect immunofluorescent assay procedure for the detection of EHV-4 antigens in infected cells was performed on the ED cell. The cells were grown on coverslips in 6-well microtiter plates (Nunc). Before reaching confluence, the monolayer was infected with the virus isolate. After observing the appearance of CPE, coverslips were washed with phosphate-buffered saline solution, fixed in frozen 80% acetone solution for 5 min and dried for 30 min at 37 °C. Fixed cells on coverslips were orderly incubated with EHV-4 polyclonal antisera and anti-rabbit IgG conjugated with FITC (Sigma), for 1 h at 37 °C. The coverslips were washed 3 times in PBS and observed specific reactivity using fluorescent microscope. For sequencing of EHV isolate, viral DNA was extracted from nasal swabs using a DNeasy mini kit (QIAGEN) according to the manufacturer's instructions. Extracted DNA was stored at -20 °C before use. The first and nested PCR was performed according to the OIE protocol using the Hotstart master PCR kit (QIAGEN). The amplicon was excised from the gel and purified by using a Gel extraction kit (QIAGEN). The purified DNA was sequenced. Sequence analyses were carried out using CLC Main Workbench software and gB sequences of EHV4 1942 strain previously reported. A Korean EHV-4 strain K001 was isolated in inoculated cells with the supernatant from the nasal swab sample collected from a Thoroughbred. The cells were observed with characteristic viral CPEs after 3 and 4 days of postinoculation by microscopy and exhibited bright intracellular fluorescence. The sequence of the PCR products amplified from the isolate K001 exhibited 99.8% identity in nucleotide and was 99.5% identity in amino acids to the published EHV-4 sequence (M26171). An EHV-4 was isolated in nasal swabs collected in a horse showing respiratory clinical signs. ED cells inoculated with the sample were observed with characteristic viral cytopathic effects after 3 days of postinoculation and the infected cells exhibited bright intracelluar fluorescence by indirect immunofluorescence assay. At the nucleotide level, the partial glycoprotein B gene of K001 isolate had 99.9% identity to 1942 strain (M26171). To author's knowledge, the report describes the first isolation and partial characterization of EHV-4 in Korea. The virus can be used for further study of EHV-4.

Keywords: Equine herpesvirus-4, indirect immunofluorescent assay, sequencing, virus isolation

A Comparative Study of Equine Adipose and Bone-Marrow Derived Mesenchymal Stem Cells

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Recently, significant populations of race horses are put aside because of the chronic sport-related injuries. Several non-cell-based and cell-based methods have been applied to overcome this problem. Considering the mesenchymal stem cells (MSCs) multi-lineage differentiation ability, immunomodulatory properties and growth factor supply, they are promising therapeutic cell sources for a variety of equine diseases (e.g. orthopedic disorders). Although bone marrow is an interminable source of these cells, other sources such as adipose tissue, which contain MSCs, provide preferable advantages. Autologous therapy with MSCs is widely used because it does not result in any significant deleterious effect. However, long MSCs expansion times required for acute injuries treatment or the challenges of quality-quantity paucity of MSCs for elderly patients appear as limitations of treatment with autologous MSCs. In the present study, we aim to reveal the potential of Bone Marrow derived MSCs (BM-MSCs) versus Adipose tissue derived MSCs (Ad-MSCs) in regenerating an equine orthopedic injury. Before proceeding to equine MSC bank, these two cell sources were fully characterized. Cell surface markers such as CD90, CD44, CD45 and CD31 were determined by flow cytometry for both cell types. Adipogenic and osteogenic differentiation abilities were assessed by incubating cells in osteogenic and adipogenic mediums, respectively and evaluated by specific staining. Real-time quantitative PCR was further used to quantify gene expression of differentiation markers before and after subjecting the cells to differentiation medium. We investigated the proliferation of mismatched lymphocytes by Mixed Lymphocyte Reaction (MLR) assay and then analyzed suppressive properties of MSCs in the reaction. Moreover, the migration potential was assessed by spheroid culture. Beyond 85% expression of CD90 and CD44 and no expression of CD45 and CD31 in both cell types were confirmed by flow cytometry data. Gene expression analysis in Ad-MSCs and BM-MSCs indicated that while the adipogenic differentiation in Ad-MSCs was higher than BM-MSCs, osteogenic differentiation ability of BM-MSCs was stronger than Ad-MScs. Both cell types decreased proliferation of mismatched lymphocytes approximately 40% in comparison with control group. The migration potential analysis showed no significant differences in both cell types. Immunophenotypic characterization analysis of the cell surface markers in Ad-MSCs and BM-MSCs allowed us to consider them as progenitor cells. Considering anti-inflammatory properties of Ad-MSCs and BM-MSCs and their ability to differentiate, they are promising candidates that can be used in equine regenerative medicine. But dissimilarities in adipogenic and osteogenic differentiation between them may influence on therapeutic use.

Keywords: Cell surface markers, gene expression, horse, MSC, regenerative medicine

Topographical Study of the Brachiocephalic Trunk in Donkey

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The donkey or ass (Equus Asinus) is a domesticated member of the horse family, Equidae. The wild ancestor of the donkey is the African wild ass, E. africanus. The donkey has been used as a working animal for at least 5000 years. In the donkey, Brachiocephalic trunk originates from the craniodorsal of the convex part of the aortic arch. It supplies the head, neck, forelimbs and cranial part of the thoracic cavity. In this research topographical location of brachiocephalic trunk arising from the aorta and its ramifications have been studied. Ten donkey over four years were used in this study. Animals were first anesthetized with a mixture of ketamine and xylazine. Animals were then killed. Seven donkey were embalmed and others were studied in fresh condition. The thorax was dissected and the topographical location of brachiocephalic trunk was determined relative to the ribs and intercostals spaces. The distances between branches were measured using ruler and caliper. The results showed that there are general similarity and some topographical variation between donkey and horses.

Keywords: Arteries, brachiocephalic trunk, donkey, equus asinus, ramifications

Distribution of Gastrointestinal Disorders in an Austrian Equine Hospital Population (2003-2014/15)

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Gastrointestinal (GI) problems are one of the most common disorders of horses.Objectives and Methods: In this study, equine GI cases from 13.03.2003 till 02.02.2015 kept in the Orbis® Vetware Database of the Vetmeduni Vienna were analysed. Diagnosed disorders had been entered by the clinicians using the SNOMED (Systematized Nomenclature of Human and Veterinary Medicine) code. In total 1852 horses were referred to the equine hospital, 141 of them were hospitalised on two or more occasions for a GI disorder. Results: On average this is about 13 new GI-cases per month and the total average monthly cases load was 14. The gender of the population was of 885 (48%) females and 967 (52%) males, mainly geldings.Mean age was about 11 ± 7 years. With a prevalence of 65% non-strangulated colic was most common. In about 55% of all GI disordersa large bowel obstruction or strangulation was involved, while in another 12% both small and large bowel seemed to be involved, mainly obstructed.In just less than 3% small bowel obstructions or strangulations were involved.Gas colic, mainly ceacal tympani was found in less than 6% of the GI cases.Horses with functional gastric disorders or EGUS comprised in total 300 primary cases, this was 16% of the total population with GI problems. Next to primary diagnosis, gastric problems, however, were often diagnosed as second or third diagnosis in the course of an episode of colic.Only 1 % of the submitted cases had oesophageal problems.Spontaneous and iatrogenic ruptures and other trauma made less than 4% of the GI cases, whereas 3.5% of the submissions were diagnosed with enteritis, colitis and entero-colitis. Nearly 49% of the cases were warm blood type horses. The lowest case-load was in February with 5% of all cases, whereas the highest load was in July and October with both about 10% of all cases. The differences between the other months were not more than 3%. By June 44% of the annual GI cases had been submitted to the hospital, while the small majority of the cases were referred in the 2nd part of the year. In conclusion, The large data baseprovidedsuitable general information. Small bowel problems were less common compared to large bowel problems. This study showed that there were no obvious differences in gender distribution and also horse type distribution agrees with the common horse population around Vienna. Except for February, monthly differences in case load were small. For identification of risk parameters this data base provided too little information and needs to be supplemented with more case details.

Keywords: Gastrointestinal disorders, horse, orbis®, SNOMED, vetware

The First Report of Systemic Infection with Halicephalobus Gingivalis in Two Lipizzaner Horses from Southeastern Europe: Clinical, Pathological and Molecular Characterization

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Halicephalobus gingivalis (H. gingivalis) is a facultative parasite of horses that has the potential to be pathogenic. Two Lipizzaner stallions, a 14-year-old and 10-year-old respectively, from two different national stud farms were euthanized on separate occasions due to severe and rapidly progressive neurological symptoms, suggesting multifocal lesions: behavioral abnormalities, then severe ataxia and multiple cranial nerve deficits, progressing to reduced mentation status, with no response to any symptomatic treatment. The bodies were submitted to Pathology department of UASMV Cluj-Napoca (Romania) for necropsy. In both cases, the kidneys were bilaterally enlarged with multiple, large, firm, white to gray, coalescing, varying from 2-7cm in diameter nodules replacing more than 70% of the renal parenchyma. Urine samples from both horses contained numerous rhabditiform nematodes consistent with H. gingivalis. Severe, multifocal, chronic necrotizing and granulomatous nephritis, lymphadenitis and pneumonia, multifocal cerebral malacia and encephalitis with intralesional H. gingivalis had been found at histopathological examination. H. gingivalis was also confirmed by PCR. Pathological findings allowed to establish a post mortem diagnosis of systemic infection with H. gingivalis in horses, which to the author's knowledge is the first report of this disease in southeastern Europe. The monitoring of this neurohelminthiasis worldwide might be important due to the possibly fatal risks posed to horses and humans.

Keywords: Horse, h. gingivalis, PCR

Analysis of Coexistence of Heart Valve Insufficiencies and Arrhythmias in a Clinic Population

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We aimed to determine the characterisation of coexistence of heart valve insufficiencies and arrhythmias in a clinic population. A total of 357 horses in 11 years were examined for cardiac arrhythmias and heart-valve defects at the University of Veterinary Medicine, Vienna, Austria. Case histories were screened and data from 348 horses could be used for further analysis. In total, these 348 animals suffered from 477 different cardio-pathological disorders. Nine patients have been excluded because reporting failures. The data of the remaining 348 horses were categorized into groups with regards to breed, age and gender in order to determine potential risk factors for heart-valve defects and certain cardiac arrhythmias. This study population contained 256 male horses, 91 females and 160 were of adult age. Mitral valve insufficiency (MVI) was reported in 124 animals (63%), aortic valve insufficiency (AVI) in 101 (51%), tricuspid insufficiency (TVI) in 78 (39.6%) and pulmonary valve insufficiency (PVI) in 17 animals (8.6%). Cardiac arrhythmias were found in 130 horses (37%). The horse types that were most frequently affected by a cardiac-valve insufficiency were the warm bloods with a prevalence of 68%, followed by Thoroughbreds with 23%. The prevalence (46%) was highest among animals between 6 and 18 years of age. There was a significant negative relationship between the occurrence of MVI and AVI (Odds ratio: 0.29). AVI was significantly negatively associated with TVI. The most frequent coexistence between of atrial fibrillation and a valve disorder was for MVI (10 out of 38), followed by the TVI (8 out of 38). Findings were significant for the subgroup with cardiac arrhythmias, but were not significant in connection with the entire study population of 348 horses.

Keywords: Arrhythmia, analysis horse, heart valve insufficiency

Dissociative Anaesthesia in Foals for Umbilical Herniorrhaphy under Field Conditions

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In foals, one of the several cases that require treatment under general anaesthesia on the farm and in the field, is umbilical hernia. Many researchers have used the tiletamine-zolazepam combination in horses and foals. Nonetheless, the majority of these studies has been conducted in a clinical environment with no surgical intervention performed in the animals. Thus, the cardiorespiratory effects of the preparations were determined in healthy horses and foals. The aim of the present study was to investigate the effects of a dissociative anaesthetic combination of xylazine-tiletamine-zolazepam, administered for the umbilical herniorrhapy of foals under field conditions, on certain cardiorespiratory and clinical anaesthesia parameters. Eleven foals diagnosed with umbilical hernia, of 4-7 months of age (mean age 5.73±0.91 months) and 130-175 kg body weight (mean body weight 152.55±14.35 kg), 7 of which were female and 4 were male, and 8 of which were Arabian horses and 3 were English horses, constituted the material of the study. The anaesthesia protocol was xylazine (1.1 mg/kg iv), tiletamine-zolazepam (1.65 mg/kg iv) and half of the indicated doses after observation of the first signs of recovery 3 times at 8-10 minute-intervals for sustainment, together with the subcutaneous injection of 12 ml of 2% lidocaine into the periphery of the hernial sac in a circular pattern for local anaesthesia. In all foals after last drug injection; anaesthesia induction time, operation time, anaesthesia time and standing time were recorded. Quality of induction, anaesthesia/analgesia and recovery were evaluated. Heart rate, respiratory rate, body temparature, arterial oxygen saturation values, mean arterial blood pressure were evaluated before anaesthesia (as a baseline) and after induction of anaesthesia at 15, 30, 45, 60 and 90 minutes. It was determined that, under field conditions, the anaesthesia protocol applied to the foals for umbilical herniorrhapy did not cause any mortality. Excluding recovery from anaesthesia, the anaesthesia protocol followed did not cause any adverse effect on the clinical anaesthesia parameters of the foals. During recovery from anaesthesia, it was observed that the foals made multiple attempts to regain standing position. After induction of anaesthesia cardiopulmonary parameters and body temparature were decreased below baseline values in first stage of anaesthesia and then they were reached to baseline values in late stage of anaesthesia. In previous studies carried out in horses and foals by administering different doses of the anaesthetic combination of xylazine-tiletamine-zolazepam for the induction of general anaesthesia; anaesthesia induction time was reported to range between 34-75 seconds, the duration of anaesthesia between 22.5-35.7 minutes, and the period of regaining standing position (standing time) between 30-50 minutes. Furthermore, it has been indicated that, if required, the duration of anaesthesia could be prolonged by administering half or one-third of the initial doses of the anaesthetics used for sustainment, yet, in such cases, complications such as serious hypoxaemia, myositis and delayed recovery from anaesthesia could be encountered as a result of cardiovascular/pulmonary depression. In the present study, the clinical anaesthesia parameters resulting from the anaesthesia protocol applied was found to be in compliance with the findings of the studies referred to above. It was ascertained in the present study that, the supplementation of the combined use of xylazine-tiletamine-zolazepam in foals under field conditions with local anaesthetics induces an anaesthesia of adequate depth for umbilical herniorrhaphy, and sustaining doses enable the prolongation of the anaesthesia period with cardiorespiratory adverse effects remaining within acceptable limits.

Keywords: Foals, herniorrhaphy, umbilical

Recombinant Production of the p26 Protein from Equine Infectious Anemia Virus: Its Use for Diagnostic Tools

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Equine infectious anemia (EIA) is a disease produced by a lentivirus (EIAV) with an almost worldwide distribution. Lentiviral genomic and antigenic variation is the major obstacle to EIA vaccine development, so the diagnosis is the essential tool for controlling the disease. Cuban Entrepreneurial Group LABIOFAM, produces the antigen-antibody kit for EIA diagnostic by Agar gel immunodiffusion (AGID). The antigen is obtained from the spleen of experimentally infected horses with the EIAV Wyoming strain, which is an expensive production. The major viral capsid protein p26 is a highly conserved and immunogenic molecule, used as antigen for diagnosis of EIA. In this work, the cDNA encoding p26 was amplified by RT-PCR using total RNA from the spleen of one experimentally infected horse with EIAV, Wyoming strain. The cDNA encoding p26 protein was inserted into a vector designed for the expression in Escherichia coli. The recombinant protein p26 was purified by affinity chromatography on metallic chelate with a purity greater than 85%. The evaluation of recombinant protein p26 as antigen for the diagnostic by AGID showed a 100% of diagnostic specificity and sensitivity (kappa index=1) in comparison with the antigen of the diagnostic kit LABIOFAM. These results indicated that the recombinant protein p26 could be useful for diagnostic purposes and also we will introduce a new technology of antigen production more economical and efficient.

Keywords: Agar gel immunodiffusion, equine Infectious anemia virus, escherichia coli, recombinant p26 protein

Stress Responses of Stallions during Long Transport Period: the Variations of Complete Blood Count and Serum Biochemistry

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Horses throughout their lives may have been transported for several reasons involving breeding, military and/or ceremonial actions, competitions, pleasure, scientific purposes and slaughter. Transformation of the modes in transporting horses has progressed up to date and shipping or railway is the most preferred transportation methods. A satisfactory and suitable transportation environment for a horse provides for, physical and thermal comfort minimal disease/maximum health conditions, and physiological and behavioral requirements. Each of these four areas can be a potential source of stress for the horse. The veterinary literature of documents an elevation in trailering accidents, ailments, and other related stress occuring during transporting horses. In the present study the objective was to determine the effect of one transport journey on some stres indicators in horses. Eleven mature horses (all stallions) were enrolled to assess the pato-physiological responses of 19 h of transport in a commercial van under Nevsehir, Turkey summer conditions. Travel commenced on June 2007, for 19 h, with a total of 867 km travelled. Blood samples were withdrawn before and after journey on each horse, respectively. Horses were weighed and rectal temperatures recorded. Total and differential leucocyte counts, serum biochemistry panels (glucose, BUN, creatinin, total protein, albumine, total bilirubine, ALT, AST, ALP, CK, LDH and GGT), Na, K, Cl, Ca and serum cortisole levels were measured in 11 stallions of dressage horses. After 19 hours of transport RBC, Hb and Hct levels exhibited variations with increases (P<.05) and MCHC exhibited variations with slight decrease (P<.05). Mean daily glucose total protein, albumine, AST and CK levels showed increases (P<.05). Mean serum cortisol levels, Na, K and Cl levels showed variations with increases (P<.05), whereas Ca levels showed significant decreases after transportation (P<.05). These data clearly showed stres responses of stallions undergoing 19 hours of transport period including the variations of complete blood count and serum biochemistry.

Keywords: Biochemistry, hematology, stallion, stress, transport

Antibiotic Susceptibility and Molecular Identification of Antibiotic Resistance Genes of Staphylococci Isolated from Bovine Mastitis in Algeria

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The objective of this study was out to investigate the phenotypic and genotypic identification of in vitro antimicrobial susceptibility of 21 Staphylococci (10 Staphylococcus aureus and 11 Coagulase Negative Staphylococci) isolated from bovine mastitis to 12 antimicrobial drugs frequently using in veterinary medicine in Algeria. Isolates were tested for antibiotics with disc-diffusion method according to the National Committee for Clinical Laboratory Standards guidelines in the Mueller-Hinton agar, and resistant genes mecA, blaZ, aac-aph, ermA, ermC, tetK and tetM were detected by PCR. Staphylococci isolates showed high resistance to penicillin (95.23%), oxacillin (80.95%), clindamycine (80.95%), and erythromycin (76.19%) but, no resistance of all these strains was detected for gentamicin. Among 21 isolates of Staphylococci, 20 were found to be methicillin and multidrug resistant. The distribution of antibiotic-resistant genes was mecA (100%), tetM (100) followed by blaZ (42.85%). In the present work, the significant determination was the high prevalence of methicillin-resistant Staphylococci. The finding of methicillin-resistant staphylococci (MRS) from bovine mastitis is the first report in Algeria and revealed the status of resistant isolates in herd that might be helpful in treatment, controlling of resistant strains and for deciding culling of cows.

Keywords: Antimicrobial susceptibility, bovine mastitis, resistance genes, staphylococci

Effect of Catechin Administration Together Diet of Belgian Malinois on Performance, Fecal Quality, Mineral and Malondialdehyde Levels

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This study was performed to determine the effects of catechin administration on performance, fecal quality, mineral, and malondialdehyde (MDA) levels in Belgian Malinois puppies. In this study, 12 Belgian Malinois puppies at 6 months of age were used. In the study, the addition of catechin to diet was not observed a significant effect on live weight, feces pH, DM, diethyl ether extract, crude cellulose, ash, and feces score (P>0.05). Catechin administration significantly decreased plasma MDA concentrations (P<0.05). Blood serum Ca, Zn, Cu, Co, Fe, Mg, Al, K, Cr, Mn, and As levels were not affected by the catechin (P>0.05); however blood serum Se levels were determined at a higher level (P<0.05) compared to the control group. The levels of Zn (P<0.05), Mg (P<0.01), and Cu (P<0.05) in the feces significantly increased but there were no statistical differences in the levels of other minerals (P>0.05). As a result, administration of catechin together with the diet of Belgian Malinois puppies at 6 month of age revealed antioxidant effects which did not have any negative effects on body weight, feed intake, fecal quality, and measured serum mineral levels, with the exception of Se. However the concentrations of Zn, Mn, and Cu minerals in the feces of dogs were increased by catechin supplementation.

Keywords: Antioxidant, belgian malinois, catechin, feces, mineral

Effect of catechin supplementation on body weight, fecal characteristics, and malondialdehyde levels of plasma

	Control group	Catechins group	
Parameters	Mean±SEM*	Mean±SEM	P value
Initial body weight, g	11.54±0.20	12.10±0.18	0.430
Final body weight, g	15.32±0.31	15.20±0.45	0.270
Fecal pH	5.98±0.14	6.26±0.16	0.230
Fecal DM, %	23.30±0.92	24.37±2.43	0.550
Fecal diethyl ether extract,%	1.77±0.59	1.51±0.27	0.730
Fecal CC,%	1.45±0.21	1.63±0.11	0.245
Fecal ash,%	6.19±0.68	5.73±0.88	0.684
Fecal score	2.4±0.20	2.2±0.40	0.342
Fecal score >2.5**	2	1	
MDA, nmol/ml (in plasma)	3.21±0.22	2.48±0.16	0.018

**SEM = Standard error of mean, n=6 **Numbers of dogs in each treatment group (n=6) above the ideal fecal score range In the study, adding catechin to the diet did not have a significant effect on body weight, fecal pH, DM, diethyl ether extract, crude cellulose, ash, and fecal score (P>0.05). Addition of catechin to the diet significantly decreased plasma MDA concentration (P<0.05)*

Effect of catechin supplementation on mineral content of serum

	Control group	Catechins group	
Parameters	Mean±SEM*	Mean±SEM	P value
Ca, ppm	17.57±3.06	21.59±1.84	0.294
Mg, ppm	7.65±1.02	6.70±1.26	0.576
Fe, ppm	2.46±0.61	2.38±0.71	0.936
Zn, ppm	0.43±0.08	0.35±0.10	0.513
Cu, ppm	0.51±0.04	0.37±0.07	0.125
Se, ppm	0.048±0.01	0.112±0.01	0.016
Cr, ppm	0.02±0.007	0.02±0.004	0.776
K, ppm	156.98±30.10	216.10±17.85	0.130

**SEM = Standard error of mean, n=6*

Measurment of Aflatoxin M1 Concentration in Milk of Dairy Farms in Varamin,Tehran Province

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Milk contaminated with aflatoxins is a potential danger to human health. Aflatoxins are a major group of mycotoxins (Mycotoxins) which are produced following the growth of some species of Aspergillus flavus and Aspergillus parasiticus, particularly in agricultural products. Aflatoxin B1 is the most toxic member of the family, and is considered as the most abundant aflatoxin in feed. B1 is metabolized in the body and changes to M1. Aflatoxin M1 is secreted to milk and remains there since the process of pasteurization and sterilization has little effect on this metabolite. The maximum allowable limit of 50 ng/L L is accepted by the European Union, which is accepted by Iran as well. In the present study, 221 samples of raw milk were collected from four dairy farms in Varamin region, Tehran province. The samples were transferred to Central Veterinary Laboratory, rapidly. The samples were placed in a refrigerator for 2 hours and then were centrifuged at 2000 g for 5 min. The fat layer was removed and M1 levels were measured using ELISA kits from Euro Clone, Italy. M1 levels were measured in the milk samples from the four studied farms as follows: 2.5 ± 0.7ng/L and 54.6 ± 13.7 ng/L, 11.3± 2.5 ng/L, and 6.6±1.3 ng/L, respectively. ANOVA showed that there was a significant differnce ($P<0.05$) between the M1 concentrations of the studied farms. Dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and proteins. According to results obtained, the M1 concentration was above the recommended maximum allowable value offered by Institute of Standards and Industrial Research of Iran. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination and animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control.

Keywords: Aflatoxin, M1, milk, varamin

Study on Infestation of Ready to Cook Hamburgers to Sarcocystis spp. in Savadkoohs' Delis

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Sarcosistis is one of the common protozoa in cow. Since the human as a final host of this protozoa become infested when undercooked, cyst-laden meat and meat products is consumed. The prevalence of intestinal sarcocystosis in humans is low and, except in immunocompromised persons who ingest large numbers of sarcocysts. Illness in human associated with nausea, stomach ache, and diarrhea. In Iran, beef meat is used for preparation of 70 % of hamburgers and infestation of cattle to sarcocystosis was reported in many investigations. in this study the abundance of sarcosystis spp. has investigated. 83 frozen raw hand made and industrial hamburgers which was ready to cook were sampled from two town of Savadkooh region, Polesefid and Zirab deli. Samples obtained from March to August 2014. After thawing, samples were tested with Dab smear method and Acid-pepsin digestion technique. Results analyzed with MSTAT-C software. Results showed that infestation of hand made hamburgers (50.00%) more than industrial (39.075%). On the other hand, testing of sample with Acid-pepsin digestion technique Showed more infestation (70.00%) than Dab smear method (15.625%). Comparing of results concluded that there was significant difference between two methods and two towns and two kinds of hamburgers (P<0.05). This study showed that Acid-pepsin digestion technique is more accurate and attributable than Dab smear method.Also infestation of hand made hamburgers more than industrial.

Keywords: Acid-pepsin digestion technique, dab smear method, hamburgers, sarcocystis spp., Savadkooh

Figure 1

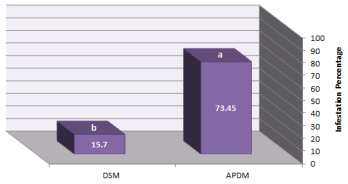

Figure1-Comparison of infestation percentage of Sarcosystis spp. in ready to eat hamburgers of Savadkooh's delis by using of two detection methods.(APM=Acid-Pepsin Digestion Method and DSM= Dab Smear Method) (P<0.05)

Figure 2

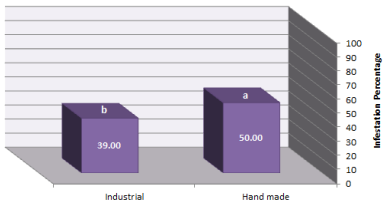

Figure 2-Comparison of infestation percentage of Sarcosystis spp. in ready to eat hand made and industrial hamburgers of Savadkooh's delis.(P<0.05)

Multiplex Real-Time PCR Method for Rapid Diagnosis of Sepsis at Meat Inspection Center

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In meat inspection, cases difficult to judge only with gross inspection are put in hold and subjected to microbiological inspection, in which sepsis is the most common diagnosis. The most common bacteria separated and identified from animals suspected for sepsis and others at our center included *Streptococcus suis*, followed by *Trueperella(Arcanobacterium) pyogenes*, the genus *Streptococcus*, the genus *Staphylococcus*, and *Erysipelothrix rhusiopathiae*. For rapid and concurrent detection of these three bacterial species and two genera, we devised an inspection method based on multiplex real-time PCR, by utilizing a method to identify amplification products by melting temperature (Tm), and incorporating an internal amplification control (IAC) required to prevent pseudo-negative results. In this study, we evaluated this method. Setting the three major bacterial species and the two genera that as the target bacteria, we established a multiplex real-time PCR system using two primer sets: Set A for the detection of the three bacterial species and an IAC, and Set B for the detection of the two genera and the IAC. For the evaluation of this method, we performed multiplex real-time PCR for 25 strains of 10 bacterial species from our culture collection and measured the Tm values. Then we practically applied this method for bacteria separated through biological inspection of 10 specimens (four cows and six pigs) from suspected animals of sepsis, and compared the results with those from identification kits. As a result of melting curve analysis using the bacteria from the collection, we obtained following Tm values: 74.45-74.83 °C for *E.rhusiopathiae*, 82.01 °C for *T. pyogenes*, 87.47-87.59 °C for *S. suis*, and 78.32-78.59 °C for the non-target bacterium (IAC) in Set A, and 81.66-82.60 °C for genus *Streptococcus*, 85.43-86.25 °C for genus *Stapylococcus*, and 77.32-78.19 °C for the non-target bacterium (IAC) in Set B. In each set, the Tm value for each of the bacterial species, genus, or non-target bacteria was apart from those of others by more than 2.5 °C, allowing clear discrimination from each other. The results obtained from our method and those obtained from identification kits regarding the isolated bacteria from 10 suspected sepsis specimens were consistent with each other, which means our method was fully capable of making a judgment using isolated bacteria from specimens on hold. In microbiological inspection in meat inspection, the diagnosis of sepsis is made when an identical bacterium is isolated from multiple organs and others of an animal through minimum biochemical identification tests, by the second day after the start of the inspection. For the final identification of bacterial species, it takes more than five days from the start of the inspection. Our new concurrent detection analysis method for five target genes of causal bacteria of sepsis, which is based on multiple real-time PCR with melting curve analysis, is a rapid test method just requiring less than three hours and simple operation. Thus, this method will serve as a proper diagnostic tool for meat inspection laboratories.

Keywords: Causative bactera, internal amplification control, meat inspection, multiplex real-time PCR, sepsis

Molecular Typing of *CLOSTRIDIUM Perfringens* Isolated from Turkey Meat by Multiplex PCR

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Clostridium perfringens is sporeforming pathogen that responsible for gas gangrene, food poisoning, and diarrhea in humans as well as for enterotoxemia and hemorrhagic gastroenteritis in animals. It's virulence is related with the toxins which are used for the typing of the bacteria. The alpha (α), beta (ß), epsilon (ε), and iota (ι) toxins are the major lethal toxins produced by the organism. In addition to the major lethal toxins, a few number of *C. perfringens* strains produce a *C. perfringens* enterotoxin (CPE), which is responsible for the *C. perfringens* type A food poisoning. The objective of this study was to determine the toxin gene profiles of *Clostridium perfringens* isolated from turkey meat samples. For this purpose, a total of 22 *C. perfringens*, isolated from 180 turkey meat samples collected from different supermarkets located in Ankara over a year period. Isolates were analyzed for the detection of alpha (*cpa*), beta (*cpb*), beta 2 (*cpb2*), epsilon (*etx*), iota (*iA*) and enterotoxin (*cpe*) toxin genes by multiplex PCR. All 22 turkey meat *C. perfringens* isolates were found to carry the *cpa* gene, so all the isolates were confirmed as *C. perfringens.* However, the *cpb, cpb2, etx, iA* and *cpe* genes were not detected in any of the isolates. According to the multiplex PCR analysis, results showed that all the isolates were *C. perfringens* type A and *cpe* negative. In recent years, many authors have focused on the molecular typing of *C. perfringens* by the detection of toxin genes using multiplex PCR. Several studies reported that type A is the predominant type in poultry. This study is the first about the detection of toxin genes of *C. perfringens* using multiplex PCR in turkey meat samples in Turkey. Our results indicate that, *C. perfringens* type A is the most common type in turkey meat. Also multiplex PCR is effective and rapid method for the detection of toxin genes of *C. perfringens*. In our study, all types of isolates were negative for *cpe*, this result might be due to the rare detection ratio (0–5%) of *cpe* positive *C. perfringens*.

Keywords: Clostridium perfringens, toxin genes, turkey meat

Prevalence Study on Diseases Observed on Sheep for Slaughter and on the Causes of Exclusion of the Meat for Human Consumption

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The goal is to collect clinical and anatomic-pathological evidences in the slaughterhouse and analyse the capacity of the inspection to reveal diseases and other conditions recognized as a ground for exclusion of sheepmeat from human consumption. This study investigates the role of the slaughterhouse as an epidemiological observatory. The study has been conducted for 45 months on sheep slaughtered at the plant IT034MCE current in Italy in the Province of Teramo from 01.04.2011 to 31.12.2014. The data of the results of inspections and decisions concerning live animals and meat have been recorded according to the requirements of collection provided for by EC Reg. 854/2004 in a database. There have been measured the prevalence of diseases clinically detected during the ante-mortem and the post-mortem inspection, and that of the other conditions of the exclusion of the meat for human consumption. The observed sheep population is composed of 45846 sheep. The origin of the animals is due to the territorial area of the slaughterhouse only in a small proportion (25-30%), the rest has national or EU origin. For each condition, in addition to the absolute number of cases, it has been calculated the prevalence compared to the animals inspected and the results are summarized in Tables 1 and 2. There have been considered "cases" all those ones resulting from the inspection and reported by the official veterinarian according to the EC Reg. 854/2004 subject to the exclusions from the human consumption. Ante-mortem inspection: it has identified dead animals or those with clinical signs of generalised disease or emaciated animals in no case it was zoonosis nor any disease of sheep for which there are provided for animal health measures. Post mortem inspection: it has allowed the identification of animals suffering from hydatid disease (zoonoses) and other diseases, none of those for which there are veterinary police measures. Decisions concerning the meat: the total exclusion from the consumption concerned the dead animals and those with generalised disease or emaciated animals, while for other diseases localized to individual organs or systems the exclusion was partial. In 1968 cases the exclusion from the consumption of the meat concerned technological errors. The Tables 1 and 2 summarize the quantitative results of the controls in both: the first column indicates the disease or the reason of the exclusion and the second one the number of cases (absolute / prevalence). The slaughterhouse is still a point of epidemiological observation essential to the safety food system, but it reflects the epidemiological situation of populations of heterogeneous origin. The routine inspection as contemplated in the Reg. EC 854/2004 together with the food chain relevant and complete informations effectively pursues the objectives of consumer protection. However, only in the case of animals suspected of disease that may adversely affect human health or animal health, there are a detailed examination and collateral laboratory tests to obtain an etiologic diagnosis. In other cases the decisions are taken on the basis of clinical and pathological diagnosis, this reduces the role of epidemiological observatory of the slaughterhouse compared to non-zoonotic diseases or for which there are no measures of veterinary police. This reveales that control in slaughterhouses is an effective tool for protection against zoonoses and diffusive animal diseases, although about 48% of slaughtered sheep proves suffering from some disease, however, of these 98% had localized processes (76.50% no parasitic zoonotic and 21.50% results of nonspecific inflammatory processes).

Keywords: sheep, meat, inspection, slaughter

sheep carcasses

the health mark is applied only to sheeep having undergone ante-mortem and post-mortem inspection

Inspection results

1. favorable outcome inspection	23733/51,08%
2. cases of animals with disease	22113/48,02%
2.a. generalised disease (septicemia, pyaemia, toxaemia or viraemia)	13/0,02%
2.b. emaciated animals	1/0,01%
2.c. hydatid disease (zoonosis) Echinococcus granulosus	251/0,54%
2.d. distomatosis liver (Fasciola aepatica, Dicrocelium lanceolatum)	5451/11,84%
2.e. parasitosis by nematodes	11464/24,93%
2.f. nonspecific acute pneumonia	105/0,22%
2.g. nonspecific acute hepatitis	67/p.0,14%
2.h. nonspecific chronic inflammatory processes of the lung and / or the pleura	3122/6,80%
2.i. nonspecific chronic inflammation of the liver and / or the capsule of Glisson	1161/p.2,50%
2.j. nonspecific chronic inflammation of the heart and / or pericardium	66/0,13%
2.k. chronic degenerative processes of the liver	391/0,84%
2.l. other nonspecific diseases	21/0,05%

Column 1: Inspection results on 45846 sheep; Column 2: number cases/prevalence

Decisions concerning meat

1. derives from animals which are dead before slaughter	5/0,02%
2. derives from animals affected by a generalised disease (septicaemia, pyaemia, toxaemia or viraemia)	8/0,03%
3. derives from animals affected by: hydatid disease/Echinococcus granulosus	251/1,13%
4. exhibits parasitic infestation	15184/68,5%
5. indicates patho-physiological changes, anomalies in consistency, insufficient bleeding or organoleptic anomalies	4696/21,5%
5.a. Insufficient bleeding	1/0,004%
5.b. degenerative changes of the liver	391/1,70%
5.c. outcomes of chronic inflammatory processes of the lung and / or pleura	3122/14,11%
5.d. outcomes of chronic inflammatory processes of the liver and / or capsule of Glisson	1161/5,25%
5.e. other patho-physiological changes	21/0,09%
6. derives from emaciated animals	1/0,004%
7. shows soiling, faecal or other contamination	1968/8,81%

Column 1: Meat declared unfit for human consumption: causes (on 22113 cases of animals with disease); Column 2: Number cases/prevalence

Investigation of Meat and Meat Products for Pathogenic Microorganisms and Detection of Certain Bacteriocin Genes with PCR

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In this work, investigation of pathogenic/ indicator microorganisms and bacteriocin producing lactic acid bacteria in various meat and meat products was aimed. 129 Turkish sausage (sucuk), Frankfurter type sausage, salami and ground meat samples were obtained from the producers in Izmir province and were classified according to criteria such as brand, packaging and season.In these samples, Mesophilic aerobic bacterial count, Staphylococcus aureus count, Bacillus cereus count, Yeast-Mold Count, Coliform bacteria count, Clostridium perfringens count with Salmonella spp., and Listeria monocytogenes analyses were carried out. Apart from these, antimicrobial and bacteriocinogenic activity of lactic acid bacteria have been investigated. Bacteriocin coding genes for enterocin-AS 48, Nisin and Pediocin AcH/PA-1 were amplified with PCR. As a result of these analyses; the mean in sucuk samples were 2.8x107 cfu/g for Total Aerobic Mesophillic Bacteria Count, 1.3x103 cfu/g for S. aureus count, 1.7x103 cfu/g for B. cereus count, 4.7x104 cfu/g for Yeast- Mold Count, 71 MPN/g for Coliform Count, and 1.0x108 cfu/g for Lactic Acid Bacteria Count. For Frankfurter type sausages these were found to be 5.3x105 cfu/g, 3.9x102 kob/ gr, 9.8x101 cfu/g, 4.4x101 cfu/g, 30 MPN/g, and 1.3x102 cfu/g for Total Mesophillic Aerobic Count, S. aureus count, B. cereus count, Yeast Mold Count, Coliform Count and Lactic Acid Bacteria Count respectively and for salami these were 9.9x104 cfu/g for Total Aerobic Mesophillic Count, 2.2x102 cfu/g for S. aureus count, 7.7x101 cfu/g for B.cereus count, 1.4x102 cfu/g for Yeast Mold Count, and for Lactic Acid Bacteria count; 6.5x102 cfu/g. In salami samples, Coliform bacteria were found to be below detection limit. 2 of the sausage and salami samples were found to be positive for Salmonella spp. and one of the salami samples was found to contain Listeria sp. Also one of the non-packaged sucuk samples was found to be positive for L. monocytogenes. Ground meat counts were respectively 5.8x107 cfu/g, 9.0x102 cfu/g, 1.2x103 cfu/g, 4.6x103 cfu/g, 126 MPN/g,and 5.0x102 cfu/g for Total Aerobic Mesophillic Count, S. aureus count, B. cereus count, Yeast-Mold Count, Coliform Count, and Lactic Acid Bacteria Count. Cl. perfringens count average values were found to be under the detection limit for sucuk, Frankfurter sausage, salami and ground meat samples. The difference between packaged and non - packaged products and branded and non-branded products were statistically different for certain criteria. Bacteriocin activity was detected only in 3 lactic acid bacterial isolates (Lactococcus lactis, Enterococcus faecalis and Enterococcus faecium) which were also positive for bacteriocin producing genes. Apart from these, one Lactococcus lactis isolate from a Turkish sucuk sample which did not show any anti-microbial effect on indicator microorganisms was found to be positive for nisin genes with PCR. As a result, antimicrobial activity of lactic acid bacteria was decided to be a result of various factors along with bacteriocins.

Keywords: Bacteriocin, lactic acid bacteria, PCR, pathogens

Investigation of Aflatoxin B1 in Animal Feed and Animal Feed Raw Material with HPLC-FLD

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Aflatoxin is the most common mycotoxin. Aflatoxins consist of 4 major metabolites as aflatoxin B1, B2, G1, G2, which are synthesized by *Aspergillus flavus*, *Aspergillus paraciticus*, *Aspergillus nomius* and some species of*Penicillium* and *Rhizopus*. This study was conducted to determine aflatoxin B1 levels in animal feed and various animal feed raw material that were consumed by animals in Konya, using high-performance liquid chromatography - fluorescence detection (HPLC-FLD) and post-column photochemical derivatization method. In this study, totally 112 specimens that were collected from towns and city of Konya, including dairy feed (n=24), stock feed (n=28), poultry feed (n=25), coarse feed (n=10) and various animal feed raw material (n=25), were analyzed for aflatoxin B1. Aflatoxin B1 was extracted in these samples by a methanol/water (80/20 v/v) mixture and then cleaned up with an AflaTest™ immunoaffinity column. The samples were analyzed with a simple and highly sensitive method for the aflatoxin B1 in animal feed and animal feed raw material using high-performance liquid chromatography coupled to online postcolumn photochemical derivatization and fluorescence detection (HPLC-FLD). Level of detection (LOD) was determined as 0.16 µg/kg, and level of quantitation (LOQ) was 0.5 µg/kg. Recovery was calculated as 91%, repeatability (RSD_r) was 8.1%, and reproducibility ($\%RSD_R$) was 8.9%. There was no aflatoxin B1 at detectable levels in 89 (79.5%) of the animal feeds and animal feed raw materials. There was aflatoxin B1 residue at levels ranging between 0.6-9.6 ppb in 12 (13.8%) of the animal feed sample; there was aflatoxin B1 residue at levels ranging between 0.8-18.6 ppb in 11 (44%) of the animal feed raw material. None of the detected levels of aflatoxin B1 was over the maximum acceptable residual level that is permitted in the Declaration on Unwanted Material in Animal Feed (2014/11) by Ministry of Food, Agriculture and Livestock. In conclusion, although residues of aflatoxin were detected in feed and feed raw material that is consumed by stock, dairy and poultry animals in Konya, it can be stated that it would not cause a toxic effect, since their level did not pose a health risk for animals, and thus, for humans.

Keywords: Aflatoxin B1, Animal Feed, HPLC-FLD

Investigation of Urea Toxicity in Ruminants

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This study was performed to determine the urea fertilizer toxication in Ruminants and to detect the urea fertilizer in Ruminant liver, kidney, rumen, intestinal, feed, feedstuff, and suspected material (drink water) samples at Konya and the surrounding provinces (Afyonkarahisar, Adana, Aksaray, Antalya, Burdur, Isparta, Karaman, Konya, Mersin and Niğde). A total of forty ruminants (n=40), forty livers (n=40), thirty-three kidneys (n=33), twent-eight rumen fluid samples (n=28), twenty-six intestinal fluid samples (n=26), five drink water samples (n=5), fourteen ruminant feeds/feedstuffs samples (n=14) were analyzed in terms of existence of the urea fertilizer. The ruminant samples that were brought by producers (ruminant farms) to the Konya Veterinary Control Institute Toxicology Laboratory between 08 Nowember 2004 and 22 October 2012 were analysed toxicologically. Water extraction was performed during preparation of samples. The samples were analyzed in terms of urea fertilizer by using High Performance Thin Layer Chromatographic (HPTLC) method. A volume of 10 µl extractions were applied to a HPTLC plate in a 5 mm spot, 12 mm from the lower edge of the plate by using a pipette.The plate was developed at room temperature in an unlined glass tank containing absolute ethanol and 13.5 M ammonia (99:1, v/v). The mobile phase was allowed to run a distance of 80 mm. After solvent evaporation, the plate was sprayed with a solution containing 0.5% (w/v) of p-dimethylaminobenzaldehyde and 0.5% (v/v) of sulphuric acid in absolute ethanol by using a sprayer. The urea fertilizer was determined at nineteen samples (n=19) of ruminant liver (47.5 %), sixteen samples (n=16) of kidney (40 %), eleven samples (n=11) of rumen fluid (27.5 %), sixteen samples (n=16) of intestinal fluid (40 %), four samples (n=4) of water (80 %) and five samples (n=5) of ruminant feeds/feedstuffs (35.7 %). Conclusion: The urea fertilizer was determined in twenty-seven ruminants (at least one sample of liver, kidney, rumen fluid, intestine, feed/feedstuff, drink water) and was determined at nine provinces (Adana, Aksaray, Antalya, Burdur, Isparta, Karaman, Konya, Mersin and Niğde) except one province (Afyonkarahisar).

Keywords: Feed, feedstuff, HPTLC, ruminant, urea

Aflatoxin Occurrence in Ruminant Feeds and Feedstuffs from Konya and the Surrounding Provinces

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This study was performed to determine the aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) in Ruminant feed and feedstuff samples from Konya and the surrounding provinces (Afyonkarahisar, Aksaray, Antalya, Burdur, Isparta, Karaman, Konya and Niğde). A total of fifty-six ruminant feeds (n=56) and fifty-nine ruminant feedstuffs (n=59) were analyzed with in terms of aflatoxins (AFB1, AFB2, AFG1, and AFG2). The samples were brought by producers (ruminant farms and feed industries) to the Konya Veterinary Control Institute Toxicology Laboratory between 15 June 2009 and 30 August 2012 for toxicological analyses. The samples were analyzed for aflatoxin using Thin Layer Chromatographic (TLC) and semi-quantitative method for detection and quantification. A florisil column was used during preparation of samples. AFB1 was found in 16 samples of ruminant feed (28.6 %) and 9 ruminant feedstuff (15.3 %) whereas AFB2 in 2 ruminant feed (3.6 %) and 3 ruminant feedstuffs (5.1 %). AFG1 and AFG2 were detected in only one sample of ruminant feedstuff (1.7 %). It was found that AFB1 levels in ruminant feed samples ranged from 1 to 20 mg/kg and were 0,5 to 60 mg/kg in ruminant feedstuff samples. However AFB2 levels in ruminant feed samples ranged from 0,2 to 2 mg/kg and were 0,5 to 15 mg/kg in ruminant feedstuffs. Levels of AFG1 and AFG2 in ruminant feedstuff samples were detected 0,5 mg/kg and 1 mg/kg, respectively. All ruminant feed samples for AFB1 levels are below (<20 mg/kg) the maximum permissible levels for aflatoxins established by the Ministry of Food, Agriculture and Livestock. Only two samples of ruminant feedstuffs for AFB1 levels (60 ppb AFB1 and 35 ppb AFB1) exceeded the maximum permissible levels for aflatoxins. Ruminant feed samples and ruminant feedstuff samples for other aflatoxins (AFG1 and AFG2) the maximum permissible levels not established by the Ministry of Food, Agriculture and Livestock.

Keywords: Aflatoxin, feed, feedstuff, ruminant, TLC

Detection of Different Meat Species in Meat and Meat Products with ELISA Technique and Histological Analysis

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Meat and meat products have importance for human diet because of the high nutritional value and the terms of flavor. Detection of different meat species in meat and meat products is very important for certification of the meat quality and the protection of human health. In this study it is aimed to detect different meat species in meat products to prevent meat fraud, consumer cheating and unfair competition. In this study, 606 meat and meat products which had been sent from different provinces of Turkey to Veterinary Control Center Research Institute in 2014 were analysed with ELISA-TEK® Raw Meat Species Kits (ELISA Technologies; Inc. Florida/ U.S.A) to detect different meat species in meat and meat products. Also 1-3 mm diameter, 5 mm depth pieces were cut from the surface of the samples for histological analysis. Fixing, Following, embedding paraffin and hematoxylin eosin staining were done respectively and these preparations were examined under the microscope. At the end of study; from the total number of 454 samples which are tested for meat species, 433 (95.4%) beef and 21 (4.6%) beef-chicken mixture were detected. Horse and pork meat wasn't detected. In the histological examination of 606 samples, primarily cartilage and bone tissue, including tissue epithelium of the skin, plenty of connective tissue (tendons, disaster, ligaments) and cellular structures of internal organs have been identified in 62 (10.2%) samples and were found incompatible with the national standards in terms of histological analysis. 21 of 454 samples (4.6%) was containing different types of meat from the tag information. According to this study; usage of different animal species' meats and also cartilage tissue, skin epithelial tissue and various internal organ cells was detected. Some meat products were not appropriate to national standards. For this purpose, control of meat and meat products should be carried out more frequently and regularly.

Keywords: Histological analysis, ELISA, meat, meat products

Rapid Confirmatory Method for Analysis of Nitrofuran Metabolites in Egg by Liquid Chromatography-Mass Spectrometry

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This study has been devoloped and validated a quick method for confirmatory analysis of nitrofuran metabolites (3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ). 1-aminohydantoin the (AHD) and semicarbazide (SEM) according to the European Commission ecision 2002/657/EC requirements. Nitrofuran metabolites in egg were acidic hydrolised followed by derivatisation with nitrobenzaldehyde in ultrasonic bath at a 40 min and liquid-liquid extracted with ethylacetate and analysis by liquid chromatography/electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS) in the positive ion mode. Nitrofuran metabolites were identified using the precursor ion and at least two product ions, meeting the qualitative and quantitative criteria set by the European Commission in the Decision 2002/657/EC. We calculated mean drug recoveries, CCα and CCβ of the method, and reported data on specificity and within-laboratory reproducibility. The method avoids the use of clean-up by SPE and should be performed quickly. The obtained validation results indicate the accordance of the method with Decision 2002/657/EC. The repeatability and within-laboratory reproducibility (precision) of the method are less than 9.86% for egg samples. The advantage of the method which could be detected low levels and quantitatively confirmed in egg samples.

Keywords: Egg, LC-MS/MS, nitrofuran, residue

Determination of Indicator Polichlorinated Biphenyl Residues in Salami, Sodjouk and Sausage by Gas Chromatography - Mass Spectrometry (gc-ms)

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Polychlorinated biphenyls (PCBs) were ubiquitously found in the environment and in many kinds of food since they were previously used in various industrial applications widely. These chemicals were subjected to restrictions internationally due to their adverse health effects including endocrine disruption. PCB residues in food present a concern in food safety programs. Simple and sensitive analytical methods are needed to monitor the residues and ensure that the food is safe for consumption. Salami, sodjouk and sausage are fermented and air-dried meat products, originating from one or a variety of animals. Since they can be stored at room temperature for periods of up to 30-40 days once cut, supplementing a possibly meager or inconsistent supply of fresh meat, these products are widely comsumed and has a traditional value in Turkey. In the current study, we evaluated indicator PCBs (PCB 28, 52, 101, 138, 153, 180) and PCB 118 residues in 60 samples of bovine meat products (salami, sodjouk and sausage) collected from five different regions of Turkey, between January and March 2015. Fat from each sample (10 g) was melted in an oven at 60 °C, and one gram of it was weighted in a 50 mL polypropylene centrifuge tube and extracted with 7 mL acetone for 10 min, using ultrasound system at 35 °C. The sample was then centrifuged at 3500 rpm for 30 min at -20 °C and dried by rotary evaporator. Dry extract was collected by 2 mL acetonitrile and 400 mg PSA and 600 mg magnesium sulphatewere added. The tube was then shaken vigorously for 30 second and centrifuged for 10 min at 4500 rpm. Finally the sample was dried and collected by 100 µL isooctane and applied to GC-MS. The optimized procedure was validated. Meat products samples free of PCBs were spiked at 6 concentration levels (0; 2,5; 5,0; 7,5; 10,0; 25,0 µg kg-1) of selected PCBs, and used to prepare a series of matrix-matched calibration curves. The samples were measured using this optimized procedure. The linearity was satisfactory in all cases with correlation coefficients >=0.995. The limits of determination and the limits of quantification were 0,230 (+0,152) µg L-1 and 0,768 (+0,508) µg kg-1, respectively. The recoveries at 3 spiking concentrations (2,5; 7,5 and 25 µg kg-1) were in the range of 88,853 % to 104,553 % and the relative standard deviations were less than 5,3 %. This validated method were found to be more economic and ecofriendly, since it uses less amount of extraction solvents which are less toxic as well.The validated method has been successfully applied to the analysis of selected PCBs in meat products with satisfactory results. These results indicate the presence of PCBs in some meat product, on the other hand the levels were all found to be below maximum level established for animal origin food products in Turkey (40 mg kg-1 fat) in accordance to EU levels. Overall, this method provides a sensitive, convenient and ecofriendly process for determining PCBs in salami, sodjouk and sausage samples and could be implemented for further legislation strategies in the following years.

Keywords: GC-MS, PCBs, residue, salami, sausage, sodjouk

Preliminary Evaluation of Slaughtered Cattle Carcass Quality Based on Aerobic Colony and *Enterobacteriacea* Counts

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The aim of this study is to determine the Aerobic Colony Count (ACC) and *Enterobacteriacea* Count (EC) of cattle carcasses by an internationally recognized standard culture method for the preliminary evaluation of their hygienic quality according to the Turkish Food Codex Regulation of Microbiological Criteria Annex 2. For this, a total of 135 carcasses were sampled between 2013-2014, in 4 different periods (Period I: Jan-Jun 2013; Period II: Jul-Dec 2013; Period III: Jan-Jun 2014; Period IV: Jul-Dec 2014) by following the ISO 17604:2003 Microbiology of food and animal feeding stuffs - Carcass sampling for microbiological analysis (ISO 17604:2003), non-destructive, sponge sampling method. All samples were collected from the most consistently contaminated sites (brisket, forerib, flank, flank groin and round-lateral) of each carcass by covering a total area of 100 cm2 (10×10 cm) as indicated in Annex A of ISO 17604:2003. The mean ACC and EC in Periods I to IV were found as 1.7 x 105, 2.4 x 105, 5.6 x 105, 6.7 x 104, and 9.6 x 102, 1.1 x 103, 6.8 x 102, 8.9 x 101 cfu/cm2, respectively. Evaluation of individual ACC according to Turkish Food Codex Regulation of Microbiological Criteria Annex 2 indicated that out of 135 carcasses 5 (3.70%) has Satisfactory, 82 (60.74%) has Acceptable and 48 (35.56%) has Unsatisfactory ACCs. Accordingly, 27 (20.00%), 58 (42.96%), and 50 (37.04%) of 135 carcasses were Satisfactory, Acceptable, and Unsatisfactory from the aspect of EC, respectively. Results indicate that 63-64% of all the carcasses had ACC and EC within the Satisfactory/Acceptable microbiological criteria range, whereas approximately 36-37% of the carcasses had Unsatisfactory ACC and EC levels. Application of good slaughter hygiene practices outside and within premise, and observation of post slaughter hygiene rules are strongly advised in order both to reduce high carcass contamination rates and to produce safer red meat for consumption.

Keywords: Aerobic colony count, cattle, carcass, enterobacteriacea

Detection of *Salmonella* spp. in Slaughter Cattle Feces by ISO 6579/A1: 2007 Method

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Improper slaughter hygiene practices in premises, which lack minimum technical and hygienic requirements, can lead to primary and secondary contamination of cattle carcasses by fecal content, consequently with*Salmonella* and other pathogens. Such meat with high microbial load can carry enteric pathogens; *Salmonella* in particular, and when treated insufficiently with heat and served under improper conditions, is a food poisoning risk for consumers. According to the European Union's 2010 Zoonoses, Zoonotic Agents and Foodborne Outbreaks Summary Report, *Salmonella* was indicated as the most prevalently reported agent in zoonotic foodborne infections and outbreaks, with contaminations primarily from poultry and secondarily from red meat. Presence of only a few, sample number/type - currency - methodology - restricted prevalence studies in our country up to our knowledge, determination of *Salmonella* presence in fecal content of slaughter animals with an internationally recognized standard method formed the basis for this study. This study aimed to determine the prevalence of *Salmonella* in cattle feces by the internationally recognized Gold Standard method ISO 6579/A1: 2007. For this, a total of 105 cattle fecal samples were randomly collected from slaughtered animals of different herds between January-December 2014 from private slaughterhouses in Bursa, Turkey. Samples were transferred to the laboratory in cold chain and *Salmonella* isolation and identification was performed by following the requirements of ISO 6579/A1: 2007- Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* – Amendment 1: Annex D: Detection of*Salmonella* spp. in animal feces and in environmental samples from the primary production stage. Two (1.90%) out of 105 the cattle feces samples were found positive for (S.F133, S.F158) *Salmonella* spp. This finding is important as it reflects both the current *Salmonella* spp. prevalence in our country, and it was determined by ISO 6579/A1: 2007 for the first time.

Keywords: Cattle, feces, salmonella, prevalence

Determination of Lead in Cow Raw Milk in the Northwest Region of Iran

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Milk contamination with heavy metals is considered to be of great concern to public health of heavy metals toxic effects on human. Lead is harmful to humans, particulary to chideren under the age of six. According to WHO information, exposure of pregnant women to high levels of lead can cause stillbirth. Furthermore, lead can cause cancer in experimental animals. 100 cow raw milk samples were collected randomly from 52 milk collection centers and dairy stores in Khoy region from October to November 2014. For lead detection in milk, Fast Sequential Atomic Absorption Spectrometer(Varian) was used. The result showed that the mean concentration of lead in milk was 8.35±0.52 µg/L. In 1% of samples the lead concentration exceeded from the maximum tolerance limit accepted by FAO/WHO(20 µg/L). Consequently, there is no high level of lead contamination in milk in the northwest region of Iran.

Keywords: Lead, milk, northwest, Iran

Occurrence and Antimicrobial Resistance of Host Nonspecific *Salmonella* Species in the Eggs and Poultry Products

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This study was carried out in order to present the occurrence and antimicrobial resistance profiles of host nonspecific *Salmonella* spp. which are *Salmonella* Enteritidis and *Salmonella* Typhimurium. For this purpose, totally 200 samples consist of eggs (n=100), chicken nuggets (n=20), chicken sausages (n=20), chicken salami (n=20), gizzards (n=20) and chicken livers (n=20) were examined in the current study. In the isolation process, egg shell surface and egg content were examined separately. The method proposed by ISO 6579 was used for *Salmonella* spp. isolation and the suspicious isolates were confirmed by PCR. The mPCR method was performed for the determination of host nonspecific *Salmonella* species obtained from the study. The antimicrobial susceptibility testing was performed by disc diffusion method. In the test, ampicillin (10 µg), tetracycline (30 µg), amoxicillin-clavulanic acid (30 µg), cephazolin (30 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (10 µg), nalidixic acid (30 µg), enrofloxacin (5 µg) and trimethoprim/sulphamethoxazole (25 µg) disks were used. In the study, 30 *Salmonella* spp. were isolated from 200 poultry related products were investigated. *Salmonella* spp. was found in 5 out of 100 egg shell samples analyzed. None of egg contents contaminated with*Salmonella* spp. Totally 25 samples including, 10 chicken livers, 10 gizzards samples, 3 chicken sausages and 2 chicken nuggets were determined to be contaminated with *Salmonella* spp. Fourteen isolates (7 liver, 4 gizzard and 3 sausages) of 25 poultry samples and 3 of 5 egg samples were identified as *Salmonella* Typhimurium. None of the *Salmonella* isolates obtained in the study were determined to be *Salmonella* Enteritidis. In this study, all strains isolated from eggs and poultry products, were resistant only to erythromycin (100%). However, all egg isolates were susceptible to tetracycline, trimethoprim-sulfamethoxazole, gentamicin and enrofloxacin (100%). In addition, all poultry isolates were susceptible to trimethoprim-sulfamethoxazole (100%) and most of the isolates were susceptible to ampicillin (83%), amoxicillin-clavulanic acid (83%) and gentamicin (75%). In conclusion, the detection of high prevalence of host nonspecific *Salmonella* spp. than that of host specific is important for public health. For this reason, eggs and poultry products need to use hygienic practices in handling and processing operations.

Keywords: Antimicrobial resistance, eggs, host nonspecific salmonella species, poultry products

Control of Dry Sausage, Salami, Sausage and Hamburger Meatball Produced in Meat Production Facilities Applying ISO Food Security System in Terms of Food Pathogens

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In this study, fermented sausage, salami, sausage and hamburger meatball produced by 6 companies from Marmara Region of Turkey which apply and do not apply ISO 22000 food security system were compared in terms of secure food production. In the samples, the total mesophilic aerobic microorganism (TMA), *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* quantities and existence of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 were researched. The samples were divided into pieces with a sterilized lancet aseptically. 25 g. samples were weighed for each pathogen. Different diluting agents of 225 ml were put in the sterilized bags of mixer for each microbiologic analysis. They were smashed in stomacher for 1 or 2 minutes. Decimal dilutions were contained in diluents of 9 ml for the microorganisms to be detected and culture process was realized. Standard FDA/BAM methods were used for examination and biochemical tests (Biomeriux, API®) were applied to the colonies obtained. As a result of the study among 96 samples, *Salmonella* spp. contamination was detected in 3 samples (3.12%), *L. monocytogenes* contamination was detected in 17 samples (17.7%) and *E. coli* O157:H7 contamination was detected in 4 samples (4.16%). The fact that 20 meatballs out of 24 being detected to be contaminated with zero tolerance microorganisms and that 9 of these meatballs, *L. monocytogenes* and *E. coli*O157:H7 contaminations detected in 2 of sausage samples 1 out of 2 *L. monocytogenes* contaminations detected in salami samples and that 2 out of 4 *E. coli* O157:H7 contaminations detected in the samples being produced in companies with ISO certificate gave an impression that up to now ISO system has not created its effect in meat products in terms of food security and public health. It is considered that all applications related with food security and consumer health are important in all stages of supply chain starting from the general health conditions of animals to the delivery to conscious customer. Taking into consideration the beefs and slaughterhouses as the main *E. coli* O157:H7 contamination source, it was concluded that significant deficiencies were present in applying ISO certification and raw material contamination since two of the four contaminated samples were produced by ISO certified firms. Besides, some deficiencies were demonstrated to exist in hygiene and training of the personnel as well as inappropriate or unsatisfactory applications of sanitation and disinfection.

Keywords: ISO 22000, food pathogens, food safety, meat products

Comparison of Milk Composition, Fatty Acid Profile and Conjugated Linoleic Acid Content in the Milk of Ewes or Does of Indigenous Breeds in the Region of Epirus, Greece

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Objective of the study was to compare chemical characteristics of milk from ewes or does of indigenous breeds in the region of Epirus, Greece. The investigation included five sheep flocks and five goat herds in the upland or hill areas of the region of Epirus in Greece. Sheep and goats grazed on pasture land for at least 8 hours daily and also received a concentrate supplementation of 0.5 kg daily. Milk samples were collected from the farm's milk tank thrice throughout a lactation period. First sample was collected on the first month after lambing/kidding, second on the 5th month of the lactation period and the third on the 8th month of the lactation period. The following parametres were measured in milk samples: pH, fat content, protein content, lactose content, total solid content (by means of Milkoscan FT120) and fatty acid content (by means of a gas chromatographer with flame ionization detector). Chemical composition and fatty acid profile of ewes' or does' milk are in Tables 1 and 2, respectively. We found increased fat, protein and total solids content in ewes' milk. We also found increased conjugated linoleic acid content, eicosenoic acid and linolenic acid in ewes' milk fat, whilst we found increased capric acid content in does' milk fat. Differences in milk content between sheep and goats reflect the effects of differing physiological parametres between the two species (e.g., rumen metabolism and fatty acid synthesis in the mammary gland), as well as differences in their management (e.g., feeding behavior). The results also present the characteristics of ewes' and does' milk, which need to be taken into account in the preparation of the various dairy products.

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Keywords: Dairy product, fatty acid, milk composition, goat, sheep

Table 1

	Ewes	Goat
Milk (mL per day)	711.1	869.0
pH	6.82±0.15	6.75±0.12
Fat (g per L)	83.76±23.50	43.19±14.56
Protein (g per L)	59.52±7.6	37.56±6.18
Lactose (g per L)	45.99±4.87	42.53±4.64
Total solids (g per L)	197.73±25.36	131.95±16.1

Chemical composition of ewes or does milk from ovine and caprine milk of indigenous breeds in the region of Epirus, Greece

Table 2

	Ewes	Goats
Fatty acids (g 100g -1)		1.47±0.72
C4:0; butyric	1.38±0.91	1.47±0.72
C6:0; caproic	2.26±1.37	2.80±2.12
C8:0; caprylic	2.23±1.10	3.18±1.90
C10:0; capric	6.51±1.08	9.92±2.17
C11:0; undecylic	0.26±0.16	0.20±0.11
C12:0; lauric	3.82±1.36	4.75±2.25
C14:0; myristic	10.38±1.80	9.94±1.64
C14:1; myristoleic	0.45±0.13	0.39±0.13
C15:0; pentadecylic	1.40±0.20	1.03±0.37
C16:0; palmitic	25.40±2.61	26.91±4.51
C16:1; palmitoleic	0.95±0.56	0.52±0.38
C17:0; margaric	0.61±0.24	0.64±0.23
C18:0; stearic	12.93±2.92	14.09±3.94
C18:1n9t; elaidic	2.42±1.17	1.44±0.78
C18:1n9c; oleic	23.33±4.88	18.96±4.34
C18:2t; conjugated linoleic acid	0.43±0.10	0.23±0.09
C18:2n6c; linoleic	2.57±1.36	1.89±0.62
C20:0; arachidic	0.10±0.17	0.11±0.03
C18:3n6; γ-linolenic	1.38±0.79	0.96±0.37
C20:1; cis-11-eicosenoic acid	0.21±0.09	0.05±0.03
C18:3n3;linolenic acid	1.07±0.55	0.52±0.31

Table 2. Fatty acid composition in fat of milk of ewes or does of indigenous breeds in the region of Epirus, Greece

Meat Inspection - Challenges for the Future Perspective for the Republic of e Kosovo - Pass (Access) to Europe

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Meat Inspection is a special task of veterinary epidemiology in public health. The surveillance of hygiene and safety of food of animal origin is should be in the hands of veterinarians. Veterinarians play a central role for the control of the operational self-control. Officials are responsible to protect the people from health hazards and to protect the health and well-being of animals. Regulation (EC) No 882/2004 of the European Parliament and of the Council sets out principles for the uniform and transparent implementation of the supervision and awareness of international standards for quality management, in particular DIN EN 45004, DIN EN ISO 9000 and DIN EN ISO 19011 aligned. Homogeneity, trace-ability and transparency of the systems facilitate their acceptance and trust among consumers as well all people in the food chain. The Presentation will give an overview about the specifications and requirements of meat inspection designing and implementation of these and focus on the advantages and disadvantages of such a system in the field in Kosovo. The slaughter process, starting with opinions in stunning, removal and disposal of SRE"s, processing hygiene, GMP in slaughter process, unification of pathological findings designation and disposal of confiscates due to the absence of rendering plant in Kosovo and the region, are described. Furthermore, Presentation is illustrating the Implementation in the Republic of Kosovo and in other EU Member States. First results and experience in Implementation are discussed and an outlook on current changes is given. In summary, the presentation gives an overview of consumer health protection under the responsibility of veterinarians in the Veterinary Public Health Sector.

Keywords: Challenges, meat Inspection, perspective, EU, Kosovo

Investigation of the Antimicrobial Effect of Ginger Essential Oil on *Campylobacter Coli*

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Most *Campylobacter* species are pathogenic and can infect humans and other animals. *Campylobacter jejuni* and *Campylobacter coli* are mainly responsible of *Campylobacter* infections. Essential oils are defined as aromatic liquids obtained from plant materials. The interest of consumers to antimicrobial effects of essential oils is increasing. In addition to their antimicrobial properties, most of them have also antioxidant properties. The objective of this study was to investigate the antimicrobial activity of ginger essential oil against *Campylobacter coli* by using agar dilution method. Clinical *Campylobacter coli* isolate and a commercial ginger essential oil were used in this study. Antimicrobial effect of ginger essential oil on *Campylobacter coli* was determined by measuring minimum inhibition concentration (MIC). MIC values were determined by using agar dilution method. By using this method, the growth is observed by the addition of different concentrations of essential oils into the medium. MIC value is defined as minimum essential oil concentration that inhibit visible growth of microorganisms. While some studies reported higher antimicrobial effects of essential oils and plant extracts against Campylobacter spp., on the other hand some studies determined the resistance of *Campylobacter* spp. to essential oils and plant extracts. According to the results of this study, the MIC values of tested essential oil for *Campylobacter coli* was of 7.5 µL/L. There are limited studies related to antimicrobial activity of ginger oil against *C. coli*. There are different methods to reduce *Campylobacter* spp. especially in chicken meat. These results suggest that ginger essential oils may be used as a natural preservative in food against growth of*Campylobacter* spp. as an alternative to chemical preservatives.

Keywords: Antimicrobial activity, campylobacter coli, essential oils

Effects of Essential Oils on Pathogen Microorganisms

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Essential oils are antimicrobial agents that might be used to control food spoilage and foodborne diseases. Using of essential oils as an alternative to chemical preservatives in foods is an increasing demand. The objective of this study is to review researches releated with effects of different essential oils on pathogen microorganisms and their potential applications in the food industry. In the literature, studies about antimicrobial effects of essential oils on different bacteria such as *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* spp. are available. In this study we will focus on to review studies about antimicrobial effects and summarize the differences among the investigation of antimicrobial activities. The antimicrobial activities of essential oils are widely studied by different researchers. As a result of these studies, one of the most studied essential oils is origanum. Other essential oils studied by researchers are thyme, rosemary and clove. Most effective essential oil components are carvacrol, eugenol and thymol. There are different methods to determine the antimicrobial effects. Generally inhibition zone determination by agar-well diffusion and disc diffusion method and determination of minimum inhibition concentrations (MIC's) are used to express the antimicrobial effects of these essential oils and essential oil components. Some studies mentioned that gram negative microorganisms are more resistant to essential oils than gram positive microorganisms. On the other hand some studies reported the similar effects of essential oils to gram negative and gram positive microorganisms. These findings can be useful for the antimicrobial effects of commercial esential oils. Usage of essential oils can be combined with other preservation techniques to maintain green safe food production.

Keywords: Essential oils, food borne pathogens

Study of the Additional Input of Hormones into the Human Food Chain by the Slaughtering of Pregnant Cattle

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The presence and metabolism of endogenous steroid hormones in meat-producing animals has been the subject of much research over the past 40 years. While significant data are available, there are no official or published studies in the EU concerning hormones in meat of pregnant animals. The continued increase in incidence of some hormone-related cancers worldwide is of great concern. Although estrogen-like substances in the environment were blamed for this increase, the possible role of endogenous estrogens from food has not been widely discussed. We are particularly concerned about slaughtered pregnant cattle' meat, which may contain a considerable quantity of estrogens. The determination of the prevalence of pregnant slaughter animals, together with their characterization, would allow the estimation of an additional hormonal exposure to humans. The additional excess intake of steroid hormones by the consummation of meat from pregnant cattle was evaluated in this study on the basis of (i) the total concentration of steroid hormones in different tissues of pregnant cattle, and (ii) the total concentration of these substances in the meat of non-pregnant heifers and bulls (with and without an additional hormone implant). 6690 culling cows were examined. The prevalence of pregnancy amounted to 5.3% for female cattle and the average month of pregnancy was 5. The theoretical excess intake of natural steroid hormones by consumption of meat from pregnant cattle (last third of pregnancy) is calculated to amount up to 442 ng/person/day in the case of estrogens. This would correspond to the fivefold quantity of excess intake of estrogen by the consumption of meat from hormone-treated animals, as determined by JECFA (Joint FAO/WHO Expert Committee on Food Additives). The additional intake of testosterone compared to non-pregnant cattle reaches up to 370 ng/person/day. This quantity corresponds at least to the double quantity of excess intake of testosterone by the consumption of hormone-treated beef meat. The additional intake of progesterone can be up to 6.8 ng/person/day compared to the content in the musculature of cattle in the luteal stage of the sexual cycle and 13.3ng/person/day of cattle in the follicle stage. In conclusion, more data are urgently needed on the input-levels of hormones due to slaughter of pregnant animals in Europe. For this purpose, we recommend further studies on the prevalence of slaughter of pregnant animals including the quantification and characterization of hormones in relevant edible tissues of these animals.

Keywords: Estrogens, hormones, pregnant cattle

Unauthorized Tissues in Heated Meat Products in Iran: Histological Evidences

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Due to public health-threatening consequences, intentional adulterations in meat products industry including addition of illegal animal tissues have caused great concern. The aim of this study was to detect unauthorized tissues in heated meat products in Iran based on histological examination. 23 samples of different types of processed meat products were randomly collected from supply centers in Iran. Each sample was divided into three equal parts and one sample was taken from each part. The samples fixed in 10% formalin, sectioned by a microtome and stained with hematoxylin and eosin for histological assessments. Plant tissues and skeletal muscle were observed in 100% and 98.86% of samples, respectively. Moreover, cartilage, bone, skin, hair follicle, salivary gland, soft palate and aorta were found in 44.32%, 29.55%, 2.27%, 2.27%, 1.14%, 1.14% and 1.14% of the samples, respectively. Our finding revealed the presence of unpermitted animal tissues in heated meat products in Iran. Therefore, histological examination as a cost effective method could be used for precise monitoring of foodstuffs hygiene and safety.

Keywords: Histology, Iran, meat product, unauthorized tissue

Prevalence and Antimicrobial Resistance Profile of Enterococci in Cheese in Turkey

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Enterococci is an emerging cause of nosocomial infections worldwide. Consumption or mishandling of contaminated foods of animal origins including milk, milk products and meats could pose a public health problem. In this study, it was aimed to assess the presence and antibiotic susceptibility profile of enterococci in different variety of cheeses (n=100) sold in Hatay province, Turkey. Material and Enterococci strains were isolated by suspending 10 gr of each sample in sterile buffered peptone water (90 ml) and then incubated at 37 oC for 18 to 24 h. After enrichment, 100 µl of overnight growth suspension was added into the Enterococcosel Broth and further incubated at 37 oC for 24 h. At the end of the incubation, 100 µl of the suspension was plated on both VRE agar and VRE agar with vancomycin (6 mg/L). A total of 139 isolates were recovered and species identification was carried out by using VITEK 2 system (Biomeroux). Antimicrobial susceptibility profiles of enterococci isolates was assessed by the disc diffusion method against 15 antibiotics. E. faecalis was the most frequently detected species (58.3%), followed by E. faecium (15.1%), E. gallinarum (12.9%), E. durans (5%), E. avium (2.9%) and E. casseliflavus (2.9%). A total of five vancomycin resistant (two intermediate resistant) enterococci strains were recovered. The results indicated that 88.5% and 84.2% of isolates exhibited resistance to lincomycin and kanamycin, respectively. Only E. faecalis (8.2% and 2.4%, respectively) and E. gallinarum (5.6% and 5.6%, respectively) isolates displayed high level resistance to streptomycin (300 µg) and gentamicin (120 µg). 47.1 % of E. faecalis were resistant to tetracycline whereas only one E. faecium strain showed intermediate level of tetracycline resistance. Resistance to chloramphenicol was relatively low (3.6% and 6.5%, resistance and intermediate resistance, respectively). However, all isolates were susceptible to penicillin and ampicilline. Conclusion, the findings of this study shows that most enterococci isolates were found to be resistant to a number of clinically important antibiotic agents which might play a role to spread antimicrobial resistance to food-borne pathogens through food chain. We are currently carrying out more studies in order to determine the resistance mechanism.

Keywords: Antibiotic resistance, cheese, enterococci

The Food Chain Information in Opinion of Polish Veterinarians

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The aim of the study was to evaluate veterinarians' feedback rating on the usefulness and functioning of the food chain information (FCI). During the study the desk research and quantitative and qualitative empirical study were used. Desk research covered the analysis of the existing Polish and EU legislation and the analysis of the organization and functioning of the FCI system. Quantitative and qualitative empirical study were implemented with the use of interview techniques to investigate the experiences and attitudes in groups of 50 private veterinarians and 50 veterinary officials. The survey was performed on February and March 2014 and has shown mediocre or poor functioning of the FCI (83% of respondents). None of veterinarians rated the current arrangements positively, 17% awarded a satisfactory grade. 78% of respondents indicated unreliability of information provided by breeders. 74% of official veterinarians had difficulties in the analysis of the information provided. Only 5% of private veterinarians had been informed of the anomaly to the health or welfare of livestock. Providing and collecting food chain information is a legal requirement imposed on food operators. The FCI is a public health protection tool ensuring the safety of food of animal origin, applicable under EU Regulations 853/2004 and 854/2004. The FCI includes data on the health and welfare of animals arriving at a slaughterhouse. Information is referred to slaughterhouse by owner of animals. The data provided are a basis for operator and official veterinarian to organize activities at the slaughter. Official veterinarian uses FCI to make correct decisions during pre- and post-mortem inspection. Ante- and post-mortem inspection findings should be transferred to the farmer. The farmer and private veterinarian are supported by transferred data in improvement of the health and welfare of animals. According to Polish official veterinarians, FCI in its current form is not a useful tool for supporting slaughter animals inspections. Paper form of the FCI makes it difficult to analyze the data provided and therefore they can't be fully used by official veterinarians. Pre- and post-mortem examination data are not transferred to a farmer and private veterinarians. Despite compliance with legal requirements the FCI remains nonfunctional. Both official and private veterinarians perceives FCI as additional bureaucratic burden with no added value for meat inspection and animal health management on farms.

Keywords: Animal health, food safety, FCI, food chain

Detection of Alaria Alata Mesocercariae in Meat

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Alariosis is a re-emerging zoonotic disease caused by infection with larval stages of trematodes of Distomum musculorum suis (DMS). The mesocercarial stage of the trematode Alaria alata, can cause severe damages within their hosts, and since several reports about cases of human larval alariosis have been published, it became apparent that infected game animals and in particular wild boars are a potential source of infection for both humans and animals. A final statement concerning the health risks for consumers could not be given due to the lack of information about both the prevalence of DMS and the suitability of Trichinella inspection methods to detect this parasite in wild boar meat. Against this backdrop the aim of our work was to develope, and valide an alternative method for the detection of this parasiteand to assess its distribution within the paratenic hosts. Furthermore it was imperative to analyse the survivability of A. alata mesocercariae in the meat and meatproducts. As a new approach a larval migration technique, the Alaria alata mesocercariae migration technique (AMT), was developed. This new method offers an impressive sensitivity of 96% when used for the detection of A. alata mesocercariae and persuades furthermore by its easy handling and high robustness. 142 wild boars were analyzed using AMT in order to elucidate the distribution of the mesocercariae within its hosts was evaluated by analysis of the parasites' distribution patterns in wild boars. The analyses of the distribution pattern of A. alata mesocercariae in the body of its hosts showed a highly heterogeneous distribution of this parasite which suggests a body migration. Therefore the choice of possible predilection sides is highly important in practice.

Keywords: Alaria alata, alariosis, AMT, wild boars

Seroprevalence of *Toxoplasma gondii* in sows from farms located in Mazowsze and Wielkopolska Province, Poland

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Toxoplasmosis is a widespread disease of humans and animals (mammals, birds) caused by the protozoan parasite *Toxoplasma gondii*. It is estimated that about 1/3 of the world's population may be infected with this parasite. In immunocompetent individuals in 80% of cases infection is asymptomatic. Only 3-20% of infected persons show mild, non-specific symptoms that sometimes are confused with symptoms of other diseases. Nevertheless, infection may cause serious consequences in immunocompromised patients and in women after primary infection during pregnancy. *T. gondii* has an indirect life cycle with domestic cat and other felids as definitive host (source of oocysts in the environment) and human and many other species of warm-blooded animals, including pigs, as intermediate host. In the intermediate hosts occurs the development of tissue cysts containing bradyzoites. Consumption of raw or undercooked meat or offal containing invasive tissue cysts is considered to be the most important route of infection for human which is responsible even for 30 to 63% of *T. gondii* infections in Europe. Therefore, monitoring of the infections should be focused mainly on meat and meat products. In Poland pork is one of the most widely consumed type of meat and therefore monitoring of *T. gondii* infection should be focused on the pigs. The aim of the study was to determine the prevalence of antibodies to *T. gondii* in sows kept on farms located in Mazowsze and Wielkopolska Province and to determine on this basis the safety of consuming meat derived from those animals. The material (serum samples) was obtained from sows originated from one farm located in Mazowsze Province and seven farms located in Wielkopolska Province. On farm located in Mazowsze sows were kept in confinement whereas on farms located in Wielkopolska pigs had limited contact with natural environment. In total 65 serum samples were examined using a commercial ELISA test (PrioCHECK® Toxoplasma Ab porcine) for detecting antibodies to *Toxoplasma gondii* in serum, plasma and meat juice samples obtained from pigs. Among 65 examined serum samples 43% (28/65) showed the presence of antibodies to *Toxoplasma gondii*. The higher percentage of positive results we observed in sows from Wielkopolska Province (46%, 28/61 pigs) than in those from Mazowsze (0%; 0/4 pigs). Lower seroprevalence to *Toxoplasma gondii* in sows from Mazowsze (0%, 0/4 seropositive pigs) than in animals from Wielkopolska (46%, 28/61 seropositive pigs) was associated with maintaining of the animals on farm in Mazowsze without contact with natural environment. The possibility of contact with the environment increases the risk of infection with *T. gondii* by ingestion of sporulated oocysts or by eating of intermediate host (e.g. rodent or bird). The seroprevalence of *T. gondii* in sows is relatively high which is associated with a longer life span of these animals (more situations conducive to infection). This is important because the meat derived from these animals is often used for the production of sausages or cured meats and the processes used during their production not always provide the inactivation of the parasite. Must be kept in mind that meat products are often mixed products and the meat from one infected animal can contribute to the contamination of the whole batch of the meat product.

Keywords: Antibodies, pigs, sows, toxoplasma gondii, toxoplasmosis

Molecular Characterization and Determination of the Antibiotic Resistance of Campylobacter Jejuni Strains Isolated from Chicken Meats and Offals Sold in Erzurum Province of Turkey

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Campylobacteriosis is commonly seen all around the world at last century and causes zoonose enteritis. Campylobacters are carried in the intestinal tract of wild and domestic animals, especially poultry. Also Campylobacteriosis is an infection which can be transmitted via consumption of contaminated meat and milk or infecting with contaminated products of them. This study is aimed to isolation and identification of Campylobacter jejuni in chicken meats and offals and determination of antibiotic resistance. In this study, 200 samples including 22 pieces of drumsticks, 4 pieces of neck, 57 pieces of leg, 7 pieces of liver, 3 pieces of skin, 72 pieces of breast, 33 wings, 2 pieces of gizzard. The Samples were transferred randomly to the laboratory by keeping cold chain according to the procedures as specified in the OIE Manual and examined immediately. After 48 hours of enrichments onto Preston Campylobacter Selective Broth, each samples transfered two loopfuls of enrichment broth to THE Campylobacter Selective Agar for isolation. Plates Protect from light and incubated for 48 hours at 420C in the microaerophilic environment provided by C02 gas kits. Bacterial growths on plates were then evaluated for their colony formation and microscopic characteristics. The colonies having Gram staining of small, curved or seagull-winged, spiral, faintly staining, gram-negative rods were accepted as seen in Campylobacter spp. which was separated on suspicious and identification tests were performed. The pre-diagnosis was achieved on colony suspected of Campylobacter spp., by using VITEC II COMPAQ system (Biomerieux). The PCR assay was performed using four different primers specific to each species ceuE gene. For determination of antibiotic resistance by using eight (8) different antibiotics was determined by disk diffusion method. In this study total of 200 samples of chicken meats and offals from the example at 80 (40%) of Campylobacter jejuni was isolated and identified. All strains were resistant to oxitetracycline while 76% of the strains were sensitive to erythromycin. The results of this study indicated that PCR assay may successfully be applied for the identification of these species as an alternative to conventional methods, due to its accuracy and speed. High contamination of Campylobacter jejuni was found in chicken meat and offal samples taken from chicken meat sales point in Erzurum province and Campylobacter jejuni strains showed high resistance to different antibiotics.

Keywords: Antibiotic resistance, campylobacter jejuni, PCR

Assessment of Probiotic Activity of Lactic Acid Bacteria for Safer and Healthier Cheese Productions

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The beneficial effects of probiotics in food on human health and safety are increasingly recognised by the scientific community. The term "probiotic" is referred to live microorganisms that are intentionally added in foods and that address the following characteristics: resistance to gastric pH and bile, adhesion to intestinal cells and activity against bacterial pathogens (1). The scope of our work was to isolate Lactic Acid Bacteria (LAB) from an Italian typical sheep milk cheese at different curing stages in order to assess their probiotic properties. The selection of LAB strains able to survive to stress factors typical of human body and of cheese manufacturing and with anti-bacterial properties could enhance food quality when applied directly into the chain productions. A total number of 93 LAB were isolated in MRS media from sheep milk, curdle and cheese samples cured up to 150 days. Colonies were firstly screened by Gram stain, catalase and oxidase tests. Furthermore all strains were identified by DNA sequencing. Strains of genus *Lactobacillus* were subjected to amplification and sequencing of the hypervariable region of the first 500 bp of the 16S rRNA coding gene. A PCR was performed to distinguish between *Lactobacillus paracasei* and *Lactobacillus casei*. Following these analysis, a group of 17 LAB were selected on the bases of the ability to survive after a long curing period (up to 150 days). They undertook other tests in order to be assessed for resistance to pH (2.5 and 3), bile concentrations (0.3%, 0.5% and 1%), adhesion activity to Caco-2 cell lines, determination of the presence of genes coding for bacteriocins (9 genes) and biogenic amines (11 genes; multiplex and simplex PCRs) and bacteriocin production (deferred antagonism test). Among 93 isolates, 82 were identified as LAB, related to the genus *Lactococcus* and *Lactobacillus*, 17 *Lactobacillus* strains were furthermore selected for their resistance to a long curing period. They showed different pH/bile tolerance and adhesion properties but, on the basis of the results of gene detection for bacteriocin production, only two of them also undertook the test for the bacteriocin production. The strains were identified both as *Lactobacillus paracasei* subsp. *paracasei*. In particular one of them showed a percentage of surviving cells of 95,44% at pH 3, of 16.32%, 45.87% and 80.23% at bile concentrations of 1%, 0.5% and 0.3%. Moreover it was able to adhere to Caco-2 cells for 0.032%. The latter showed a percentage of surviving cells of 100% at pH 3, 33.09% at pH 2.5, 8.34%, 18.81% and 36.69% at bile concentrations of 1%, 0.5% and 0.3%. Moreover it showed adhesion capacity of 0.079%. Both the strains produced an inhibition zone against *Listeria monocytogenes* of 2 mm, but no antibacterial activity against *Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* VTEC was observed. Two strains of *Lactobacillus paracasei* subsp. *paracasei* have been identified as potential probiotics to be applied into the cheese production in order to enhance cheese quality and safety against *L. monocytogenes*.

Keywords: Cheese, Lactic Acid Bacteria (LAB), probiotic

Addition of Natural Anti-Oxidative Agents for Oxidative Stability of Poultry Meat and Eggs

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Poultry products like meat and eggs are readily available sources of protein for humans. In recent years, people have become more conscious about their food and health. People like to consume balance and enriched food which can fulfill their nutritional needs properly while not causing any adverse effect on health status. Poultry products are the desired and focused target which can be enriched with needed supplements which the certain population demands. Designer eggs and enriched poultry meat is now getting popularity. Eggs and meat can be enriched with different nutraceutical, health promoting substances or desired unsaturated fatty acids like n-3 fatty acids and/or vitamins. Unsaturated fatty acids have proved to exert beneficial effects on cardiovascular system and overall health status. It has been reported that while enriching eggs and meat with these health promoting substances, these can exert the negative effect on meat and eggs quality. This may result because of higher susceptibility of unsaturated fatty acids to oxidation which can deteriorate the quality and taste of the product. Oxidation can be prevented and controlled by the use of anti-oxidative agents in the feed of birds. Synthetic anti-oxidative agents or preservatives are not much liked by the consumers. For this purpose natural anti-oxidative substances are more getting more popularity in terms of consumer preference and improved product quality. Keeping in view the above points, this review is focusing on the use of natural anti-oxidative agents (herbal extracts/essential oils) and their benefits on eggs and poultry meat quality by improving oxidative stability in a consumer friendly way.

Keywords: Antioxidant agents, essential oils, eggs, meat, unsaturated fatty acid

Survey of Aflatoxin Residue in Milk and Feed Samples in Kirikkale Province, Turkey

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The aim of this study is to detect whether there is total aflatoxin (AF) and AFM_1 contamination in feed and milk samples obtained from the dairy cow farms in Kırıkkale region. A total of 154 dairy cow feed and 154 raw milk samples were obtained from the villages (Delice, Keskin, Sulakyurt, Bahşılı, Yahşihan, Çelebi, Karakeçili, Balışeyh) and central of Kırıkkale province, Turkey, between years June 2012- August 2013. The quantitative analysis of total AF, and AFM_1 in the samples was carried out using an enzyme-linked-immunoassay (ELISA) with commercial kits (HELICA biosystems inc., HELICA for total aflatoxin-981AFL01LM-96, HELICA for aflatoxin M_1-961AFLM01M-96). Mycotoxin extraction and tests were conducted according to the instructions of manufacturer. In all the feed samples total AF were detected, also AFM_1 contamination was found in all of the milk samples. In 5 of 154 feed samples the total AF were above 20 µg/kg. None of the milk samples were found to be above the legal limit. The mean residue of total AF level for concantrated feed was 6.43±0.57 µg/kg, and found to range from 0.20 µg/kg to 28.80 µg/kg. The mean residue of AFM_1 for milk samples was 1.73 ±0.18 ng/L, and found to range from 0.08 ng/L to 10.11 ng/L. Consequently, although all of the milk samples were found to be contaminated with AFM_1, these amounts were determined within the legal limits that are allowed in milk. On the other hand % 3.25 of the feed samples were above 20 µg/kg. Based on the results of this study the occurance of AFM_1 may not be considered as a possible risk for public health. Finally it is recommended to continue the strategies and monitoring programs to prevent aflatoxin contamination.

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Keywords: AFM_1, ELISA, feed, raw milk, total aflatoxin

Seroprevalence and Risk Factors of Leptospirosis in Indigenous Bulls in Sudan

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Leptospirosis is of puplic health important and worldwide distribution. It is caused by spirochetes of the genus *Leptospira*. *Leptospira borgpetersenii* serovar Hardjo that is considered the serovar maintained by cattle, but infections by other serovar like *Leptospira interrogans* serovar Pomona have also been associated with economic losses on dairy farms. Leptospirosis causes serious economic loss to the cattle industry evidence by decreased milk production, abortion, stillbirth, infertility and mortality. Humans are usually infected by contact with urine and tissue of an infected host, contaminated drinking water and soil. The objective of this survey was to detect anti-*Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona antibodies in indigenous bred bulls sera in four localities in Sudan by Enzyme- linked immunosorbent assay (ELISA). One hundred and one serum samples were collected from apparently healthy indigenous bred bulls (46 cattle butana bulls ecotype and 55 cattle kenana bulls ecotype) from different localities in Sudan (Khartoum, Gezira, Sennar and River Nile States). All serum samples were investigated for specific antibodies against *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona using ELISA kit and were performed according to manufacturer's instructions (Biovet, Canada). Fisher's exact test was used to examine the significance of the associations between leptospirosis seroprevalence and risk factors (breed, age and location). A P value < 0.05 was considered significant, as determined by the Statistical Package for the Social Sciences (SPSS, version 18). The seroprevalence of *Leptospira borgpetersenii* serovar Hardjo were found 19.8 (20/101) at animal level and 31.6 (12/38) at herd level. Only one animal was found positive for specific antibodies against *Leptospira interrogans* serovar Pomona. The lowest seroprevalence 10.5% (4/38) was found at River Nile and the highest 32.6% (14/43) was found at Sennar States. All sera collected from Khartoum State were found negative for *Leptospira borgpetersenii* serovar Hardjo antibodies. The associations between leptospirosis seroprevalence and risk factors (breed, age and location) was found statistically significant (P < 0.05) by Fisher's exact test. It could be concluded that leptospirosis was prevalent in cattle (bulls) in three localities in Sudan. Therefore, further investigation of leptospirosis in other farm animals and man at the country level is important to monitor and determine the magnitude of the disease and to estimate the economic impact and potential threats to human health.

Keywords: Bulls, leptospirosis, seroprevalence, Sudan

Animal Toxoplasmosis in Mogadishu City of Somalia

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Toxoplasmosis is one of the more common parasitic zoonoses worldwide. It is caused by a protozoan parasite, *Toxoplasma gondii*, in both animals and humans. Due to the little information about the disease in the country and its public health importance, this study was conducted to contribute a recent data base on zoonotic diseases and increase public awareness about the zoonotic potential of the disease. One hundred fifty one serum samples from different animals (camels 64, cattle 28, sheep 29 and 30 goats) were tested by using Latex Agglutination Test (LAT). 15.9% (24 out of 151) of studied animals were positive to antitoxoplasma antibodies. The sero-positive of each species was: 6.3%, 7.1%, 34.5% and 26.7% in camels, cattle, sheep and goats respectively. About 5.3% of 45 males and 10.6% of 106 females of studied animals were infected with *T. gondii*. More researches of the diseases using different diagnostic tests in the country and impacts of these findings on Somalis people is suggested.

Keywords: Camel, cattle, goat, sheep, somalia, toxoplasmosis

Effects of BCRP and P-gp Modulators on the Penetration of Aflatoxin B1 into the Mouse Brain

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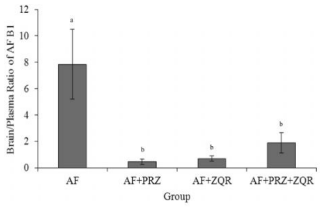
Aflatoxin B1 (AFB1) is a substrate of BCRP. Often the substrates for BCRP tend to overlap with P-gp. There are not data whether AFB1 is a substrate for P-gp. The aim of this study was to determine whether the plasma and brain concentrations of AFB1 are affected by inhibitions of P-gp and BCRP using zosuquidar (ZQR) and prazosin (PRZ), respectively. In this study, a total of 40 healthy adult male Balb/C mice (32±3.7 g) were used. Animals were randomly divided into 5 groups of 8 animals per group. Group 1 was used in the studies for method validation. Group 2 (AF) received intraperitoneal AFB1 at the dose of 20 mg/kg of body weight. Groups 3 (AF+PRZ), 4 (AF+ZQR), and 5 (AF+PRZ+ZQR) received 20 mg/kg AFB1 intraperitoneally at 30 min following intraperitoneal administrations of prazosin (0.3 mg/kg), zosuquidar (25 mg/kg), and prazosin+zosuquidar (0.3 mg/kg+ 25 mg/kg), respectively. Blood and brain samples were taken from animals 6 hours after the administration of AFB1 for Groups 2 to 5. AFB1 concentrations were determined using an HPLC system with fluorescence detection. Effects of prazosin and zosuquidar on plasma and brain concentrations of AFB1 following IP administration of AFB1 at the dose of 20 mg/kg of body weight in mice are presented in Table 1. Single and simultaneous administrations of prazosin and zosuquidar significantly decreased brain concentrations of AFB1, compared to the single administration of AFB1 ($P<0.05$). Ratios of brain concentrations to plasma concentrations of AFB1 in mice are shown in Figure 1. The brain/plasma ratio in the AF group was higher than the groups (AF+PRZ, AF+ZQR and AF+PRZ+ZQR) that were administrated prazosin and zosuquidar (Figure 1, $P<0.05$). Inducers of transmembrane proteins such as PRZ can be life-saving in acute poisoning with AFB1, according to overall health status and brain concentrations of AFB1 in mice. The results of in vitro studies should be confirmed with in vivo study of native animals.

Keywords: Aflatoxin B1, bcrp, brain, mice, p-gp

Ratios of brain concentrations to plasma concentrations of aflatoxine B1. AF; aflatoxine B1, PRZ; prazosin, ZQR; zosuquidar. a,b Different letters are statistically significant ($P<0.05$).



The plasma and brain concentrations of aflatoxin B1 at 6 hours after the intraperitoneal administration of aflatoxin B1 at a dosage of 20 mg/kg of body weight in mice (n=8).

Group	Plasma (ng/mL)	Brain (ng/g)
AF	23.45±13.84ab	181.23±89.40a
AF+PRZ	15.53±10.10b	6.69±2.81b
AF+ZQR	31.96±14.28a	24.07±19.64b
AF+PRZ+ZQR	13.37±9.50b	24.85±11.66b

a, b Different letters in the same column are statistically significant ($P<0.05$). AF; aflatoxine B1, PRZ; prazosin, ZQR; zosuquidar.

GC-MS Analysis of Specimens for Pesticides in Suspicion of Intoxication

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Pesticides are substances that are used to kill harmful organisms which are present, or live on or around human and animal body, plants, and non-living things that give harm to or decrease the nutritional value of food stuffs during their production, storage and consumption. In this study, it is aimed to evaluate the GC-MS analysis results of specimens that are sent to Konya Veterinary Control Institute Toxicology Laboratory for suspicion of pesticide intoxication. Between March 2014 - March 2015, specimens [liver (n=118), kidney (n=97), gastric content (n=105), intestinal content (n=97) and questionable material (n=7)] belonging to 132 cases (dog, cat, cattle, calf, goat, sheep, yeanling, lamb, chicken, pigeon) that were suspicious for intoxication taking place in Konya, Aksaray, Niğde, Karaman, Afyon, Burdur, Antalya, and Isparta provinces, which were sent to Konya Veterinary Control Institute, were analyzed for the presence of 45 different pesticides. Extraction of pesticides from the materials was performed by modification of the methods defined by Mills (1959) and Pelosi et al. (2002); analyses were carried out in GC-MS qualitatively. In 20 (15.15%) out of 132 cases that were applied for analysis, various pesticides were detected. Out of the total 424 specimens that were analyzed, presence of various pesticides was detected in 55 specimens. Of these 55 materials, 15 were gastric content (27.27%), 11 were intestinal content (20%), 12 were liver (21.81%), 11 were kidney (18.18%), and 7 were questionable material (12.72%). Within the specimens with positive pesticide results, detected pesticides were carbamate group (Methomyl, Aldicarb, Carbofuran) in 45 (10.61%), organic phosphorus compounds (Malathion, Dimethoate, Chlorpyrifos) in 10 (2.35%), organochlorides (Alfa-endosülfan, Beta-endosülfan) in 2 (0.47%) and pyrethroid group (Deltamethrin) in 2 (0.47%) of them. Pesticide poisoning is an important problem threatening the health of animals in Turkey. In suspicion of poisoning, it is important that detailed evaluation of anamnesis, clinical condition, autopsy findings and epidemiological data is carried out by veterinarians in the first place, and specimens are sent to laboratory with this information in order to achieve accurate results in short time and also for providing aid for animal health in a timely manner. In conclusion, it can be stated that methomoyl that is present in carbamate group of pesticides is commonly responsible of pesticide intoxication in animal cases.

Keywords: Intoxication, pesticides, GC-MS

Comparison of *In Vıtro* Efficacy of Toxin Binders Used in Poultry Feeds

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In this study, it was aimed to determine the effectiveness of organic and inorganic toxin binders (TB) used in poultry feeds to bind aflatoxin B1 *in vitro* conditions. Prior, *in vitro* medium of poultry, which had certain conditions such as pH, temperature and duration, was carried out in order to evaluate the effectiveness of toxin binders in binding of aflatoxin B1. Then, a total of 20 TB, which were most used in poultry industry, were divided into three groups as organic, inorganic and mix according to their contents. For each TB *in vitro* poultry mediums were used both in pH 3 and pH 6,8. Control and treatment groups were formed for each study. Control groups were formed from standard of aflatoxin B1 and its buffer solutions; and the treatment groups were also formed from TB, standard of aflatoxin B1 and its buffer solutions. In this study a total of 80 samples were analyzed by the method of Vicam aflatest method in HPLC-FLD after extraction and filtration processes. Toxin binding activity of TB's used in poultry industry in *in vitro* conditions were compared by analysis of variance. The binding capacity of TB's used in poultry mediums: Inorganic TB's were found the highest 98% at pH 3, and organic TB's were the lowest 40% in *in vitro* conditions. At pH 6,8: The binding capacity of inorganic TB's were determined the highest 93%, and organic TB's were the lowest 43% in *in vitro* conditions. The rise of pH values made decline in the binding activity of inorganic TB's, whereas increase the binding activity of organic TB's at the same rate. The binding capacity of mix TB's to aflatoxin B1 was found 96% at pH 3, and decreased to 88% at pH 6,8 in *in vitro* conditions. It was concluded that the inorganic TB's, which were used to reduce the unfavorable effect of aflatoxin B1 in poultry feeds, were found more effective to bind aflatoxin B1 among TB's. The organic TB's were found to be not sufficient to bind aflatoxin B1. However, these findings should be supported by *in vivo* experimental trials.

Keywords: In vitro, poultry feeds, toxin binder

Investigation of Mycotoxin Residues in Total Mixed Rations (TMR) of Cattle and Poultry Feeds by LC MS/MS Methods

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In this study, it was aimed to determine the levels of mycotoxin contamination in total mixed rations (TMR) of cattle and poultry feeds in Konya and the surrounding provinces. A total of 147 ready to consume feed samples, which are 74 TMR and 73 poultry feeds from animal farms in Konya, Afyonkarahisar, Karaman, Aksaray, Nigde, Antalya, Isparta and Burdur province were collected. Homogenization and extraction were performed for each of feed samples. Analysis kits of Zivak Technologies were used in the extraction process. Samples were analyzed for Aflatoxin (B1, B2, G1 and G2), Ochratoxin A, Zearalenone, T-2 Toxin, Fumonisin and Deoxynivalenol (DON) levels by LC MS/MS method. The levels different mycotoxins in cattle and poultry feeds obtained from cattle and poultry farms, the proportion of positive samples and the percentage of presence in the feeds were compared in terms of mean values. A general screening of cattle and poultry feeds was performed in terms of mycotoxin presence and contamination levels. According to Turkish Food Codex (TGK 2014/11) the exceeded rates was found 30% for aflatoxin B1(>=5ppb), 20% for aflatoxin B1(>=10ppb) and 3% Ochratoxin A in TMR; and 1% for aflatoxin B1 in poultry feeds. T-2 Toxin has no legal limit in TGK and the presence rate was found 9% in whole analyzed feeds with the levels of 0,3 to 12 ppb. It was concluded that there was no contamination in poultry feeds in the studied area in terms of legal limits; according to exceeded levels TMR should be stored in more favorable conditions; and the T-2 Toxin was detected at very low levels, which cause no adverse effects in animals.

Keywords: LCMSMS, mycotoxin, poultry feeds, total mixed rations (TMR)

Identification of Apiair Component and to Investigate the Pharmacological Effects

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Api-air (hive air) has gained great importance to define for apitherapy applications. To investigate the api air components and intended to identify theirs pharmacologic effects for human health. Apiair applications are neded, to clean, quality field, study areas suitable for organic or natural beekeeping practices., 1- Apiary and materials: Average 100 hives bee-location an organic bee breeding field of application 2- Apiair application cabinet: 10-20 m2 room in a 9-10 active beehive, 3- Api (Hive)air sampling device for measuring components 4- To be Analysed by GC-MS / GC-MSMS systems Apiair applications: In apiair applications system have a slowly taking systematic mechanism with a fan motor inside the hive, is based on inhalation. In this application, apiair were transferred to the tube-mask with a regulator to the speed of the fan assembly adjustable in beehives. Apitherapy, "bee (bee products) treatment" bee products produced not only himself, even its habitat are effective even apitherapy. First applied in Germany in the World "Bee hive air or air" to a new bee products utilized in the treatment studies apitherapy added. There are important and aerosol containing volatile substances that are high quality microclimate to ownership means. The hive atmosphere of saturated water vapour in 36 ºC temperature comprises providing a continuous air flow and is regenerated by bees. Isoprenoids carotenoids, terpenes and essential oils are also included within the hive air with varying levels in other bee products. In addition, hormones, pheromones, phyto hormone, essential wax components, higher polyhydric alcohols, bees of mandibular gland secretions, aerosol, propolis welded aerosol, trace elements, enzymes, a choline in microclimate in hives. In other words, some active ingredients are located with honey, pollen, bee wax, the royal jelly, propolis and apilarnil as nanoscale in apiair Apiair is applied in individuals are determined accordingly to relax, relaxation, sleep uninterrupted and high quality, the expansion of lung capacity and breathing relaxed definitions Bronchitis, asthma, allergies, COPD (Chronic Obstructive Pulmonary Disease), emphysema, pseudocroup, immune system deficiencies, migraine, it is seen that the air in the bee diseases such as depression is extremely effective. As a result, promising results obtained by the application of apiair to patients with low cost, without side effects, of regaining their health with the support of a reliable method of treatment, healthy people also reveals more energetic and quality should be possible to continue their life. Apiair application, not alternative medicine, seems worth noting that in supportive. A bee products as medical treatment "complementary" should be considered as a factor should not be seen as a miracle and a short-term solution. A greater number of apiair components can be measured on the patient and on the type of disease. There is a need to do multidisciplinary studies with pharmacological parameters

Keywords: Apiair, component, identification, pharmacological effects

Research of the Pharmacologic - Toxicological Parameters in Apitheraphy Applications

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Apitherapy Definition: Bee products are used to support the treatment of some diseases and order to obtain a more robust performance. In this study, to identified of pharmacologic-toxicological effects in apithrerapy and take care of applications methods are aimed Bee-hives, honeybee and using of their materials for beekeeping Bee Products; the products getting secretions of bee from its body: Royal Jelly, beewax, beevenom, apiair, apilarnil, honey, polen, propolis and apivoice. Some components as pharmacologic effects in bee-products These products are using to apitherapy, as their structure and different rate mixing each others Bee Products; The products getting secretions of bee from its body: Royal Jelly, beewax, beevenom, apiair, pilarnil, bee voice, - The products collecting from plants and adding partly body secretion by honeybee; Honey, polen and propolis.1- Honey, the most discussed on as natural health source is miraculous honouring from bee to us Plant nectar has max. 80% sugar that the sugar ratio is usually 20%-40%. Sugar makes honey as viscous and hygroscopic. The bee picking up feed comes back to its hive and gives the ingredients (about 50 mg) of its honey sac to other bees in the hive. Honey is included different useful substances for health which are vitamins, minerals, flovanoids, enzymes, amino acids and others. 2. Pollen is a micro-spore (male sperm cell) of plant. Pollen is collected by beekeepers with trap put on hive and after drying or freshly kept in deep-freezer – 18 C. 3. Royal Jelly secreted in the pharyngeal gland of young employer bee is a homogenous, an off-white colour, a sharp smell, a sourish taste, a water-soluble substance. Propolis is sticky, has a special smell,its colour range is from light brown to dark red. It is collected from leaf, seed, branch, and tree shell by bees. After, they transport it to their own hive and mix ıt with their enzymatic secretions and wax. 5. Api (Hive) Air: In cancer treatment, in order to strengthen general state of the patient a one week session under a doctor observation, very interesting progresses are got. However, repeated apiair therapies are required for these kinds of patient.those are continued to obliterate or stabilize cancer agent. 6. Api Larnil: Apilarnil is obtained by lyophilisation of the act of male bee larvae. Some researcher reported that apilarnil used to increase in sperm rate and performance 7. Bee Voice The successful results were stated in the application made as supportive treatment for muscle and skeletal system, 8. Apitoxin (Beevenom): This is the most generally known and the best applied therapy methods. Especially for immune system and MS diseases, very successful results are get. Apitherapy applications must be supported by specific studies on the subject. Application to support the treatment of bee products and apitherapy, the effect of pharmacologic - toxicological parameters must be well-defined. You will also have a healthy lifestyle and a more detailed study of the effects of apitherapy useful application for sporty performance.

Keywords: Apiteraphy, applications, parameters, pharmacologic, toxicological

Bovine Lactoferrin Suppresses LPS-induced Acute Phase Response in Holstein Calves

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The acute phase response (APR) induces systemic non-specific changes in response to various stimuli, including inflammation and infection. The APR is primarily regulated by inflammatory cytokines such as tumor necrosis factor-alpha (TNF). The release of these mediators results in alternations in the plasma concentration of hormones and metabolites. Lactoferrin (LF) is an iron-glycoprotein that can be detected in most physiological fluids of mammals such as plasma, milk, saliva, and mucous secretions. This protein is known to have a role in iron absorption and is believed to be an important component of host defense. There is evidence also the LF modulates host defense responses, acting through the modulation of cytokine production and inhibition of lipopolysaccharide (LPS)-mediated activation of neutrophils. Therefore, we report the modulatory effect of LF on the plasma concentrations of TNF, metabolites, and hormones in the LPS-induced inflammatory response in preruminant calves. Thirty clinically healthy Holstein male calves were used in this study. At 4 day of age (-10 day), each calf was randomly assigned to one of three treatment groups (n = 10), matched to body weight. The treatments were the oral administration of LF 1g/day, 3g/day, or 10 mL of saline/day (control) for 10 days (-10 day – -1 day). The LF or saline was added to whole milk daily for the LF or control group, respectively. The day after the end of LF feeding for 10 days (0 day), the calves received an i.v. injection of LPS (50 ng/kg BW, E. coli 055:B5) at 09.00 hour. There was a marked, transient increase in plasma TNF at 2 h after the LPS administration in all groups. Plasma TNF concentrations at 2 h after LPS treatment were lower ($P < 0.05$) in LF 1g/day-fed claves compared with LF 0g/day (control) calves. Similarly, the concentration of plasma TNF in the LF 3g/day group tended to be lower than in the control group, but did not differ among groups. There was no effect of LF feeding on plasma concentrations of haptoglobin (Hp) between -10 day and 0 h in all calves of three groups. In LF groups, plasma Hp concentrations slightly increased after LPS injection, but those levels at 6 – 24 h were lower ($P < 0.05$) than in the control group. The LF treatment inhibited ($P < 0.05$) the reduction of plasma ferrin concentration in calves following LPS challenge. After LPS injection, plasma aspartate aminotransferase (AST) concentration increased at 6 h in all groups, and AST concentration was higher ($P < 0.05$) in control calves than in LF-groups. Thereafter, AST level in LF groups was lower ($P < 0.05$) than in the control group between 24 and 96 h. The concentration of plasma insulin-like growth factor-1 (IGF-1) in all groups was decreased by LPS treatment while, in LF groups, the IGF-1 level was higher ($P < 0.05$) than in the control group. Plasma insulin concentration in LF groups was lower ($P < 0.05$) than in control calves at 2 h after LPS injection. In the present investigation using preruminant calves, we have shown the repeated LF given orally as feed could lead to the reduction of metabolic and hormonal disturbances in LPS-induced inflammatory response. These data suggest that LF has a substantial anti-inflammatory effect on the modulation of the host defense system in preruminant calves.

Keywords: Acute phase response, calves, lactoferrin, lipopolysaccharide

Methicilline Resistance Determination of Staphylococci Isolated from Dogs and Their Owners

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Staphylococci are present in the skin and mucosa of humans and animals as commensals, and they may cause life threatening pathological changes. The antibiotic resistance that may crop up in these bacteria elevate the problem to more serious levels. Presently, methicilline resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudointermedius* (MRSP) strains are commonly isolated from humans and animals. This study aims to identify the presence of MRSA and MRSP and their coa gene profiles from dogs and their owners. To this end, swab specimens were taken from both dogs and their owners (as 33 cases) and isolation and identification were carried out by conventional culture methods. Fourtyfive out of 98 (45.91%) strains that were isolated were identified as *Staphylocoocus* spp. by 16S rRNA gene targeting PCR. Eight (17.77%) of these isolates were established as *S. aureus* by nuc gene targeting PCR. Twentythree of the remaining 37 (82.23%) strains of the staphylococcus isolates were identified as *S. pseudintermedius* by PCR-RFLP. Methycilline resistance of the isolates were determined by Kirby-Bauer disc diffusion method and mecA gene targeting PCR. According to these, 10 of the (22.22%) staphylococcus strains were defined as MRS. Five of the isolates were defined as MRSA (11.11%) and 2 as MRSP (4.44%). The remaining 3 isolates were determined as other MRS's. One of the MRSP isolates was found to be isolated from a human. In this study, the coagulase gene profile of the strains were determined by coa gene targeting PCR. According to this, 23 out of all staphyllococci (51.11%) are positive regarding the coa gene, however 3 of the *S. aureus* and 8 of the *S. pseudintermedius* strains were found to be negative in this regard. Ten different coa groups were determined in all staphylococcus strains. While the ratio was low, it was considered to be possible that MRSP that is commonly present in dogs as commensal was also isolated from humans, which may pose a health risk to humans. Also there was a polymorphism regarding to the coa gene between strains and coagulase negative variant *S. aureus* strains were found. The results of this study shows that precautions may need to be taken against contamination of MRSA and MRSP to both humans and animals.

Keywords: Dogs, dog owners, methicilline resistance, MRSA, MRSP

Model Municipality in Control of Zoonotic Diseases in Homeless Cats and Dogs

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In Turkey, in scope of the Animal Protection Law, animals for which no home can be found after rehabilitation at Temporary Animal Shelters are released to the streets. These rehabilitation efforts conducted by municipalities include vaccination, sterilisation and marking. These efforts are important to control the zoonotic diseases vectored by homeless animals living among humans in the city life. This study aims to point out that preventative veterinary services are one of the most important functions of municipalities. In scope of this study rabies vaccinations and internal and external parasite screenings conducted on homeless animals by Çiğli Municipality of İzmir Province. The data used in the study were gathered by examination of the Çiğli Municipality homeless animal register, examination protocol register and the communications regarding this issue.
It is found that approximately 6000 cats and dogs were taken in for rabies vaccination between 2010 and 2015, and 4000 animals were routinely treated for parasites in scope of the same process.In addition to these efforts, it is also seen that efforts were exercised in regard of animals presenting a suspicion of rabies infection. Municipality efforts on homeless animals should be assessed not only in view of animal health, but also as a public health issue. In scope of the concept of "one medicine one health", it is very important for professional chambers and veterinary doctors working for municipalities to relate the importance of their activities to the public. It is our conclusion that the data obtained from this study can provide a model for all municipalities and the results derived from such studies can help in creating statistical databases on national level.We believe scientific opinions and findings of professional chambers should be referred to and detailed statistical studies on public health and zoonotic diseases should play a determinative role in creation and application of legislations regarding homeless animals.

Keywords: Control, homeless animals, municipality

Characterization and Spatial Distribution of Dog and Cat Bites in Humans in Jaboticabal, Brazil

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Animal biting is an important form of zoonosis transmition. The aim of this study was to characterize dog and cat bites in humans and to estimate their spatial distribution in the city of Jaboticabal, São Paulo, Brazil. The database consisted of 183 human rabies post exposure prophylaxis records collected in 2014 from January to July. The spatial distribution of the aggressions was estimated in the MapInfo professional 7.5 SCP software. There were 92 (50%) notifications of bites in adults, 98 (54%) in men and 85 (46%) in women. It was registered 150 (82%) dog bites, 27 (14.8%) cat bites and 6 (3.2%) aggressions involving other species. The number of attacks caused by biting was 168 (92%) and caused by scratching/licking, 15 (8%). Concerning the injury type, 66 (36%) were deep, 104 (57%) were superficial and 13 (7%) were from other category. The number of healthy animals was 155 (85%) and the number of unknown, lost or deceased animals was 29 (15%). The spatial distribution analysis showed the central region of the city contained the greatest number of bites, with a total of 20 (11%). This might be due the fact that the great proportion of the animals in this region lives confined inside the house. This behavior increases the contact between the animals and their owners. The regions with at least one case of animal biting were those with the greatest number of animals that live in the streets. Other studies also found similar results and concluded that semi-confined animals presented a territorialism behavior that increased the risk of biting. In conclusion, there is a lack of knowledge about the behavior of housed dogs and cats. The regions with the greatest number of habitants and domestic animals with free access to the streets are the ones with greatest risk of biting. It is necessary to know the animal and human populations of each region of the city to create adequate strategies that aim to reduce animal biting. Responsible ownership attitudes prevent dog and cat bite accidents and ensures resource savings for their care. Thus, resulting in better public and animal health.

Keywords: Aggressions, public and animal health, rabies prophylaxis, spatial analysis

Spatial Distribution of Rabies Cases in Brazil, from 2001 to 2012

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The aim of this study was to characterize the vulnerability of the Brazilian states and evaluate the spatial distribution of the human rabies cases occurrences, from 2001 to 2012. The dataset consisted of 127 records of human rabies cases. This data were obtained from SINAN (Diseases Information System Notification), which belongs to the DATASUS database (Department of Informatics of the Unified Health System - Ministry of Health, Brazil). The data pre-processing was carried out using the Microsoft Office Excel 2010. The cases reported in 22 states of Brazil were evaluated by the software MapInfo Professional 7.5 SCP. From the total, 7 brazilian states (Acre, Tocantins, Minas Gerais, Goiás, Rio Grande do Norte, Mato Grosso and the Federal District) had 1 (1%) recorded case of rabies in each. The states of Sergipe and Pernambuco had 2 (2%) and the State of Piauí presented 3 (2%) reported cases. The states of Rondônia and Alagoas presented 4 (3%) and 5 (4%) reported cases, respectively. The states of Bahia and Minas Gerais presented 6 (5%) cases. each. The State of Ceará presented 12 (9%) cases. Finally, the states of Pará and Maranhão had 37 (29%) and 39 (31%) reported cases, respectively. The spatial distribution allowed to observe that the states of Pará and Maranhão together had 76 (60%) cases of rabies notification; and sequentially Ceará State with 12 (9%), Bahia and Minas Gerais with 6 (5%) reported cases. In Brazil, the states that are in the Northeast, such as Ceará and Bahia, are classified as endemic for rabies and due to the constant presence of the disease, there is a greater concern on the part of the population, to seek medical care after accidents involving animals attack, where there is a risk of contracting the rabies virus. So, this is the reason of the high number of cases reported in these areas. Thus, it is important to emphasize the demand for medical care, especially in cases involving animal attacks, contributing to the notifications, which allow to view the true situation of rabies in Brazil, assisting in the control and prevention of disease and helping with the activities of the Health Surveillance Services of brazilian municipalities.

Keywords: DATASUS, geoprocessing, medical care, rabies

A Risk Assessment of Heavy Metal Concentrations in Fish and an Invertebrate from the Gulf of Antalya

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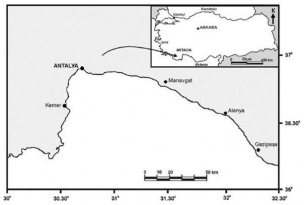
The objective of this study was to determine the concentrations of some non-essential (Pb, Cd) and essential (Cu, Zn) heavy metals in bioindicator seafood species and health risk assessment based on consumption habits. A total of 105 muscle samples of *M. barbatus* (Mb), *M. Cephalus* (Mc) and *P. semisulcatus* (Ps)] were collected from the Kemer (KE), Centre of Antalya (CA), Manavgat (MA), Alanya (AL) and Gazipaşa (GA) stations in the Gulf of Antalya (fig. 1). The metal levels determine by ICP-OES and statistically eveluated. The methodology of estimating a target hazard quotient (THQ) used to evaluate the potential health risk. The THQ values can be assessed with the following equation: $THQ=(E_F \times E_D \times F_{IR} \times C) / (R_{FD} \times W_{AR} \times T_A) \times 10^{-3}$ E_F is the exposure frequency (365 days/year); E_D is the exposure duration, equivalent to the average lifetime expectancy (70 years); F_{IR} is the food ingestion rate (g/person/day) of fish varying by dietary habits which were assumed to be 23 for Turkey; C is the metal concentrations (mg/kg dw); R_{FD} is the oral reference dose ($4x10^{-2}$ mg/kg/day for Cu, $3x10^{-1}$ mg/kg/day for Zn, $1x10^{-3}$ mg/kg/day for Cd and 4x10-3 mg/kg-day for Pb); W_{AR} is the average body weight (70 kg); and T_A is the average exposure time to no carcinogens. A THQ value of <1 indicates a potential risk. The average concentrations (mg/kg) [Pb (0.29 ± 0.14), Cd (0.02 ± 0.03), Zn (5.64 ± 1.58) and Cu (1.64 ± 1.02) for Mb; Pb (0.22 ± 0.12), Cd (0.02 ± 0.01), Zn (7.66 ± 2.29) and Cu (1.33 ± 0.82) for Mc; and Pb (0.25 ± 0.16), Cd (0.04 ± 0.05), Zn (13.33 ± 3.34) and Cu (5.15 ± 2.09) for Ps] were below the legal limits (Fish: Pb: 0.30, Cd: 0.05, Prawn Pb and Cd: 0.50). According to the evaluation of worldwide and Gulf of Antalya proved that Zn level is higher, even though relatively low compared with other studies on Turkish and European coasts of Mediterranean Sea. Considering the studied metals, trends of bioconcentration in species were in the descending order of Mc<Mb<Ps. The differences among the bioaccumulated metals in the species are due to their habitats. Mb (demersal) and Ps (benthic) live and feed on or near the bottom of the sea, therefore they exposed to heavy metals more heavily. THQ average value is 2.4E-02 in Mb average, 1.8E-02 in Mc, 2.1E-02 in Ps for Pb, 6.6E- 03 in Mb and Mc, 1.3E-02 in Ps for Cd, 6.2E-03 in Mb, 8.4E-03 in Mc, 1.5E-02 in Ps fo Zn and 1.3E-02 in Mb, 1.1E-02 in Mc, 4.2E-02 in Ps fo Cu. There are no THQ values above 1 caused by the consumption of the studied seafood species, suggesting that people will not experience significant health risks if they ingest individual heavy metals only from these sources. Concentrations were below the legal limits for Pb and Cd, although the Pb in Mb (0.29) was close (0.30). Therefore, the samples posed no risk to human health. Additionally, despite national and international policies intended to protect the environment and public health, and the level of Zn is increasing throughout the Gulf of Antalya. Finally, the origins of fishery products should be indicated on sales labels because the metal levels can differ by the region in where it was caught.

Keywords: Heavy metals, mediterranean sea, mullus barbatus, mugil cephalus, panaeus semisulcatus, target hazard quotient (THQ)

Fig. 1 Stations where the samples caught



Investigation on Tularemia in potential reservoirs' of Anatolia

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Tularemia is a zoonotic infection caused by Francisella tularensis and has gained renewed importance since there has been a recent increase in the number of human cases in several countries across the world. The aim of this study was to investigate the existence of F. tularensis in rodents and in water by culture and PCR techniques. In addition, spesific antibodies for F. tularensis were investigated by serological methods in sheep. A total of 445 samples, including rodents, sheep blood sera were collected from 8 different residental areas in Turkey and 4 water samples from Ankara/Beypazarı. Isolation of the agents from the rodent and water samples: In order to detect the rodents that were bacteremic with F. tularensis, liver and spleen samples from 40 rodents were inoculated into the Francis medium supplemented with antibiotics and defibrinated sheep blood. Microagglutination test: Francisella tularensis specific agglutinins were examined by using microagglutination test (MAT) with antigen (0.004% including spleen-O) prepared with F. tularensis strains. PCR analysis: For the purpose of molecular diagnosis, conventional PCR (Polymerase Chain Reaction) was used in water samples, rodent liver and spleen samples and to confirm isolates. At the end of the study, F. tularensis was isolated from one water sample by culture and PCR techniques, on the other hand, 27.6% seropositivity was detected in the blood samples of sheep.

Keywords: Francisella tularensis, rodent, sheep, water, zoonoses

Production of Suspansion BHK21 Cell Cultures in Differend Mixing Systems

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Cell culture is the process by which cells are grown under controlled conditions, generally outside of their natural environment.Cell culture systems are generaly defined as monolayer and suspension cell culture.In the production of large scale Foot and Mouth Disease vaccine, BHK21(Baby Hamster Kidney) cell cultures are produced in suspension systems. Optimum media conditions and mixing systems are very important in suspension cell culture production. In our Institute, hole plate with vibro motor mixing system is used in suspension cell culture production. In this study,hole platewith vibro motor and impeller with magnetic motor mixing systems are compared in our bioreactors. cells were produced In both systems parallelly and checked in terms of morfology, viability, number of cell and proplifiration speed. As a result, any big difference in terms of cell production between two mixing system was not found. The cleaning, operating and maintanence of impeller with magnetic motor mixing systems are easier than hole plate with vibro motor mixing system and further studies are needed.

Keywords: BHK21(Baby Hamster Kidney), FMD vaccine, mixing, suspension cell culture

Detection of Minute Virus of Mice Contamination in BHK-21 Cells

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Manufacturing of Foot and Mouth Disease Vaccine is a complex process and requires large volumes of suspention cell cultures. This process is prone to contamination by numerous agents of bacteria, fungi and viruses. Contaminations are heavy burdens for vaccine producers by means of cost, labour and time. Mouse parvoviruses are one of the most important patogens of mouse colonies. Minute Virus of Mice (MVM) is a parvovirus of murine. Unenveloped spherical small particles contains single strand 5.1 kb DNA. This genome encodes two non-structural proteins NS-1, NS-2 and two structural proteins VP-1 and VP-2. For replication MVM requires the S phase of cell cycle. There are two serologically indistinguisable strains of MVM, namely MVMp and MVMi. It was found that BHK-21 suspension cells can be infected with a virus serologically related to MVM. The virus isolated from large scale cultures as well as storage tanks of different laboratories. The source could not be identified. PCR is a powerful technique for detection of contaminants in cell culture. In 2011, FMD Institute Ankara/Turkey faced a persistent death of cells in the large scale BHK cell cultures used in FMD vaccine production. Cells could not be grown in fermentors and eventually died. Repeats to grow from master cell stocks resulted in failure. Exact reason of death could not be identified for long time. After an extensive search for extraneaous agents, MVM genome was detected in cells. Conventional PCR has been used for the detection of MVM genome. Moreover a commercially available real-time MVM detection kit has been used for confirmation. Hundreds of samples from master and working cell stocks and suspension of cells from fermentors tested and positives eliminated from production. Mass disinfection methods including fumigation, aerosol peroxidase and UV lighting have been utilized. After elimination of the contamination from production a positive control is designed by using a plasmid vector containing VP-2 insert of MVM genome.

Keywords: BHK-21, cell culture, contamination, minute virus of mice

Research of Neonicotinoid Group Insecticides Toxications for Honeybee

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In this Project; Neonicotinoid group pesticides will be examined the effects of on honeybee health and be able to determine whether cause any toxicity. Investigate of these group pesticides levels on sunflower and cotton plants and can be determined how they affect the process by which the honey bees. The field study will be examined the effects of this group pesticides on the behavior of honey bees. To develop methods for the analysis of neonicotinoid gr.pesticides in our laboratories will be also be extended to other institutions laboratories. This method is a sensitive method to provide to analysis of nenonicotinoid insectisides residues in honey, bee, sunflower, cotton and soil samples by LC-MSMS. This method is a multirersidue analysis method that neonicotinoid group pesticides which are Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam First, sunflower, cotton and soil samples were collected from the Marmara region and planting areas in the Mediterranean during two sessions. In Laboratory step, at the beginning, neonicotinoid pesticides standards are analysed and validated with LLE method by LC-MSMS system Then, Samples are extracted and predicated every samples collect sessions and storage in deepfreeze (-18 ºC) Neonicotinoid group of insecticides are used for a limited number of products such as potato pest etc. as licensed to fight against pests in agricultural production in our country. Neonicotinoids are a class of insecticides which act on the central nervous system of insects with lower toxicity to mammals. Neonicotinoids are among the most widely used insecticides worldwide, but recently the uses of some members of this class have been restricted in some countries due to a possible connection to honey-bee colony collapse disorder. End of first year, some samples of sunflower cotton and soil are analysed and detected some positive results in sunflower crown especially first 10 days. Also some positive results were obtained cotton samples and soil samples. The worker bees neglecting to provide food for larvae by some to account and and possibly leading to. Beekeepers and the observations of experts under the influence of neonicotinoid pesticides, 1 - Worker bees could not feed the larvae in the colony to the task of disrupting, Worker bees could not feed the larvae after effected with this pesticides and caused to be effect larva toxicosis should be research definitely 2-Caused to lose navigational abilities of the bee, 3- And that creates Stress 4- Colony Collapse Disorder cause of they believe. The use of beekeeping activities in developed countries where prohibited by these groups should be further investigated and the effects of pesticides on bees should be tested experimentally. Effects of neonicotinoid class pesticide are required to investigate thoroughly on the bee deaths and colony losses in these areas. In addition, the group should be examined in the neonicotinoid pesticide residues in sunflower oil and textiles. Because, the effects of the their metabolites and their half-life may be important particularly.

Keywords: Honeybee, imidocloprid, insecticide, neonicotinoid, toxication

Effects of Desication through Freeze Drying Procedure on the Stability of Brucella Vaccines

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B. melitensis Rev.1 and B. abortus S19 vaccines are the most efficient vaccines using in Brucellosis control and eradication programs worlwide and suggested by the international organizations such as, OIE, FAO, WHO. Commonly used anti-brucella vaccines are live, attenuated and freeze-dried. These vaccines should be stabile, good quality with enough germ and have at least one year shelf life. Desication by Freeze-drying is a preferable method of preserving vaccines since the long-term viability is excellent and the storage and transportation requirements are simple. The aim of this study was to detect effectes of freeze-drying process in survival rates of brucella vaccines indicating to stability and shelf life of vaccines. For this purpose the survival rates after drying were analyzed as of comparatively three different freeze-drying techniques. B. abortus S19 and B. melitensis Rev.1 vaccine strains were inoculated to bioreactors containing trypton medium. Growth cultures were harvested and concentrated by adding carboxymethylcellulose. Viability control (enumeration) was performed by living bacteria after plating dilutions on solid medium and incubation 5 days at 37 ºC. World Health Organization (WHO) medium, consisting of 5 % sucrose, 2,5 % casitone and 1% sodium glutamate, was prepared and sterilised by passing trought 0,2 µ capsule membrane filter. This is the recommended stabilizing medium for Brucella vaccine production and lyophilization. According to enumeration results, concentrated bacterial suspensions were diluted with a WHO medium to a concentration of approximately 200 x 109 cfu/ml. For 2 ml fill volumes, 6R borosilicate glass vials with 20 mm openings were filled with 2 ml of the diluted bacterial suspension. Filled vials in lyophilization trays were partially closed with silicone treated, split, butyl rubber stoppers, after that vials were lyophilized (Model Benchmark 4000 SLR-60,Virtis Co., Gardiner, NY, U.S.A.). This procedure was repeated for three different freeze-drying cycles. In a same way as done prior to lyophilization, enumeration were conducted just after lyophilization on three randomly selected vials from each lyophilization. Also accelerated stability tests were performed and residuel moisture content was determined by Karl Fischer method. When compared to three lyophilization tecniques Rev1 and S19 vaccines could not be lyophilized efficiently in two trials. Lyophilization losses were about 65 %, survival rate was 35 %, cake appearance was not good becase of the collaps, residuel moisture content was 8 % and accelerated stability results were poor. In spite of this, the results of third trial were promising. Lyophilization losses were about 25 %, survival rate was 75 %, cake appearance was very good, residuel moisture content was 1,7 % and accelerated stability results were satisfied. Freeze drying process damages to bacteria and effects survival rate of it. Detrimental effects of dessication can be prevented by using appropriate stabilizer medium and convenient freeze-drying cycle. That kind of cycle can be summed up this way, the shelves of lyophilizer must be precooled up to -50 ºC this creates small ice crystals. But small ice crytals can cause to slow sublimation, in order to avoid it, annealing should be implemented. Annealing process provides larger por size in ice crystals and also prevents vial breakage. During the drying process high pressure poor vacuum implementetion is vital principle.

Keywords: Brucella vaccines, lyophilisation, stability

Effects of Kefir on Blood Parameters and Intestinal Microflora in Rats

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A probiotic product of kefir is widely consumed by human beings but there are inadequate number of studies with Kefir in animal nutrition. The purpose of this research was to investigate the effects of kefir on blood parameters and intestinal flora in rats. A total of 24 female rats were used in this study. Rats were randomly allocated into one control group and three treatment groups each containing 6 rats. During 35 days of experimental period, rats were fed with a commercial diet having 23% crude protein and 2800 kcal/kg metabolizable energy. Feed in pellet form and water were provided ad libitum. Kefir was given at the levels of 10 ml/kg, 20 ml/kg and 30 ml/kg with oral gavage to the first, second and third treatment groups, respectively. Kefir was not given to the control group. The number of yeast was found to be 1,65x107 and the number of lactobacilli was found to be 4x 108 in kefir. At the end of the experiment blood samples were taken from all rats. Blood serum parameters and intestinal microflora were investigated. Initial and final body weights were not different among the groups. No differences were observed among the groups in total protein, albumin, uric acids, cholesterol, SGPT, SGOT, alkaline phosphatase and phosphorus in blood serum. The serum triglyceride levels in the second and third of the experimental group were lower than those of control group and the first experimental group ($p < 0.05$). No differences were observed in the intestinal pH levels among groups. Although total bacteria number of intestinal microflora was not changed in groups, the number of enterobacteria and coliform bacteria in the third experimental group was lower than the other groups ($P<0.001$). The number of Lactobacilli and the yeast level in the intestinal contents were increased by the usage of kefir ($P<0.001$). Kefir usage reduced the serum triglyceride level. Positive effects of the usage of kefir were observed in intestinal microflora with increasing the number of benefical bacteria and decreasing harmful bacteria. It was concluded that kefir has a positive effect on the health of the animals.

Acknowledgements: This research was supported by Mustafa Kemal University Scientific Research Projects (Project no:13321)

Keywords: Blood parameters, intestinal microflora, kefir, rat, performance

Experimental Model of Pathogenesis of Abortion by Leptospirosis

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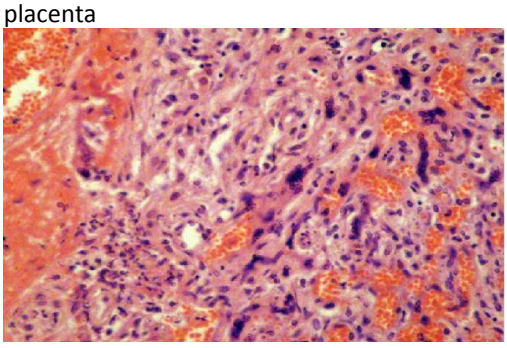
The pathogenesis of abortion by Leptospira sp. may have a multifactorial origin and a wide array of factors could be involved. Domestic animals abort frequently due to leptospirosis, however, the physiopathological mechanisms still are unknown. To better understanding of these facts in vivo assays have been done in order to study such mechanisms in guinea pigs. These animals are very sensible to leptospirosis infection and could be an excellent model for study this disease. The objective of this experimental trial was to study the changes and mechanisms involved in abortion caused by an infection of Leptospira spp. Our aim was to visualize lesions produced by infection in the placenta, therefore, understand more the causes of abortion. Samples of placenta from aborted animals infected by Leptospira interrogans serovar Pomona were collected and fixed in 10% buffered formalin, embebbed in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (HE), Warthin-Starry, Periodic acid-Schiff (PAS) and Masson`s trichrome modified by Lillie. Observations were focused both on blood vessels and perivascular areas. Masson-stained tissues exhibited fibrinoid material and granular proteinaceous deposits on the vessel walls, whereas PAS colored ones showed thickened basal membranes as well as rupture and disorganization of other vascular components. Placental changes consisted of vacuolar degeneration of the arterial endothelium, disseminated vascular coagulation, neutrophilic histiocytic vasculitis, fibrinoid degeneration of blood vessels and trophoblast necrosis. At the uteroplacental union were observed necrosis, neutrophilic infiltration and haemorrhage together with degenerative change of large arteries including proliferation of sincytotrophoblasts that invaded both wall and lumen. Warthin-Starry placental tissues showed aggregations of leptospiras adhering to the vascular endothelium. This study leads to a better understanding of the pathological consequences of Leptospira infection. Direct participation of these bacteria has been suggested as a crucial factor in cell lesion, characterized by the presence of inflammatory cells, endothelial damage by proteinaceous deposits and adhesion of leptospires to the vascular wall of the placenta. The demonstration of fibrinoid material, granular deposits and the thickened of the basal membranes in the placental vessel are factors which interfere with the mother- fetal exchange. Thus, it is cause of disrupting the mother- fetal nutrient flow mostly in the later pregnancy states, thus finally triggering hypoxia and cell death. These events lead generally to fetal death. We hope that these findings can contribute to understanding of the pathogenesis of the abortion by leptospirosis.

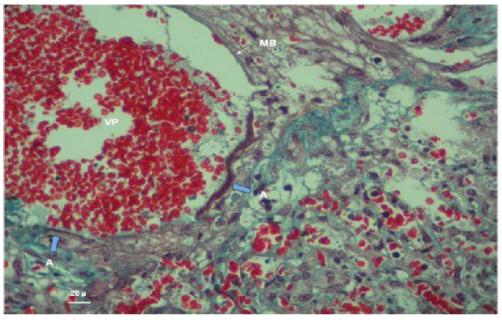
Keywords: Abortion, guinea pigs leptospirosis, pathogenesis

placenta



Neutrophilic infiltration and haemorrhage. (H&E).

placental vessel



MB) basal membranes. A)fibrinoid aterial and granular proteinaceous deposits. Masson-stained

Prevalence and Antimicrobial Resistance of Arcobacter Species Isolated in Some Animal Faeces with 16S rDNA-RFLP Method

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In this study, we investigated the presence of Arcobacter species was investigated in the faeces of cattle, sheep, goats, dogs and cloacal swab samples of chicken by using the 16S rDNA-RFLP method. A total of 78 (13%) Arcobacter spp. isolates were obtained from 600 samples observed in this study. Of the 78 Arcobacter isolates, 24 (30.77%), 20 (25.64%), 11 (14.10%), 8 (10.26%), 4 (5.13%), 3 (3.85%) and 2 (2.56%) were identified by 16S rDNA-RLFP as A.cryaerophilus, A.butzleri, A. skirrowii, A. cloacae, A. cibarius, A. halophilus, and A. nitrofigilis, respectively. Six of the samples (7.68%) were carrying more than one Arcobacter species. This is the first study to identify the presence of A. cloacae, A. cibarius, A. halophilus, and A. nitrofigilis species in the analyzed samples. All A. cryaerophilus isolates were found to be resistant to cloxacillin and all A. butzleri and A. skirrowii isolates to penicillin/novobiocin, cefoperazone, tetracycline and cloxacillin.

Keywords: 16S rDNA-RLFP, antimicrobial, arcobacter spp., faeces

Protective Effects of Safranal against Testicular and Spermatological Failure in Cisplatin-Induced Rats

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The aim of the present study emphasized to investigate cytogenetic and testicular damage induced by the chemotherapeutic, cisplatin in Wistar-albino rats and the effect of saffron aqueous extracts. Cisplatin which contains platin that is known as heavy metal is a strong antineoplastic agent. Safranal is known that one of the component of safran flower. In recent studies, safranal has high radical sweeper effects and can be used as a protective or anticancer agent for pharmacology and treatment of cancer on functional food, in drinks which include antioxidant. In this study, 30 rats were used and groups which were divided into five section contains six rats in the each group and the control group (K), the group which were exposed to cisplatin (CP), safranal group (S), cisplatin + safranal group (CPS), our protective safranal group (S) + cisplatin+ safranal (SCPS). Cisplatin was administered only once a time at fifth day of experiment with the dosage of 7 mg/kg via intra peritoneal route. Safranal was used at study groups with the dosage of 200 mg/kg. In CP and CPS groups, motility was significantly decreased ($p<0.05$) compared with the control group. Abnormal sperm rate were significantly increased ($p<0.05$) in group CP compared with control and other study groups. Otherwise the significant effect is improved that prior administration of safranal+cisplatin groups, results are more effective against cisplatin+safranal group. Seminifer tubul diamater was decreased significantly ($p<0.05$) group C and group CPS compared with to the control group. According to these results, safranal has a high protective effects against cisplatin's adverse reactions that are known damages sperm quality.

Keywords: Cisplatin, safranal, spermatogenic cell density, sperm, testes

Effects of Amlodipine on Spermatological Parameters and Genital Tract Weight in Adult Wistar Male Rats

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The aim of this study was to investigate the effect of calcium channel blocker (CCB) amlodipine (AML) on spermatological features and genital tract weight adult in Wistar male rats. Adult male rats were divided into two study groups, contain six rats in each group. Group I rats were fed normal diet without amlodipine served as the control, group II received 0.04 mg/daily for 30 days AML. Motility, abnormal sperm rate, left testis, right epididymis and prostat weight were significantly ($p<0.05$) decreased in amlodipine groups compared with control group. Seminifer tubul diamater and germinal cell layer thickness was not affected compared with control group. According to results emphasized detrimental effects of subacute administration amlodipine on the reproductive function of male rats. Further researches are necessarily on this field for a grand scale study to investigate the potential effect of long-term administration amlodipine on sexual impairment in rats.

Keywords: Accessory glands, amlodipine, rat, spermatogenic cell density, sperm, testis

The Effect of Lyophilized Pomegranate Extract on Sperm Quality, Oxidative Stress and Spermatogenic Cell Density in Rabbits

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The aim of this study was to investigate the effect of liophilized pomegranate juice extract on spermatological features, pathology of testes and total antioxidant/oxidant status in rabbits. Adult male rabbit were divided into four treatment groups, contains six rabbits in each group. Standart diet was received Group I, Group II was received 25 mg/kg/day+ 1 ml % 0.5 carboxymethyl cellulose (CMC), Group III was received 50 mg/kg/day+ 1 ml % 0.5 CMC and Group IV was received 100 mg/kg/day+ 1 ml % 0.5 CMC. Rabbits were sacrificed by using xylazine 5 mg/kg+ketamine 35 mg/kg anaesthesia and were euthanized by 150 mg / kg intraperitoneal thiopental sodium end of eighth week. Sperm motility, mebran integrity was increased significantly ($p<0.05$) in group II, III and IV, abnormal sperm rate was decreased significantly ($p<0.05$) in group III, IV total oksidant status was decreased significantly ($p<0.05$) in group IV when compared with to the control group. Seminifer tubul diamater was increased significantly ($p<0.05$) in all groups compared with to the control group. Germinal cell layer thickness was significant increased ($p<0.05$) in group IV compared with to the control group. Results of this study suggest that 50 mg/kg/day+ 1 ml % 0.5 CMC and 100 mg/kg/day+ 1 ml % 0.5 CMC improve sperm parameters in rabbits.

Keywords: Oxidative stress, pomegranate extract, rabbit, sperm characteristics, spermatogenic cell density

The Effect of Melatonin on Reproductive Parameters in Pigeon Columba livia Exposed to Different Photoperiodic Regimes

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The effects of a treatment with exogenous melatonin on the reproduction of male pigeons (Columbia livia) submitted to long or continue photoperiod were evaluated. A total of 36 male pigeons were randomly divided into 6 groups according to the photoperiod regimes (light for 18 hours and light for 24 hours) and to the daily oral dose of melatonin (3 or 6 mg in toto) administrated for 6 weeks. Testicle volume and plasma concentrations of glucose and thyroxin were determined at the end of the experiment. Melatonin treatment has induced significant increases in, testicle volumes and plasma thyroxin concentrations and significant decreases in glycaemia. Variations in the different parameters have been proportional to the melatonin doses in one hand and they have been more prominent when birds were exposed to a continue. These results show that melatonin stimulate the reproductive function coupled to an increased thyroxin secretion in male pigeons exposed to long or continue day length instead of photorefractorines.

Keywords: Columba livia, melatonin, pigeon, testicles photoperiod, thyroxin

The Impact of Chronic Doses of Cadmium and Selenium on Rabbit Cuniculus Lepus, Biochemical Study

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The effect of CdCl2 either alone or combined with sodium selenite on rabbit Cuniculus lepus have been investigated. Male and females were divided into 3 groups, and subjected to treatments for a period of 6 weeks. In addition to the serum urinary and reproductive parameters have been measured. Results have showed a significant variation in serum iron and total iron binding capacity between the two treated groups in both males and females. However, in males treated cadmium group, serum glucose and urinary calcium levels have been increased significantly, whereas, their concentrations have not changed in females. It seems that cadmium toxicity has also been affected by sex, where females were more resistant than males. However selenite addition to the diet has not reduced urinary calcium level in both sexes, indicating that it cannot be a suitable antidote against cadmium. In contrast to what have been observed in the groups exposed to cadmium, a significant decrease in urea and creatinine levels were recorder in the combined treatment, which means a proper kidney function, has been maintained.

Keywords: Biological parameters, cadmium toxicity, rabbit, sodium selenite

Arterial Supply of the Penis in the Persian Squirrel

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In the present study, the distributional pattern of the penile artery and the vessels joining the blood supply of the penis were investigated in the Persian squirrel. Eight adult squirrel were used in the study, latex was injected via the abdominal aorta. The samples were fixed in 10% formaldehyde solution and arteries were dissected. The penile artery is originated as a branch of internal pudendal artery, is a branch of the internal iliac artery and extern from pelvic cavity throw lesser isciatic notch. At the level of ischiatic arch penile artery divides in to four branches which form the bulbourethral, balbus, deep and dorsal penile arteries. The deep penile artery pass the tunica albuginea, and forms the arterial network of corpus cavernosum penis. Bulbourethral artery for bulbourethral gland,bulb artery for bulb penis and dorsal penis artery at the level of the attachment of preputium branches in two glans and preputium artery. The course of both arteries follows the dorsolateral surface of the penis to the glans and ends in an anastomosis. Hence, a caudal branch of the external pudendal artery which originates from the pudendoepigasteric artery joins the blood supply of the penis in the squirrel. After vascularizing the scrotum and preptium, it ends by entering the corpus cavernosum penis at the dorsolateral surface at the level of the ischiadic arch.

Keywords: Arterial blood supply, persian squirrel, penis

The Effects of Midazolam, Ketamine and Isofluran Anaesthesia on Hematologial and Biochemical Parameters in Rabbits

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The aim of the present study was to investigate the possible effect of the midazolam, ketamine and isoflurane on hematological, biochemical parameters, and to present these results to veterinary practice. Forty-eight New Zelland mature healthy rabbits were used in this study. The rabbits were allocated into 3 study groups and 3 control groups (each had 8 rabbits). Group I: Each rabbit in this group was administered 3 mg/im dose of midazolam (M). Group II; 10 minutes after following 3 mg/kg of M injection, 25 mg/kg im of ketamine (K) was administered for each of the rabbits. Group III; 10 minutes after following 3 mg/kg of M injection, 25 mg/kg im of K was administered for each of the rabbits. Ten minutes after ketamine injection, each rabbits were entubated and anaesthetized by 2% of isoflurane. Equal volume of physiological solution was administered to the same number of the rabbits in control groups. From the control and experiment group of rabbit, blood samples prior to and after anaestesia of 10, 30 min, 1, 3 and 5 hours were drained into tubes for the measurement of blood gases, biochemical and haematological values. There were significant differences for haematological, biochemical and blood gase values between the experimental and control groups of rabbits.

Keywords: Anaesthesia, ketamine, midazolam, rabbit

An Experimental Study of Systemically Used Midazolam, Ketamine and Isoflurane Anaesthetic Agents Effects on Intraocular Pressure and Tear production of Rabbits

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The aim of this study was to determine of the effects of midazolam, ketamine and isoflurane anaesthetic agents on tear production and intraocular pressure in rabbits. Twenty healty white New Zealand rabbits were used as a material. The rabbits were randomly seperated into two groups of ten animals. Before the application of anaesthetic agents intraocular pressure (IOP) and tear production were measured with rebound tonometer (TONOVET, RBT, IcareVet, Helsinki, Finland) and schirmer test strips (STT) (Vet Eickemeyer, Schimer strip, ophthalmic strips). Anaesthesia was performed with midozalam (3 mg/kg, IM) and ketamine (30 mg/kg, IM) in first group, midozalam (3 mg/kg, IM), ketamine (30 mg/kg, IM) and isoflurane (2 %) in second group. After thirty minutes of ketamine injection in first group and intubation in second group, IOP and tear productions measured again. The obtained datas were statistically evaluated. Before anaesthetic application, mean IOP values of first group were 14.2±0.75 mmHg (right eyes) and 14±0.80 mmHg (left eyes), in second group's were 12±0.75 mmHg (right eyes) and 12.2±0.80 mmHg (left eyes). After anaesthesia mean IOP decreased in both groups. The decrease was stastically significant ($p<0.05$). The mean values of tear production before anaesthesia in first group were 13.25±0.70 ml/minute (right eyes), 12.25±0.70 ml/minute (left eyes), in second group's were 7.75±0.70 ml/minute (right eyes), 8.12±0.70 ml/minute (left eyes). After anaesthesia mean tear production decreased in both groups and it was stastically significant ($p<0.05$). As a result of this study, systemically used midazolam, ketamin and isoflurane anaesthetic agents decrease IOP and tear production in rabbits were defined. The anaesthesia and anaesthetic agents choice of the animals with eye problems should be careful.

Keywords: Anaesthesia, IOP, rabbit, STT

Effects of Using Dietary Safflower and Sunflower Meals in Quail Diets on Performance and Egg Quality Parameters

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This study was conducted to determine the effects of using safflower meal (SM) and sunflower meal (SFM) in combination (SSM) in laying quail diets on performance and some egg quality parameters. A total of 192 (128 females and 64 males) eight-week-old Japanese quails (Coturnix coturnix japonica) divided into one control group and three treatment groups containing 48 quails. Each group was divided into four replicate groups each containing 12 quails. The control group was fed diet containing corn-soybean meal basis without SSM. The SSM was used at level of 10% (SSM10), 20% (SSM20), and 30% (SSM30) in treatment diets (in each treatment S and SF ratio is 1:1). The experimental period was lasted for 8 weeks. The results showed that there were no changes in terms of initial and final body weights, feed intake, egg production, feed conversion ratio and egg weight as well as shape index, shell thickness, albumen index, yolk index, Haugh unit and yolk color index in all experimental groups with SSM supplementation (p>0,05). As a result, it may be stated that the combined dietary supplementation of up to 30% of safflower-sunflower meal had no any adverse effect on the performance and some egg quality parameters in laying quails.

Keywords: Egg quality, performance, quail safflower meal, sunflower meal

Aspects of Breeding Ecologie of Waterfowl in Numidia, Illustrated by the Breeding of the Ferruginous Duck Aythya Nyroca at Lake Tonga; North-East Algeria

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Climate changes which been affecting our planete, requires the preservation of our wetlands and their biodiversity which qui represents a major priority to protect the écosystems and the species. To be effective, we must have a good knowledge of the ecologie of the species which populate the wetlands, especially the rare ones or those have a restricted distribution's area. Their decline reports most of the time a dysfunction, this is the case of the Ferruginous duck Aythya nyroca protected species, vulnerable at the red list of IUCN. Our study realised in 2011, is a contribution at the knowledge of an important phase of the life cycle of the Ferruginous duck Aythya nyroca, which is the breeding in a RAMSAR site, lake TONGA. We expose the resultats of some breeding parameters of the species (habitat selection, predation rate, abiotic parameters, etc.).

Keywords: Breeding, ferruginous duck, lake Tonga, waterfowl, wetlands

Study of Some Blood Parameters and Minerals in Akkaraman Kangal Breed of Sheep

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In diagnosing sheep diseases and efficiency increasing studies, blood parameters are often used. It is importance to determine the blood serum values of Akkaraman race Kangal sheep in that to diagnose and interpret the diseases of these animals. Because of that, the study aimed to determine some blood parameters of Akkaraman Kangal sheep which is peculiar to Sivas and clinically healthy. In this study, 40 clinically healthy Akkaraman male and equal numbers of female sheep (60-80 kg) at the age of 2-3 years were selected from Kangal and Divriği district, Sivas province in Turkey. These sheep were grazing on pasture. Blood samples were taken from each of sheep with anticoagulant from jugular vein with a needle. Blood samples were centrifuged at 4000 rpm for 10 minutes to separate the serum. Serum was stored at -20°C until analysis. Serum glucose, total protein, albumin, globulin, cholesterol, blood urea nitrogen (BUN), creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglyceride, gamma glutamyltransferase (GGT) and some minerals like calcium (Ca), phosphorus (P), magnesium (Mg) were determined via auto analyzer device. No statistical difference was found in glucose, ALP, GGT, cholesterol, ALT and albumin concentrations ($P > 0.05$). A significant ($P < 0.05$) relationship was found between male and female in terms of creatinine, total bilirubin, AST, triglycerides, total protein and globulin. Creatinin, triglyceride, total protein and globulin were significantly high in male compared to female sheep while total bilirubin, AST and blood urea nitrogen were significantly low in male animals compared to female subjects. The concentration of Mg and P was significantly high in male animals compared to female while Ca was significantly low in male animals ($P < 0.05$). The study has shown that there is statistically significant differences in some parameters between female and male Akkaraman Kangal sheep in terms of serum biochemical values and minerals. But the fact that the most of the blood values of both the sexes are within the boundaries of the reported values in sheep. These values may help as a base for future research and diagnosis of different ailments of Akkaraman Kangal sheep.

Keywords: Kangal Akkaraman, serum biochemistry, sheep, minerals

The Effect of Silage Made from Different Plant Raw Materials on the Growth Rate and Blood Biochemical Indices of Lambs

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The objective of this study was to determine the effects of feeding silage made from different plant species to lambs. The experimental materials comprised 24 Kamieniec rams weaned at 70 days of age, divided into three equal groups, and fed ad libitum three types of silage: red clover silage (RC), alfalfa silage (ALF), grass silage (GR). All lambs received also 0.5 kg ground barley per animal per day and 12.5 g premix per animal per day. The animals were kept in individual pens throughout a 60-day experimental period. The growth rate and selected blood biochemical indices of lambs were determined. At the beginning of the experiment, and on day 30 and 60, the body weights of lambs were determined and blood was sampled from the jugular vein for biochemical analyses. Following parameters of acid-base balance and ionic parameters were determined: pH, partial pressure of carbon dioxide (pCO2), partial pressure of oxygen (pO2), bicarbonate concentration ($HCO3^-$), base excess (BE), oxygen saturation of hemoglobin (O2SAT) and total carbon dioxide concentration (ctCO2) ionogram - Na^+, K^+, Cl^-. The following blood biochemical parameters were analyzed: the levels of glucose and total protein, the activity of aspartate transaminase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP) and creatinine kinase (CK), the concentrations of urea, cholesterol, triglycerides, inorganic phosphorus (Pin), calcium (Ca), magnesium (Mg). The results were processed statistically by ANOVA test for factorial designs, and the significance of differences between means in groups was verified by Duncan's test, using Statistica 9.0 software (Soft Incorp.) On day 60, the highest (34.69 kg) and the lowest (31.75 kg) body weights of lambs were noted in groups ALF and GR, respectively. The difference between groups RC and GR was also significant. The nutritional regime had no effect on blood parameters, whereas lambs fed legume silages were characterized by increased blood glucose levels, decreased total blood protein levels and significantly higher urea concentrations compared with lambs receiving grass silage. Lambs fed legume silages showed better growth performance, in comparison with those fed grass silage, which resulted from higher intake and supply of metabolisable energy and protein from alfalfa silage, and more effective utilization of nutrients contained in red clover silage. Red clover despite lower daily gains and productivity per hectare, due to the higher efficiency conservation and better output of lamb livestock appeared to be more efficient source of roughage (supplemented with barley) in fattening lambs than alfalfa whereas red fescue appeared to be a species of grass, which, due to low productivity per hectare and a worse utilization in feed ration is not a competitive source of feed for fattening lambs. Type of silage caused changes in some blood biochemical parameters, but they remained within the reference values, which shows that silages had no influence on disturbance of homeostasis.

Keywords: Blood indices, growth, lambs, silage

Body weights and daily gains of young rams

Specification	RC	ALF	GR	SEM
Body weigth (kg)				
initial	22.63	22.63	22.71	0.22
on day 30	28.45 A	28.50 A	26.50 B	0.30
on day 60	33.66 a	34.69 A	31.75 Bb	0.42
Daily gains (g)				
1-30 days	194.67 A	195.83 A	126.19 B	8.95
31-60 days	184.31	206.25	175.00	8.66
1-60 days	183.85 a	201.04 A	150.59 Bb	6.42
Feed conversion (kgDM/kg	5.21 a	5.39 a	6.36 b	0.07

RC- red clover, ALF- alfalfa, GR – grass; Means in the same line with the different superscripts differ significantly at AB (P<0.01), ab (P< 0.05)

Serum biochemical indices of rams

Specification	RC	ALF	GR	day of exper 0	30	60	SEM
Glucose (mmol/l)	3.83	3.93 a	3.75 b	3.85	3.80	3.86	0.03
Total protein (g/l)	57.11 b	58.33 b	60.92 a	58.83	59.04	59.26	0.33
Cholesterol (mmol/l)	1.54	1.68	1.51	1.50	1.59	1.60	0.05
Triglycerides (mmol/l)	0.25	0.26	0.26	0.28	0.24	0.25	0.01
Urea (mmol/l)	6.53 A	6.71 A	5.10 B	6.07	6.15	6.25	0.14
AST (U/l)	56.68	49.83	48.33	51.96	46.57	44.35	3.41
LDH (U/l)	807.83	745.33	732.71	831.43	862.52	920.91	71.68
GGTP (U/l)	53.79	55.83	45.05	50.39	50.04	55.09	1.21
ALP (U/l)	154.67	133.25	150.33	159.78	143.04	153.57	10.87
CK (U/l)	73.44	79.34	85.92	71.67 b	89.24 a	96.24 a	1.46
Ca (mmol/l)	2.46	2.47	2.43	2.40	2.45	2.39	0.01
Mg(mmol/l)	0.69	0.74	0.68	0.65	0.71	0.73	0.01
P inorganic (mmol/l)	2.04	2.32	2.20	2.22	2.15	2.29	0.05

RC- red clover, ALF- alfalfa, GR – grass; Means in the same line with the different superscripts differ significantly at AB (P<0.01), ab (P< 0.05) SEM - standard error of the mean

Molecular Investigation of Q Fever Agent *Coxiella Burnetii* in Algerian Sheep Flocks

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In order to estimate the distribution and the excretory status of Q fever in Algerian sheep flocks, a survey was conducted between 2011 and 2013 in several regions of the country where studies are few and little is known on this zoonotic disease neither in animal and human health. Around lambing or abortion period, 224 genital swabs were collected from unvaccinated females of 34 flocks with reproductive disorders antecedent history and an average of 10% of the herd size has been sampled. After DNA extraction from specimens in a biosafety level 3 laboratory, samples were tested by quantitative real-time Polymerase Chain Reaction (qPCR) assay using the IS1111 transposase multicopy gene target for *Coxiella burnetii*. A sample was considered positive if the value of the threshold cycle (Ct) of the target gene was below 40. In total, 44 tested females have shed bacteria in their vaginal secretions (19.6%), and 18 flocks had at least one shedding female (53%). Intra-herd prevalence has ranged between 0 to 100% of tested animals and most of positive cases were observed in large flocks with transhumant production system. Any relationship has been detected between bacterial shedding and ages, also among aborted and normal lambing females. Variable quantities of excreted bacteria were found and a maximum of $1.31x10^8$ bacteria per vaginal swab has been recorded on a flock in the east of the country. The high excretion rates found in our study suggest that Q fever is widespread in sheep flocks in different zones and excretory females could participate to the environmental contamination and consequently amplification of the infection. Further investigations are needed to better understand the implication of the disease in ruminants' reproduction failures and human cases. Epidemiological and molecular research could be extended to other species and other regions and these, to elaborate an adequate plan for monitoring the disease in animals, humans and environment with the collaboration of public authorities.

Keywords: Algeria, coxiella burnetii, Q fever, sheep

In Vitro Activity of Milk, from Greek Indigenous Goat and Sheep Breeds, on Cytotoxicity of Human Natural Killer Lymphocytes versus Tumor Cells

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Human natural killer lymphocytes are integral components of the immune defense system. Recent research provides evidence of both activating and inhibitory of natural killer cell receptors that have a modulatory role in their activity. Our hypothesis was that the milk from of indigenous Greek breeds of sheep and goats would increase the cytotoxic ability of natural killer lymphocytes versus K562 tumor cells. Natural killer lymphocytes (NK cells) were isolated by a random number of blood samples derived from six healthy human volunteers. The NK cells were cultured appropriately (95% O2, 5% CO2, 37°C, aseptic conditions) and then exposed to K562 tumor cells as targets in the presence or absence of small quantities of sheep or goat milk separately in Petri dishes of 96 wells. The quantity of milk was 50 µl in a final volume 250 µl of each well. Milk samples were collected from purebred animals of Karagouniko, Boutsiko and Chios sheep breeds as well as *Capra prisca* and Skopelos breeds for goats. NK cells were incubated in cellular culture conditions. Milk from cows was used as control. Small quantities of milk, which were the same for each breed, were applied under appropriate control measures that allowed the continuous assessment of their eventual direct cytotoxic action. Flow cytometry was used to determine the cytotoxic capacity of NK lymphocytes versus K562 tumor cell line. The cytoxicity of milk was estimated by the MTT test. Milk from both sheep and goat exhibited increased direct cytotoxicity to K562 tumor cells and enhanced cytotoxicity of natural killer lymphocytes (NK cells) against K562 tumor cells, in vitro. In particular, the highest direct cytotoxicity was observed for milk samples of Skopelos breed (78 ± 4 % dead tumor cells K562), followed by samples of *Capra prisca* goats (50 ± 3 % dead neoplastic cells K562), Boutsiko breed (35 ± 4% dead neoplastic cells), Chios breed (24 ± 1% dead neoplastic cells) and Karagouniko breed (24 ± 2% dead neoplastic cells). Control samples with cow's milk showed moderate cytotoxicity reaching 31 ± 0.5% dead neoplastic cells. The cytotoxicity of NK lymphocytes versus tumor cells increased after administration of 50 µl of milk, as described, with the same scale as above: Skopelos [NK lymphocyte cytotoxicity (12.5: 1) = 92%, (25: 1) = 80%, (50: 1) = 87%] average = 86,3%> *Capra prisca* [NK lymphocyte cytotoxicity (12.5: 1) = 79%, (25: 1) = 70%, (50: 1) = 65.6%] average = 71.5%> Boutsiko [NK lymphocyte cytotoxicity (12.5: 1) = 48%, (25: 1) = 48%, (50: 1) = 61%] average = 52.3% > Chios [NK lymphocyte cytotoxicity (12.5: 1) = 43%, (25: 1) = 47%] (50: 1) = 48%] average = 46%> Cow [cytotoxicity of NK cells (12, 5: 1) = 34%, (25: 1) = 46%, (50: 1) = 36%] average = 38.6%. Milk from Karagouniko breed did not exert any effects on NK lymphocytes. The results indicate that the milk samples of purebred indigenous breeds of sheep and goats exhibit mild direct antitumor effects, while simultaneously increase the cytotoxic ability of NK lymphocytes versus K562 tumor cell line.

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Keywords: Cytotoxicity, goat, milk, NK cells, sheep, tumor cells

The Pathogenecity Study of Bovine Viral Diarrhoea Virüs (BVDV) Field Strain in Pregnant Ewes

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This project was carried out to determine of the SFT cell culture adapted BVD virus pathogenicity in pregnant sheep. The tıtres of BVD TR-76 virus at passage level 9 in sheep fetal thyroid (SFT) cell culture was determined as $DKID_{50}$ 10^6/ml. In the pathogenicity studies, pregnant sheep in the 47-77 and 88-105 day's were proved as negative with regard to BVDV antigen and the presence of antibodies by ELISA and RT-PCR. These pregnant sheep were epruvated with 1 ml BVD TR-76 virus by intranasal, subcutan and intravenous inoculation. After the epruvation, along for 20 days the body temperature was measured as between 38.6 and 39.5°C and taken sera-leukocyte samples were negative against BVDV antibodies and antigen by RT-PCR and antigen detection ELISA tests as negative in terms of BVDV antigen 5,7,11,14, 21 and 35 days. In the pregnant sheep have not been observed abortion over the course of pregnancy in ewes and obtained healthy lambs that these lambs blood leukocytes and blood serum samples were determined to be negative against BVDV antigen and antibodies. The obtained from these data, ıt was resulted that BVDV TR-76 virus can not created neither persistent infected lamb birth nor both abortion cases in pregnant sheep. As a result it was concluded that this BVDV TR-76 virus can not be used as vaccine and pathogenic strain in immunity studies.

Keywords: BVDV, pathogenecity, pregnant ewes

The Use of Rabbits at Studies of Immunity and Safety of Contagious Ecthyma (CE) Vaccine

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Contagious ecthyma (CE) is a zoonotic viral infection caused by the Parapoxvirus in sheep and goats which is classified in the family of Poxviridae. The aim of this study to determine used of the rabbits with lambs and goats for immunity and safety of CE vaccine. The names and titers of strains used in this study were Pendik CE (E(P)CK_5) and vaccine (E(P)CK_{22}) and $DKID_{50}$ $10^{6.50}$/ml and $DKID_{50}$ $10^{7.0}$/ml, respectively.It was observed that there was no change in body temperature and hyperemia, vesicules, pustulles on the skin of the rabbits which had been scarified on thoracolumbar region during 15 days. It wasn't determined CE virus with PCR from scabs of the thoracolumbar region of rabbits. It was also observed there wasn't any antibody response against CE virus with serum neutralization test in the blood serum of rabbits epruvated by both vaccine and pathogen CE strains. The pathogenity studies of CE virus (E(P)CK_5) in lambs and goats, there was no rise in the body temperatures. However there was seen hyperemia, vesicules, pustulles on the skin regions since the third day of the epruvation. The observed lesions throughout the study, the healing of the scabs started from the 15'th day. At the epruvation studies of lambs and goats by CE (E(P)CK_{22}) virus was shown the protection against CE (E(P)CK_5) pathogen strain. The pathogenity studies in rabbits, which scarified by pathogen CE (E(P)CK_5) and vaccine (E(P)CK_{22}) strains, wasn't determined lesions. As a result, the use of rabbit didn't appropriate for immunity and safety study of CE vaccine post-production.

Acknowledgements: This study was funded by TUBİTAK

Keywords: CE (orf), immunity, lamb, rabbit, safety, vaccine

Investigation of the Potential to Create a Combined Hydatid/Enterotoxemia Vaccine for Livestock

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Echinococcus granulosus is a helminth parasite which causes hydatid disease in livestock animals and humans. Hydatid disease is an important cause of human morbidity and mortality in many countries of the world, including Turkey. Recombinant DNA technology provides an alternative strategy to use host protective antigens for vaccination of sheep against echinococcosis. Clostridial infections are potentially major causes of economic loss in the livestock animal industries. The objective of this study is investigation of the potential to create a multivalent vaccine to prevent these diseases. In this study recombinant proteins for E. granulosus was prepared by growth of the bacterial cultures. Recombinant hydatid vaccine (EG95) and standard enterotoxemia vaccine was used as combined vaccine. In the first part of the study 48 lambs (7 experimental groups and 1 control group) were used. Standard EG95 vaccine and its adjuvant (Quil A) was added to bivalan enterotoksemi vaccine in different combinations were tested. In second part of the study 24 lambs were used (3 experimental group and 1 control group). Saponin was replaced with Quil A as adjuvants because of appropriate price. Saponin is very widely used in routine production. After each vaccination sera were tested for specific antibodies by Enzyme Linked Immunosorbent Assay. ELISA results were shown that the group vaccinated with standard enterotoxemia and 200 µg EG95 with 5mg Saponin had the highest antibody titres. In this study EG95 vaccine was combined with enteroxemia vaccine for the first time. In conclusion, these results indicate that recombinant oncosphere antigens can protect sheep from hydatid cyst infections. We emphesize that more studies are needed to develop effective vaccination strategies in the future.

Keywords: Echinococcus granulosus, Enterotoxemia, EG95, Vaccine

Pathologic and Bacteriologic Investigations of Pneumonia in Lambs with Role of Parainfluenza 3 (PI 3) Virus in Etiology-II

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In this study, PI-3 virus infection in lambs with respiratoric problem were histopathologically and immunohistochemicaly investigated. In addition, incidence, pathomorphologic characters and the role of lamb pneumonia of this infection were determined. For this aim, brought to slaughterhouse, 100 sheep lung (6-12 months old and in different breeds- Dağlıç, Akkaraman, Merinos, Morkaraman) were used. Lungs with lesions were fixed in 10% neutral buffered formalin and sections were taken at 5 microns thick. Sections were stained with hematoxylin-eosin (H&E), Page-Green Inclusion Bodies stain and indirect streptavidin-biotin complex methods for microscopic examination. It is also made routine bacteriological examination, taking tissue samples from the lungs. Grossly, pneumonic lesions were observed linear and lobuler shaped on the cranio-ventral regions of apical and cardiac lobes and sometimes on diafragmatic lobes in lungs. Histopathological findings were described as catarrhal bronchopneumonia (CBPn), purulent bronchopneumonia (PBPn), fibrinous pneumonia (FPn), fibrinous necrotic pneumonia (FNPn) and proliferative pneumonias were described as interstisyel pneumonia (IPn) and bronchointerstisyel pneumonia (BIPn). At the end of the study, CBPn in 8 cases, PBPn in 4 cases, FPn 14 in cases, FNPn in 3 cases, IPn in 52 cases and BIPn in 19 cases were detected. PI-3 virus antigens were positive in 4 lungs of 100 in Streptavidine-Biotine Complex immunohistochemistry. Positive staining were observed in bronch and bronchial epithelium. All PI-3 positive staining were detective in BIPn group and *M. haemolytica* in 2 cases were isolated from lungs. In addition to this findings, PI-3 immunopositive staining were observed in 1 lungs with inclusion bodies. In bacteriologic studies, *M. haemolytica* 15 in cases, *E. coli* in 4 cases, *P. multocida* in 9 cases, *Pseudomonas spp* in 1 cases, *Corynebacterium spp* in 1 cases, *M. haemolytica* and *P. multocida* in 4 cases, *M. haemolytica* and *S. aureus* in 1 cases were isolated. It is concluded that immunohistochemical detection of PI-3 virus in the pneumonic lungs is practical and reliable. The isolation of M. haemolytica from in all of PI-positive lungs showed the corelation of PI-3 virus and M. haemolytica on the pathogenesis of lamb pneumonia. Detection of proliferative pneumonia in 71% of patients with pneumonia in lambs, emphasized the very importance of the viral agents which act on the pathogenesis of proliferative pneumonias.

Keywords: Bacteriology, Lamb, Pneumonia, PI-3 virus, Pathology

Serological Investigation of Sheep, Goat and Cattle Sera against Peste des Petits Ruminants

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The first objective is to perform serosurvaillance of PPR in sheep and goats for the determination of seroprevalance and vaccine efficiency. The second objective is serosurveillance in cattle that are never vaccinated against PPR, for the investigation of PPR antibodies for the indication of circulation of PPR virus in examined cattle. Serum samples (n=1046) from sheep and goats from Marmara region of Turkey in 2009 and serum samples (N=866) from cattle never vaccinated against PPR from 28 different provinces of Turkey in 2010 were collected and tested by ID Screen PPR Competition ELISA Kit (www.idvet.com) to be able to detect antibodies against PPR. Serosurveillance of PPR was carried out in sheeps, goats and cattle. The percentage of the PPR antibody negative serum was 90%, within the scanned unvaccinated population and the percentage of the PPR positive serum samples was 97,3% within the scanned vaccinated sheep and goats. On the other hand, PPR antibodies were searched in the unvaccinated cattle against PPR. Altough, PPR antibody levels were changable concerning the region, the percentage of the PPR antibody inside the investigated cattle was %14,89. The serosurveillance of PPR demonsrated that even unvaccinated animals may have antibody against PPR due to virus sirculation in the region. The study suggests that the vaccine of PPR produced in Etlik VKE has very good protection rate as 97,3%. The antibody level detected in cattle population from different provinces of Turkey might give an indication in the circulation of PPR in the scanned regions.

Keywords: Cattle, ELISA, goat, peste des petits ruminants, PPR, sheep

Propagation of Mannheimia Haemolytica (Mh) on Vero Cell Culture and Pathogenicity Studies in Mice

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Mannheimia haemolytica(Mh) is a commensal resident of the nasopharyngeal microflora of ruminant animals and is capable of causing infection when the body's defence mechanisms are impaired. The organism is mainly confined to ruminants with the most adequately characterized strains originating from cattle, sheep and goats of all ages. Inactivated vaccines are used for prevention of Pasteurellosis. Instead of target animals, mice are adviced for the potency tests of the vaccines. Unfortunately serial passage of pathogen in culture media decreases the patogenicity of microorganism and effect the potency test quality. The aim of this study was to propagate of M. haemolytica on vero cell culture isolated from lamb with high pathogenicity for the potens test which will be done in mice (BAB C) M. haemolytica isolated using blood agar from lamb lung with pneumonie syptoms was purified on blood agar. Vero cell culture was grown in GMEM medium with 10% Fetal calf serum in incubator with %5 CO2. Mannheimia haemolytica bacterial suspansion as 1 ml in the amount of 2x105/ml CFU was inoculated in to %90 monolayer vero cell culture adding GMEM medium with %10 FCS without antibiotics and incubated at 37oC, in %5 CO2 incubator for 24 hours. After incubation period Mh microorganism was harvested and 2x109/ml CFU bacteria were injected in to 10 mice by intraperitoneally(İ.P) as 0.1 ml per mouse. At same time, 10 mice were injected by İ.P route with same amount of bacteria suspansion propagated at blood agar and ten mice injected with just PBS as control. The amount of M. haemolytica reached to 8x1010/ml CFU after 24 hour period in vero cell culture. After injection of inoculum containing 2x109/ml CFU M. haemolytica propagated in vero cell culture, ten mice died in 16-20 hours, On the other hand, no mortality observed at mice which injected with M. haemolytica isolated at blood agar and mice injected with PBS as well. M. haemolytica isolated from the lungh of dead mice on blood agar again. No isolation were made from live mice inoculated PBS. In this study It has proven that while M. haemolytica isolated at blood agar is loosing its patogenicity, propagation at vero cell culture preserved pathogenicity of bacteria and much more effective for potens tets of vaccines in mice.

Keywords: Mannheimia haemolytica, mouse, propagation, patogenicity, vero cell culture

Investigation of Copper in Blood Serums of Sheep and in Samples of Water, Plants and Soil in and Around Samsun

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As in many countries of the world, metabolic diseases which cause great economic losses due to lack of nutrition occur in animals in Turkey, too. Lack of mineral matter is very common in sheep in all seasons. Taking in too much or insufficient elements of nature for the normal functions of the organism disrupts abnormal functions while unsuitable rates among elements can disrupt the health of the organism. It also acts indirectly in hematopoiesis. Copper deficiency is one of the important metabolic diseases has long been known in Samsun region. Copper insufficiency is an important problem for our area. The purpose of this study was to determine Cu levels in the blood serum of sheep in Samsun region. Based on results of these analyses, we plan to make suggestions to veterinarians and sheep breeders in that region on how to improve their performance. To this end, blood samples were taken from healthy sheep (1-4 years of age, n=423) from 8 different towns in spring (April-May) and autumn (October-November). Soil (n = 5), plants (n = 5) and water (n = 5) samples were obtained from eight different region. Levels of Cu in blood serums were examined through Atomic Absorption Spectrophotometer (AAS) Flame System and Graphite Furnace System. Ceruloplasmin was analyze by using UV Spectrophotometer. The data obtained was shown in tables as the averages of spring and autumn. Serum Cu levels were found to be lower in spring and higher in autumn ($p<0.01$). Serum ceruloplasmin levels showed statistically significant differences in terms of seasons at a level of $p<0.01$. It was observed in autumn that Cu levels of soil and plants were increase, while it was decrease in water. In light of the data collected in this study, it is clear that preventive measures can be taken to avoid some performance losses in sheep in the region.

Keywords: Blood serum, copper, sheep, soil, water

Determination of Shelf Life and Stability of Contagious Ecthyma (CE) Vaccine strain (E(P)CK22) at Different Temperature Ranges

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In this study, the stability of different temperatures and real-time shelf life of CE vaccine strain (E(PCK_{22}) that used in lambs and kids, produced by Pendik Veterinary Control Institute was determined. On the other hand, the effect of different temperature and times was exhibited on the B2L gen of vaccine strain. CE vaccine strain propagated in primer calf kidney cell cultures by passaged 17 times (EP)CK_{17}) was propagated 4 times in calf kidney cell culture and prepared as master seed virus (EP)CK_{21}) and working seed virus (E(P)CK_{22}). 3 series vaccines were produced. Each 3 series of vaccines were filled and made lyophilisation as 200 vials (totally 600) and stored in +4/+8°C for 24 months. Starting from the first month of storage period, the titers of 10 vaccines from each series were calculated according to Karber Method in every 3 months. For determination of CE vaccine strain stability at different temperature ranges, 10 CE vaccine were diluted with 10 ml PBS and incubated at 30ºC, 33ºC 37ºC for 1,2,4,6,8,10,12 hours. Titers of CE vaccine was calculated at every temperature ranges and incubation period. For identification of CE vaccine strain and the effect of different temperature ranges to B2L gene of CE vaccine strain and sequencing B2L gene of 1137 bp in full length, DNASTAR gen analyze software was used at primslect mode and primers in the selected sequence of reference genes in the NCBI gene bank were used. In the study of determination of shelf life of CE vaccine, the titer of CE vaccines maintained +4/+8°C for 24 mounts were found as TCID50106.5 at between 1 and 15 mounts and determined as $TCID50^{10^6}$ at 18, 21 and 24 mounts. For determination of CE vaccine strain stability at different temperature ranges after dilution, the The titers of CE vaccine strains dilueted with PBS and kept at at 30ºC, 33ºC 37ºC for 1,2,4,6,8,10 and 12 hours stayed stabile as $TCID50^{10^{6.5}}$ for 12 hours. It was not observed any modification at sequencing analising of B2L gene of 1137 bp in full length. As result, It has been shown that the CE vaccines made lyophilisation can be kept at +4/+8°C for 24 month and CE vaccine virus maintains it's stability for 12 hours after dilution with PBS without losing titters.

Keywords: CE, stability, shelf life, vaccine

The Detection of Pestivirus Prevalance in Sheep in Marmara Region

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Pestiviruses are RNA viruses that infect a wide range of ungulate species, such as sheep, goats, cattle, swine and other wild ruminants.The purpose of the present study was to determine the serological and virological prevalances of pestiviruses which cause persiste infection in sheep from Marmara Region of Turkey. In this study the blood and serum samples were collected from a total of 2404 sheep in 12 provinces (Bursa, Düzce, Tekirdağ, Sakarya, Istanbul, Bilecik, Yalova, Çanakkale, Kırklareli, Edirne, Kocaeli and Balıkesir) of Marmara Region. ELISA antigen positives were found in the 29 (1.2%) of tested animals. Pestivirus antigen was observed in the 7 provinces of Marmara Region. The highest positive rate was determined as 4.16% in Sakarya province. Pestivirus antibody seropositivity was detected in the 56 (2.32%) serum samples of these animals by ELISA. The prevalance varied according to provinces of Marmara Region and there were several provinces where antigens and antibodies were widely common.The obtained data showed that the prevalance of pestivirus in sheep in Marmara Region is lower than the other regions of Turkey.

Keywords: Antibody, antigen, ELISA, pestivirus, prevalance, sheep

Investigation of Prevalence in Rotavirus, Coronavirus, Enterotoksinojenic Escherichia Coli, Cryptosporidium spp. Eimeria spp. on Diarrheal Lambs and Goat Kids in Izmir Province and Surround

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This work has been carried out to determine the prevalence of bacterial, viral and parasitic agents that cause diarrhea in new born lambs and goat kids. Our aim was to reveal the etiology of scours caused by *Escherichia coli*, *Rotavirus*, *Coronavirus*, *Cryptosporidium spp*. and *Eimeria spp*. as there was a rise in the number of neonatal diarrhea cases presented to our institute in the recent years. Fecal samples from a total of 211 lambs and goat kids taken either from their origin of birth, were evaluated in our research. Fecal samples were tested with ELISA for the detection of *Rotavirus* and *Coronavirus antigens*,*E.coli* were isolated and identified bacteriologically and parasitological examination for *Cryptosporidium spp*. and *Eimeria spp.* oocyts was carried out. In this work, from the 211 fecal samples and swabs collected, 92 were from goat kids and 119 were from lambs. In 145 of these materials *E.coli* was isolated. K99 antigen was detected and tested positive for enterotoxigenicity in Rabbit Intestinal Loop Test in 8 of these *E.coli* strains. 51 of the fecal samples were found to be positive for *Cryptosporidium spp*. oocysts, 41 contained both *E.coli* and *Cryptosporidium spp*., and 76 was found to be positive for *Eimeria spp*. As a result of the virology tests carried out in the samples, no Rotaviral or Coronaviral antigen was detected. Consequently, all the enteropathogens which have detected and caused infections have effected lamb and goat kid populations significantly and care and nutrition conditions and age have an important role about formation of infections.

Keywords: Coronavirus, cryptosporidium spp, e.coli, eimeria spp, rotavirus

Serosurvey of Contagious Caprine Pleuropneumonia in Goats in Thrace Region of Turkey

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Although the presence of contagious caprine pleuropneumonia (CCPP) in Turkey was confirmed by bacteriology and molecular techniques, quantitative data on the prevalence and geographic distrubition of the disease has not been available. We aimed to determine the seroprevalence of CCPP first time in Thrace Region in Turkey. A random sample of 2400 goats was drawn from a population of 167039 goats in the region by multistage sampling. Blood sera were collected and tested by a monoclonal antibody based cELISA. The overall prevalence of CCPP was found to be 9.02 in Thrace. Provincial prevalences were 14.93, 7.31, 0, 8.17 and 5.96 for Çanakkale, Edirne, İstanbul, Kırklareli and Tekirdağ respectively. Our results show that CCPP has spread throughout the region and become endemic.

Keywords: cELISA, contagious caprine pleuropneumonia, goat, sero-prevalance, Thrace

Variations in Content of Zinc and Copper in the Wool of Sheep in Relation to Age, Region, and Stage of Gestation

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Animal dander has the property to bioconcentrate some metals and also constitutes a readily usable biological material. The bristles of goats or cattle, sheep wool are a stable biological material and easy to collect. Zinc and copper are considered as essential elements for the metabolism of the living beings, and the determination of concentrations of these two elements in wool allows to evaluate in particular deficiencies in these elements and therefore the nutritional status of the animals. That's why in this study the content of zinc and copper were determined in the wool of breed sheep Ouled Djellal, living in two deferent relief areas "mountain" and "plain". This comparative study allows further investigation of the influence of some physiological factors such as age, stage of gestation. The study was carried out in the region of "Batna". Two areas were selected, the first (A) "Arris" is a mountainous area south east of the Wilaya of Batna and the second (B) " Ouyoun El Assafer" is a plain which lies to the east of the same wilaya. The study focused on 12 lambs and 8 ewes from local breed "Ouled Djellal" of both areas A and B. The selected animals are fed exclusively with locally sourced foods and haven't suffered transhumance movement. For lambs of each area three age groups were established. The distribution of ewes was made according to the stage of gestation (beginning and end). Wool samples were taken each season (over one year).The extraction of copper and zinc of wool was performed by dry calcinations followed by an attack with nitric acid. For the determination of the two elements it was performed by atomic absorption spectrophotometry. Zinc results (between 71.58 and 111.29 ppm) reveal that copper contents are higher for sheep of the plain, but the values of the two regions remain below the physiological values. For copper contents varied between 8.08 and 9.68 ppm are similar to physiological levels and that for sheep of the two regions of study except for lambs of 2-3 months whose values are between 5.96 and 6.73 ppm which are less than standards. The contents of copper and zinc of wool vary by region, age, and stage of gestation but this change was not significant.Wool is a good biological indicator, it is considered as a preventive approach and a further review.

Keywords: Copper, sheep, wool, zinc

Effects of Challenge of the Goats with *Staphylococcus Aureus* into the Teat Duct

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Mastitis is of particular importance in dairy goat herds. It has financial significance and reduces welfare status of affected animals. *Staphylococcus aureus* is the main causative agent of the disease. Objective of the study was to elucidate pathogenetic processes in staphylococcal mastitis in goats. In total, 15 lactating goats were used in the study. Of these, 6 animals were challenged with a *Staphylococcus aureus* strain at a depth of 2 mm into one teat duct (group A), 6 animals were challenged with the same strain at 6 mm into one teat duct (group B) and 3 were challenged directly into one gland cistern (group C). The *S. aureus* used was isolated from a clinical case of mastitis in a goat; challenge dose was 1.300 cfu. The contralateral side of the udder was used as uninfected control. Initially, animals were examined clinically on two occasions before challenge, 3 and 1 days before, with special attention paid to their mammary glands. Milk samples were collected for bacteriological and cytological examination, for which standard methods were used. Then, the same examinations were performed 12 hours, as well as 1, 2, 4 and 7 days after challenge. Finally, two animals from each of groups A and B were euthanized after challenge; one animal from each group was euthanized 4 and the other 7 days after challenge. A detailed pathological examination was performed and tissue samples were collected for histopathological examination. Before challenge, all goats were clinically healthy. No bacteria were isolated from milk samples. Cytological examinations did not reveal increased cell content in milk. After challenge, no animal from group A or B developed clinical mastitis. All animals in group C developed clinical signs (abnormal secretion which contained flakes and purulent material, enlarged gland which was painful and oedematous). The difference in development of clinical disease after challenge between groups A/B and C was statistically significant ($P<0.001$). The challenge strain was re-isolated from 15/15 milk samples collected from the infected mammary gland of group C goats. In Giemsa-stained milk films, very high numbers of neutrophils were observed. Of the 6 animals in group A, 5 developed subclinical mastitis. The challenge strain was re-isolated from 21/29 milk samples collected from the infected udder side of these animals ($P=0.012$ compared to bacteriological results in group C). Increased cell content was evident in 23/29 milk samples; neutrophils predominated in Giemsa-stained milk films. Of the 6 animals in group B, 2 developed subclinical mastitis ($P=0.04$ compared to the result in group A). The challenge strain was re-isolated from 15/29 milk samples collected from the infected udder side of these animals ($P=0.052$ compared to bacteriological results from group A, $P<0.001$ compared to bacteriological results from group C). Increased cell content was evident in 9/29 milk samples; neutrophils were seen in Giemsa-stained milk films. During histopathological evaluation of the teat of the animals that were euthanized (groups A and B), a follicle-like structure was evident at the border between teat duct and teat cistern. There was increased number of lymphocytes and plasma cells present in the area. In the parenchyma, mild inflammatory reaction was evident, with hyperaemia and inter- and intra-alveolar infiltration mainly by neutrophils, as well as by some lymphocytes. It becomes evident that the teat of goats affords a significant protective effect against invasion of pathogens into the udder. The results indicate that the follicle-like structures present in the area are of protective significance.

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Keywords: Goats, mastitis, staphylococcus aureus, teat duct

The Effect on Metabolism, Some Production Parameters and Prophylaxis of Pregnancy Toxemia of Combination of Butaphosphan and Cynanocobalamin

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The effect of administration of butaphosphan and cyanocobalamin on the prophylaxis of pregnancy toxaemia in ewe was investigated. Moreover, the effects of these compounds on body weight gain and biochemical metabolism of pregnant ewe was assessed. A total of 59 pregnant Kıvırcık crossbred ewes were used in this study. Group I (n: 15) was administered butaphosphan and cyanocobalamin three times before delivery at 1-week intervals. Group II (n: 15) was administered butaphosphan and cyanocobalamin three times before delivery at 3-day intervals. Group III (n: 15) was administered 0.9% NaCl three times before delivery at 1-week intervals. Group IV (n: 14) was administered 0.9%NaCl three times before delivery at 3-day intervals. Six blood samples were taken from each ewe four times before delivery and two times after delivery. Haematological and biochemical analyses were performed. The levels of BHB and NEFA in groups administered butaphosphan and cyanocobalamin were noticeably lower but there were no statistically significance. Elevated BHB (>0.8 mmol/L), subclinical pregnancies toxaemia were identified in 56.66% in test groups and 72.41% in control groups in all ewes and this was higher in the ewes bearing multiple pregnancies 71.42 in test groups and 82.35% in control groups. Subclinical pregnancy toxaemia in pregnant ewes with twins or triplets is lower than the levels for the control groups, despite the greater lamb counts and weights of the ewes in test groups. Based on our results, it was concluded that the butaphosphan and cyanocobalamin combination could be used as an alternative treatment for the prevention of pregnancy toxaemia.

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Keywords: Ewe, butaphosphan and cyanocobalamin, Beta-hydroxybutyric acid, pregnancy toxaemia, prophylaxis

Effect of Beta Carotene Given Before and After Progesterone Insertion, and hCG Treatment on Day 12 After Mating on Pregnancy Rates in Ewes

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The present study was conducted at four different farms in Diyarbakır during transition period (1.5 to 2 month before season). A total of 306 Awassi ewes were divided into four groups before the study. As a standard, all ewes were treated with a progesterone sponge (20 mg flugestone acetate, Chronogest CR, Intervet) for 12 days, PGF2 alfa (1 ml, i.m., Estrumate, Intervet) two days before sponge removal, 600 IU PMSG (i.m., Chronogest, Intervet) on sponge removal day and 150 I.U. hCG (i.m., Chorulon, Intervet) on the day of mating. The control group of ewes (n: 91) did not receive any additional treatment. The ewes in Carotene group (n: 61) were treated twice with Beta carotene-Vitamin E 7 days before the sponge insertion and on the day of mating. The ewes in hCG group (n: 74) were treated with 150 I.U. hCG on day 12 after mating. The Caroten + hCG group of ewes (n: 80) were treated with both Beta carotene-Vitamin E and hCG, as Caroten and hCG groups. Ewes were observed for estrus signs with the aid of fertile rams (3 to 5 years old) for 4 h every morning and evening, starting at the sponge withdrawal day, for 4 consecutive days. Ewes standing to be mounted were considered at estrus and mated (ram-ewe ratio: 1/10). Diagnosis of pregnancy was made by ultrasound on the 35th–45th day after mating. In control, hCG, carotene, and carotene + hCG groups, estrus (96.7%, 100%, 98.4% and 100%; respectively), pregnancy (67.0%, 70.3%, 73.3% and 73.7%), lambing (64.7%, 68.9%, 70.0% and 71.1%) and multiple births rates (29.8%, 21.6%, 19.0% and 27.8%) were similar (P>0.05). It was concluded that the treatments with beta carotene, hCG or both, in addition to estrus synchronization during transition period in Awassi ewes did not affect the success rates.

Acknowledgements: This study was supported by Republic of Turkey, Ministry of Food, Agriculture and Livestock, Presidency of Department of Education, Extension and Publications (Project number 07.01.03.00).

Keywords: Beta carotene, estrus synchronization, hCG, sheep

Freezing of Embryos by Vitrification Method in Saanen Goats

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In this thesis, viability of freeze-thawed embryos by vitrification following superovulation is aimed in Saanen goats in breeding season. Material of the study was 2 to 3 years old healthy and fertile 15 Saanen goats and two male. Sponges containing FGA was applied to the animals via intravaginal for 11 days. Total of 200mg FSH injected with decreasing dosages twice in day during three days for superovulation was applied by IM starting 9th day. With first FSH injection, 2,2 ml PGF2α was applied by IM. Intravaginal sponges were removed on 11th day and 2 ml GnRH was applied on 12th day. Four hours after GnRH injection, natural mating was allowed twice a day. For optaining embryos, following first mating uterine flushing was done on 7th day. Uterine washing process was performed by laparotomy. After microscope examined, obtained embryos was classified according to morphological structure and development stage. Grade 1 and 2 embryos were freezed by vitrification method. During vitrification process, as equilibration solution VS1; 20% EG and VS2; 20% EG+ 10% G, as vitrification solution VS3; 25% EG + 25% G were used. Embryos waiting 5 min in VS1 and VS2 and 30 sec in VS3 was freezed in liquid nitrogen. Embryos waiting in 0,5 M and 0,25 M sucrose and then in m-DPS solution 5 minutes was thawed. For the tracking of viability, embryos were taken in 5% CO2 incubator in culture medium. Embryos (n=101) freze-thawed by vitrification of viabilities at 24th,48th and 72nd hours in invitro environment respectively was determined as 59,4%; 33,6%; 25,7%. It was detected that viability rates at 24th hour were greater ($p<0,001$) then at 48th and 72nd hours. According to development stage, viability rates at 24th, 48th and 72nd hours of embryos in morula and blastocyst period was respectively determined as 51,3%; 20,5%; 15,4% for embryos in morula period; also was determined as 64,5%; 41,9%; 32,3% for embryos in blastosist period. Viability rates at 48th and 72nd hours in embryos at blastocyst and morula stage was important ($p<0.05$). Embryos viability rates at 24th, 48th and 72nd hours of Grade 1 embryos were as 78,6%; 46,4%; 32,1%, and that of Grade 2 were 35,5%; 17,8%; 17,8% respectively. Viability in 24th ($p<0,001$) and 48th ($p<0,05$) hours of embryos at Grade 1 and Grade 2 was statistically significant. In case of prefering vitrification method to freze in vivo produced embryos in Saanen goats; based on viability after thawing 1) embryos needs to be selected regarding developmental stages and quality and 2) embryo to be selected should be grade I in quality and blastocyst development.

Keywords: Embryos, freezing, saanen goats, vitrification

Comparative Morphometry of Left and Right Ghezel Ram Testis

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Testis is one of a pair of organs that produces the male germ cells, and is contained in the scrotum together with the epididymis. Depending on the species, the testis is oval to nearly spherical and varies considerably in size. Testes of ram are releativly large, whil thos of carnivores are relatively small. In all of the vertebrates, usually left testis is larger than right testis. In this research, we studied existence significant different in size of left and right testis of Ghezel ram. 21 pair's testes of mature Ghezel ram were collected from Tabriz industrial slaughter house. Then testes removed from scrotum. 4 factors as weight, length, and thickness (left to right and cranial to caudal) were measured in each testes. Results showed which weight, length, and thickness (left to right and cranial to caudal) of left testis is more than right testes and significant different (P<0.005) was observed in all of the studied factors.

Keywords: Anatomy, ram, testes

Table 1 - Morphometric characteristic of ram testes

	Weight	Length	Thickness (Left to Right)	Thickness (Cranial to Caudal)
Left Testis	164.228±78.495	9.02±1.566	6.480±0.908	5.861±0.862
Right Testis	158.944±78.303	8.809±1.547	6.328±0.893	5.733±0.832

Pathological Examinations of Lesions Seen in Liver of the Sheep

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In this study, it was aimed to investigate the pathological findings of liver in sheep slaughtered at different slaughterhouses. For this purpose, 243 sheep livers were macroscopically examined and of the lesion seen in the sheep liver has created 60 pieces. The selected liver samples from the routine histopathological follow-up were performed. Provided preparations were examined under a light microscope and microscopic images were taken of those diagnosed. After the examination of the 243 sheep liver in 60 (24.69 %) were showed pathological findings. Circulatory disorders (hyperemia and congestion) 20%, pigment to 3.33%, liver degeneration to (hydropic degeneration and cloudy swelling) 53.33%, inflammation coupled with signs of necrosis to 31.67 %, fibrosis 26.67 percent have been found. 53.33 % of the liver lesions thirds bile duct hyperplasia, 21.67% kolongiohepatitis and 10% in abscesses were found. Parasitic infection in 44 organs (73.33 %) were observed in 26 of these infections (43.33 %) hydatid cysts were detected. In one of samples fibrosarcoma was diagnosed. Animals the care and feeding conditions, the diversity of the show, animal shelter, hygienic lack of veterinary services, the inadequacy of factors such as the animals against infection unprotected, making the liver in the various forms and distribution of disease lesions to be seen to be caused was concluded and the pathological findings was determined of liver in sheep slaughtered at different slaughterhouses.

Keywords: Liver, pathology, sheep

Effects of Cooling Rate on Membrane Integrity and Motility Parameters of Cryopreserved Ram Spermatozoa

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Although approximately 40-60% of ram spermatozoa preserve their motility after freeze-thawing, only about 20-30% remains biologically undamaged. Cold shock can harm spermatozoa in various subcellular levels. The basic damage to spermatozoa may be ultrastructural (physical), biochemical, functional or DNA integrity. Diluted semen is cooled to a temperature close to 0°C. Cooling is a process adapts spermatozoa to reduced metabolism. The cooling rate of diluted semen from temperatures above 0°C can significantly influence the post - thaw survival of spermatozoa. Rapid cooling of extended semen from +30 to about +15°C may have no effect on survival of spermatozoa, but fast cooling from +30°C to +10°C, +5°C or 0°C decreases the post-thaw motility of spermatozoa. Early studies showed that cooling spermatozoa to +5°C with high cooling rates negatively affects both the post-thaw sperm quality and motility. However in our knowledge there are no studies on the effects of cooling rates on sperm ultrastructure. The aim of the present study was to determine the effects of different cooling rates (0.3°C/min, 0.6°C/min. and 0.9°C/min.) from +26°C to +5°C on ultrastructure properties and post-thaw motility of ram spermatozoa. For this purpose semen from 6 rams was collected by electroejaculator and was pooled in a +26°C water bath. A two-step dilution was used and glycerol was added at 5 ◦C in the second step. Pooled semen was diluted with tris based extender (extender A, without glycerol) divided into three equal parts according cooling rates (0.3°C/min., 0.6°C/min. and 0.9°C/min). Cooled semen was re-extended with extender B (with glycerol) +5°C in the second step. Diluted samples were equilibrated for 1 h and then were loaded in 0.25 mL straws and freezed in liquid nitrogen vapor. After each freezing stage semen was evaluated motility with computer-assisted semen analysis (CASA). Electron microscopic evaluation was done for pooled and chilled samples. It has been observed that 0.3°C/min. cooled group had meaningfully higher values of motility and progressive motility at +5°C after equilibration and post-thaw stages when compared with the 0.9°C/min. group ($P<0.05$). When compared to the 0.6°C/min., the 0.3°C/min. cooled group had higher total motility values at after cooling to +5°C ($P<0.05$), equilibration ($P<0.05$) and post thaw stages ($P>0.05$) and had higher progressive motility at after cooling to +5°C ($P<0.05$), equilibration ($P>0.05$) and post-thaw stage ($P<0.05$). The TEM evaluation showed that at cooling to the +5°C increases the total damaged spermatozoa in all groups ($P<0.05$). In conclusion, cooling the ram semen to +5°C with a rate above 0.3°C/min. affected negatively the spermatological characteristics. Reaching the cooling rates of 0.6 and 0.9°C/min. increasingly deteriorated the post-thaw motility and progressive motility values. Also, low temperature related to ultrastructural damage was observed at the first dilution step and localized at different regions of the sperm head depends upon the processes and cooling rates.

Acknowledgements: This study was supported by TÜBİTAK (Project Number: 107 G 093)

Keywords: Cooling rate, cryopreservation, ram, spermatozoa, ultrastructure

The Effect of GnRH Injections on Pregnancy Rate in Saanen Goats Induced Estrus during the Breeding Season

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The purpose of the present study was to investigate the effect of buserelin acetate on pregnancy rate in Saanen goats induced estrus during the breeding season. For the estrus induction of all goats, ear implant containing 3 mg norgestomet was applied to the upper outside of the left ear for 12 days. Following implant removal, 500 IU eCG and 75 µg d-cloprostenol were injected i.m. (n:57). All goats showed the estrus behaviours and they were mated naturally. The goats were divided into 3 groups as group 1 (Control), group 2 (Single GnRH) and Group 3 (Double GnRH). 2 ml NaCl for goats in group 1 (n:19), 4 µg buserelin acetate for goats in group 2 (n:19) was administered i.m. immediately after mating. Goats in group 3 (n:19) received 4 µg buserelin acetate i.m. immediately after mating and again same dose was administered i.m. on the post mating 12th day. Pregnancy diagnosis was performed by B-Mode Real Time ultrasonography with a 5-7.5 MHz probe 35 days after mating. The pregnancy rate was not statistically different (P>0.05) between the groups (Control: 68.42 %; Single GnRH. 52.63% and Double GnRH: 47.37%). The pregnancy rate was 56.14% for all goats. As a result, post mating administrations of single or double GnRH (4 µg buserelin acetate) did not improve the pregnancy rate in Saanen goats induced estrus during the breeding season.

Keywords: GnRH, goat, estrus induction, pregnancy rate

Outbreak of Diarrhoea in Young Lambs Caused by Salmonella Enterica Subsp. Diarizonae

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Many pathogens can cause gastroenteritis in young lambs and are responsible for significant financial losses, due to deaths of affected animals, growth retardation and veterinary expenses. Various Salmonella spp. (e.g., ser. Montevideo, Dublin, Typhimurium, Salmonella enterica) may contribute to the aetiology of the diarrhoeic syndrome in lambs. Objective of this report is to present the findings in an outbreak of Salmonella enterica subsp. diarizonae in lambs. The outbreak occurred in a semi-intensive dairy sheep farm in Greece. Two weeks after start of the lambing season, frequent cases of diarrhoea in young (5-15 day old) lambs were reported. Incidence risk of the disease reached 17%, with a morbidity rate of 40%. Animals developed diarrhoea, loss of appetite or, in hyperacute cases, sudden death. The investigation included sample collection directly from the rectum, which were subjected to bacteriological (aerobic and anaerobic culture with selective media) and parasitological examinations. Additionally, rapid commercial immunochromotography tests for detection of Rotavirus, Coronavirus and Adenovirus were performed. Samples were also collected from ewes in the flock, although none had any clinical signs. Only the bacterial cultures yielded Salmonella spp. in pure culture, which was found to be susceptible to enrofloxacin. In view of that, enrofloxacin was prescribed at a dose of 5 mg kg-1 once daily for a 5-day course in lambs with clinical signs. Further, enrofloxacin was administered in newborn lambs at the above dose rate for a 2-day course. The situation was monitored by collection of samples from lambs and ewes at weekly intervals for up to 42 days after completion of the treatment course. Salmonella positive samples were found from affected lambs up to 21 days after initial diagnosis. Apart from lambs, Salmonella was also isolated from faecal samples collected from one ewe. The bacterial strain was ultimately identified as Salmonella enterica subsp. diarizonae serotype 61:k:1,5,(7). After treatment, progressively, rate of new cases in lambs progressively decreased. Of the 50 lambs that received antibiotic treatment, only 3 died. All the parasitological and virological examinations of faecal samples were negative. To our knowledge, this is the first report in the literature of diarrhoea in lambs caused by Salmonella enterica subsp. diarizonae. The organism is a pathogen of wild boar and reptiles; hence ewes might have acquired the infection during grazing at pregnancy. After lambing, as the result of the peri-parturient reduction of immunity, excretion of the organism in faeces might have increased and would have led to increased environmental infection in the farm houses, with subsequent infection of the newborn. Administration of antibiotics was effective in controlling the outbreak.

Keywords: Diarrhoea, gastroenteritis, lamb, salmonella

Investigation of Prevalence and Fertility, Viability and Dead Status of Hydatid Cyst in Slaughtered Sheep of Urmia city, Northwest of Iran

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Cystic Echinococosis Granulosus is one of the most prominent problems for the health of human and animals, Echinococosis Granulosus is represented as one of the factors which has crystal clear effects on economy, Echinococosis Granulosus (hydatid cyst) larval stage causes a kind of harmful disease known as zoonose)in both human and animals((,and the alternative users are canines. To see the big picture; hydatid cyst in livestock is diagnosed when sheep are sent to botchery slaughtering. This is only way to screen the disease. It is the purpose of this inquiry to denote the status of being viability dead or sterile. To be more precise; in this research, 4107 sheep were examined after butchering and separation after internal organs(liver and lung) after the witness of cysts, the symptoms were taken to the Lab. Hydatic cyst after being centrifuged were colored with eosin this is the case in witch alive cysts are achromatic, dead ones are in red and sterile ones are without any prosculecs. Results indicate information that 779(19%) examined livestock out of 4107 were infected by Echinococosis Granulosus. Meanwhile; out of 779 infected sheep, approximately about 274(35%) had infected liver and 505(65%) had infected lung. In addition the viability rates of hydatid cysts were 79.9% for liver and 81.1% for lung. Besides; the dead rates of hydatid cysts included 18.6% for liver and 16.4% for lung. What is more is that; the sterile rats of hydid cysts was 1.5% for liver and 2.5% for lung. To this research which was considered in January and February 2015 it could be concluded that should be somehow obligation for both slaughtering and the way of sheep's living and it is extremely necessary propagation of the disease in this area of controlling. One of the disease repellence factor could be shepherd dog known as the most important one.

Keywords: Echinococosis granulosus, hydatid cyst, liver, lung, sheep

Determination of *Coxiella Burnetti*, *Cryptosporidium spp*, *Mycobacterium Avium Paratuberculosis* and *Escherichia Coli* by PCR in Ovine Manure

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Manure contains many microorganisms such as bacteria, viruses and protozoa. These pathogens are capable of causing diseases in animals and/or humans. Some pathogens are able to survive for long periods of time in manure; others are susceptible to temperature and manure processing. All livestock producers have an important role in limiting pathogen movement from their operation to the environment. The objective of this study was to screen for the frequency of *Coxiella burnetti, Cryptosporidium spp, Mycobacterium avium paratuberculosis* (Map) and *Escherichia coli* in samples of dry manure and moist manure used for composting. The samples were taken of 30 ovine herds from Querétaro, México. 30 samples of dry (250 gr) and 30 moist manure (250 gr) were collected. The extraction of DNA was made with 1 gr of manure and guanidine isothyocyanate, using 20% diatomaceous earth. Primers for detection of *Escherichia coli* (662 bp), *E. coli* K99 (278 pb), *E. coli* K88 (772 bp), *Coxiella burnetti* (687 pb), *Cryptosporidium spp* (500 pb) were used by separated PCR reactions. Amplicons were detected by electrophoresis of 7 ul aliquots of each sample (1.5% agarose, 40 min and 70V), staining with ethidium bromide. For determination of Map was used a nested PCR which was characterized by a single amplicon of approximately 210 bp. All samples were negative to *Coxiella burnetti* and *Cryptosporidium spp.* 5/30 (16.6%) from dry manure were positive to Map and 1/30 (3.3%) from moist manure was positive. 27/60 (90%) were positive to*Escherichia coli* from dry manure and 30/30 (100%) were positive to *Escherichia coli* from moist manure, although were negative to *E. coli* K88 and K99. The positive results for Map indican the risk for the transmission of the microorganism through dry and moist manure in the herd ovine. It is necessary to implement of best management practices of manure.*Coxiella burnetti* and *Cryptosporidium spp* were not present in the manure samples in this study. Financing: Project FORDECYT 143064

Keywords: Manure, microorganisms, ovine

Immunohistochemical Study of Cytokeratin Expression in Normal Caprine Skin

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Cytokeratins are among the most abundant protein family that constitutes the intermediate filaments in epithelial cells. The purpose of this study was to evaluate the immunohistochemical detection of cytokeratins in normal goat skin. Skin biopsies of 10 healthy goats of 5 different body regions were studied. Human skin biopsies were also used as controls. Samples were fixed in 10% buffered formalin, embedded in paraffin and processed routinely. Serial sections from each block were incubated with anti-human monoclonal antibodies against various cytokeratins. In particular, the immunohistochemical expression of three broad-spectrum cytokeratin markers, AE1-AE3 (recognizing cytokeratins 1-8, 10, 13-16 and 19), MNF-116 (recognizing cytokeratins 5, 6, 8, 17, 19) and 34BE12 (recognizing cytokeratins 1, 5, 10, and 14) and of cytokeratins CK5/6, CK7, CK14 and CK19 was evaluated. Cytokeratins AE1/AE3 and 34BE12 are expressed in all layers of the epidermis. Cytokeratin MNF116 is expressed in the basal and the spinous layer whereas cytokeratins 5/6, 14 and 19 are confined to the basal layer of the epidermis. The epithelium of the sweat glands is stained by cytokeratins AE1/AE3, MNF116, 34BE12 and CK7. Myoepithelial cells of sweat glands express cytokeratin 5/6. Cytokeratins AE1/AE3 and 5/6 are located in the outer and inner sheath of hair follicles, whereas the outer sheath of hair follicles also expresses MNF116, 34BE12, CK14 and CK19. Sebaceous gland cells are stained by cytokeratins AE1/AE3, MNF116, 34BE12, CK5/6, CK14 and CK19. Although a number of human monoclonal antibodies have been shown to cross-react with the farmed ruminant species, the database of reagents for caprine research is limited. This study establishes the value of a panel of anti-human keratin monoclonal antibodies cross-reacting with the caprine skin, which can be applied in routine dermatohistopathology of goats. The expression of cytokeratins can be valuable for the study of epithelial differentiation and the characterization of lesions in goat skin diseases (inflammatory and less frequently neoplastic).

Keywords: Cytokeratins, caprine skin, immunohistochemistry, goats

Immunohistochemical Study of Experimental Infestation of Goats with *Sarcoptes Scabiei*

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Sarcoptic mange is one of the most important ectoparasitic diseases of goats. The aim of our study was to assess the cellular immune response of experimentally infested goats, especially in kids vs. adults. Six naïve kids (<6 months old) and 6 naïve adult goats (>1 year old) were experimentally infested at the face and the back with 50–100 mites of *Sarcoptes scabiei* var *caprae*. Four animals were used as controls. Skin biopsies were taken under local anesthesia from developing lesions on d2, d4, d8, d12, d18, d25, d33, d40, d50, d60, d75 and d90 respectively. Skin biopsies were fixed in a zinc salts fixative and then processed routinely. Hematoxylin-eosin stain was performed and additionally tissue sections were immunostained with monoclonal antibodies against T-cell subpopulations (CD3, CD4, CD8, CD21 and WC1γδ). An intense immunoinflammatory response dominated by a substantial infiltrate of lymphocytes, accompanied by eosinophils, was recorded from 2nd day post infestation to the completion of the experiment. Immunohistochemistry indicated that by 2nd day, numbers of CD3+, CD8+ and γδ+ in epidermis had increased significantly compared to controls ($p<0.05$). Moreover, there was an increased exocytosis of CD3+, CD8+, and γδ T cells located in areas with *Sarcoptes scabiei* than in areas without mites. In dermis, a progressive increase of CD3+, CD4+, γδ+ T-lymphocytes was gradually observed until d90, compared to controls ($p<0.05$). The predominant cells in the inflammatory infiltrate were at all cases CD4+ T cells. Especially in early days of experimental infestation, the CD8+ T-lymphocytes increased significantly. The distribution of all lymphocyte subpopulations in dermis was initially perivascular and throughout the time course became more diffuse. In the dermis CD4+/CD8+ ratio gradually changed from 1.92±0.53 at 2nd day post infestation to 4.09 ± 1.88 at the 90th day of the experiment. It should be noted that in the dermis of kids, γδ+ cells increased significantly ($p <0.005$) in comparison with goats from the 12th to the 90th day post infestation. There was no significant difference in the number of lymphocytes in the face and back, during the infestation both in kids and goats ($p >0,005$). Furthermore, CD21+ cells were absent. Our observations suggest that mites and their derived products cause early recruitment and substantial activation of different T-lymphocyte subpopulations (CD4+, CD8+ and γδ+ cells) in the skin, during experimental *Sarcoptes scabiei* infestation. Especially, the γδ+ T cells respond, by proliferation during early and chronic phases of infestation, in an age dependent manner. The high counts of γδ+ T lymphocytes in kids compared to adult goats, suggests that the skin immune system of young animals relies additionally on γδ+ lymphocytes as the parasitic infestation progresses.

Keywords: Goats, immunohistochemistry, lymphocytes, sarcoptes scabies var caprae, skin

Damascus Kids' Slaughter, Carcass and Meat Quality Traits in Different Production Systems Using Antioxidant Supplementation

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This study intended to investigate slaughter; carcass and meat quality characteristics of Damascus male kids reared under different production systems and antioxidant effect (Vit E). The kids, housed in pen groups and grazing groups, were equally divided for production systems and later each group was again equally divided for determination of Vit E effect. Production systems and Vit E were found to have no significant effect on slaughter and carcass traits. Differences between production systems were found significant for meat pH24, water holding capacity, cooking loss, tenderness, ether-extractable lipid and some color characteristics and concentrate feed supplemented with Vit E was effective on TBARS values. Each of the fatty acids except C18:2 n6 was affected by the production system but Vit E influence was superior on long-chain fatty acids. Grazing kids had a lower percentage of total SFA, n6, n6/n3, AI and TI ratio, while kids housed in pens had the lowest percentage of total UFA, NV and n3 ratio. On the other hand, kids that consumed supplemental Vit E had a higher percentage of total UFA, ratio of UFA/SFA, n3 and lower percentage of SFA, ratio of n6/n3, AI, TI compared to the kids fed by non-supplemental concentrate feed with Vit E. In accordance with the meat fatty acid composition, meat obtained from the kids that grazed and consumed supplemental Vit E was healthier than that of from housed in pen kids and non-supplemental Vit E consumed kids. Slaughter and carcass traits were not affected by PS and VE when the same slaughter weight was used. These results indicate that energy intake level in fattening period for slaughter and carcass characteristics may be more important than PS. It is evident from the present study that PS affected some meat quality parameters, especially related to fatty acids, and consumption of diet supplements with Vit E can be a useful source to protect meat from oxidation and to improve meat quality for human consumption. Finally, production system based on grazing and VE addition to concentrate feed can be more preferable for fattening in pen production systems for meat quality parameters.

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Keywords: Antioxidant effect, carcass traits, Damascus, meat quality, production systems

Pharmacokinetics and Bioavailability of Meloxicam in Sheep Following Intravenous and Subcutaneous Administrations

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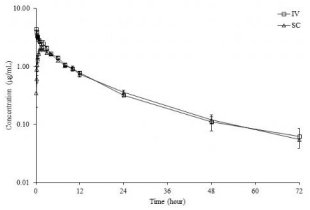
To determine meloxicam pharmacokinetic profile and bioavailability in sheep after intravenous (IV) and subcutaneous (SC) administrations of 0.5 mg/kg body weight. The study was conducted in five clinically healthy sheep according to a cross-over design (5x5) with a 20 day washout period between treatments. Meloxicam (Maxicamx4®, 20 mg/ml, Sanovel) was administered by either the IV or SC route at single dose of 0.5 mg/kg body weight. Intravenous and subcutaneous doses were injected into the left jugular vein and the left axilla region (arm pit), respectively. Blood samples were drawn by jugular venipuncture at 0, 5, 10, 15, 20, 25, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h after IV administration and SC administration. Plasma concentrations of meloxicam were measured using the reversed-phase high performance liquid chromatography. Plasma concentration–time curve of meloxicam was performed with noncompartmental analyses using WinNonlin software. Pharmacokinetic parameters and the mean plasma concentration vs. time curves of meloxicam in sheep following single IV and SC administration are presented in Table 1 and Fig. 1, respectively. The bioavailability (F) of meloxicam after the SC administration was 99±3.7%. The terminal half-life following SC administration was significantly longer from those of IV administration. Discussion: Results indicated that meloxicam had excellent bioavailability after SC administration and that plasma meloxicam concentration at 24 h following IV and SC administrations was >0.2 µg/mL, which is the concentration of meloxicam required for analgesic effects in other species.

Keywords: Bioavailability, meloxicam, pharmacokinetic, sheep

Fig. 1



Mean ± SD semilogarithmic plots of the plasma concentrations to time data of meloxicam following single intravenous (IV) and subcutaneous (SC) administrations at a dosage of 0.5 mg/kg of body weight in sheep (n=5).

Table 1. Mean ± SD pharmacokinetic parameters of meloxicam following single intravenous (IV) and subcutaneous (SC) administrations at a dosage of 0.5 mg/kg of body weight in sheep (n=5).

Parameter	Unit	IV	SC
t1/2λz (HM)	h	13.73±0.77	15.99±0.89*
AUC(0–72)	h*µg/mL	29.05±1.07	28.82±0.83
ClT	L/h/kg	16.69±0.55	-
Vss	L/kg	268±22	-
Cmax	µg/mL	-	2.08±0.07
Tmax	1/h	-	1.50±0.35
F%	-	-	99±3.7

**Statistically different according to IV administration (P<0.05). λz, the first order rate constant associated with the terminal portion of the curve; t1/2λz, terminal half-life; AUC(0–72), area under the curve from time 0 to the last detectable concentration; ClT, total body clearance; Vss, volume of distribution at steady state; Cmax, maximum plasma concentration; Tmax, time to reach peak concentration; F, bioavailability; HM, harmonic mean.*

Study of Biochemical and Parameters in Sheep Ouled Djellal in the Experimental Station ITDAS - Biskra-Algeria

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The purpose of this study was to determine the influence of physiological stage, season some blood metabolites in sheep Ouled Djellal. The research was conducted on a group of sheep clinically healthy, from arid areas of South East Algeria, divided into groups according to their physiological stage [pregnant ewes, ewes' lactation]. Circulating concentrations of glucose, cholesterol, triglycerides and total proteins were determined using specific commercial kits. Blood glucose levels were significantly lower in pregnant ewes in lactating ewes while triglycerides were significantly increased. Similarly, total protein appeared higher but due to high dispersion of values. The values of these various blood biochemical parameters on Ouled Djellal sheep dry lands were comparable to published data except cholesterol, lower. Therefore, it is important to consider the physiological stage which significantly affects several blood parameters in sheep living in arid areas

Keywords: Arid areas, biochemical parameters, sheep, oueled djellel race

Study of Survival and Motility Epididymal Spermatozoa Stored at 04°c of the Ouled Djellel Breed Rams in Algeria

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Sheep farming is an important economic and social plays a key role for certain industrial activities by providing the raw material (wool and leather) and meat that is most appreciated by the Algerian population. The use of sperm quality is one of the factors needed to increase the efficiency of artificial insemination in sheep. However, the aim of the study is to evaluate the motility and vitality of epididymal semen was late to enjoy their life after storage at 4°C for 72 hours without dilution. The study included 54 rams Ouled Djellel Arab breed, genital organs were collected after slaughter of the animal. The semen was collected after dissection of the epididymis tail and the injection of paraffin oil using a syringe inserted into the vas deferens semen after an incision at the apical region of the without dilution. Mobility and vitality were measured at 0, 24,48 and 72 h after semen collection.A drop of semen was observed by light microscopy at low magnification (×10) to assess the massal motility graded from 0 to 5. However, the vitality was assessed by counting after Eosin-nigrosin staining, to determine the percentage of live spermatozoa.epididymis tail, and the sperm was collected and stored at 4°C. We used the Software Graph Pad Prism®5.Version 5.03, to calculate the mean, the standard deviation and the standard error of the mean (SEM) and the statistical signification was set at $p<0.05$. This study showed that epididymal sperm can be used for more or less short time after storage, we can conclude that the cauda epididymal sperm stored at the above-mentioned conditions constitues, despite an obvious reduction in viability, an alternative source of gametes of meritorious parents for artificial insemination or IVF. However, for a better evaluation of the fertility or performance, rams should be tested for different trials such as scrotal measurement, semen examination, libido testing, hormonal profile and other examinations.

Keywords: Arab ram breed, ouled djellel, epididymal semen, motility, storage at 4°C, vitality

Effect of Quercetin Addition in Extender to Cooled Sheep Semen

Eunice Oba[1], *Felipe Morales Dalanezi*[1], *Luis Fernando Mercês Chaves Silva*[1], *Endrigo Adonis Braga Araujo*[1], *Sidnei Nunes Oliveira*[1], *Lorenzo Garrido Segabinazzi*[1], *Luis Roberto Pena Andrade Jr*[1], *Danilo Cunha Silva*[1], *Frederico Ozanam Papa*[1]
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The aim of this study was evaluate the effect and the optimal dose of Quercetin in addition in extender used for cooling sheep semen on sperm viability, motility and membrane integrity. Were used one ejaculate of 29 sheep of two different breeds (12 Santa Ines and 17 Texel) aging three to five years old, from a farm located in Iaras, São Paulo, Brazil. Rams were remained in similar management and nutritional conditions and selected based in yours sperm quality evaluation. Semen samples were obtained using electroejaculation, during the breeding season (July) 2014 and after collection, ejaculates were evaluated in Center for Biotechnology and Diagnosis in Animal Reproduction (CERAN), College of Veterinary Medicine and Animal Science, State University of São Paulo "Júlio de Mesquita Filho" – Botucatu – SP. The rams were only considered suitable for the experiment if their ejaculates had at least: volume ranging between 0.75 to 2 ml, sperm concentration greater than 2.5x109 sperm/ml, sperm motility evaluated for computer-assisted sperm analysis (CASA) (HTM-IVOS 12, Hamilton Thorne Research, Beverly, MA, USA) greater than 70% and abnormal morphological cells analyzed using differential interference contrast microscopy less than 10%. Each ejaculate was split into four aliquots, with final sperm concentration fixed at 50 x106 sperm/mL, corresponding with 1) Control group using Botu-Bov extender (BB; Botupharma Ltda., Botucatu, São Paulo, Brazil) which according to producers does not contain Quercetin; 2) BB with 5mg/ml Quercetin; 3) BB with 10mg/ml Quercetin; 4) BB with 15mg/ml Quercetin. The samples were evaluated immediately after dilution (hour 0) about sperm kinetics by CASA and plasma membrane integrity by epifluorescence microscopy (Leica Microsystems, DMLB, Germany). Thereafter, samples were placed at Botu-Tainer (Botupharma, Brazil) passive cooling container for transporting semen during 24 hours and cooled to 5ºC at rate of -0,05ºC/min. After cooling for 24 hours, the samples were incubated at 37ºC for 10 minutes and then evaluated sperm kinetics and plasma membrane integrity. Kolmogorov-Smirnov normality test (KS), ANOVA and Kruskal-Wallis were performed and considered significant when $p<0.05$. Differences were not observed between groups in total motility (%, 1 – 68,76±13,90; 2 - 69,80±11,46; 3 - 68,88±13,82; 4 - 70,36±12,27), progressive motility (%, 1 – 36,84±10,22; 2 - 35,60±9,39; 3 - 36,16±9,58; 4 - 37,20±9,83), percentage of rapid sperm (%, 1 – 47,76±14,01; 2 - 47,88±12,92; 3 - 47,48±13,58; 4 - 49,12±14,43) and plasma membrane integrity (%, 1 – 33,16±16,99; 2 - 32,64±16,30; 3 - 28,48±14,33; 4 - 29,4±15,43). Supplementing with Quercetin did not improve sperm kinetics and plasma membrane integrity in cooled sheep semen in this experiment. However, most studies are necessary to know the real effect of Quercetin on the semen. (Financial support conducted by the Foundation for Research of the State of São Paulo, FAPESP- Process 2012/50277-6. The authors thank the Botupharma for providing the semen extenders).

Keywords: Antioxidant, cooled semen, sheep semen

Effect of BHT (Butylated Hydroxytoluene) Addition in Extender to Freeze Sheep Semen

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The purpose of this experiment was evaluate the effect and the optimal dose of BHT in addition in extender to freeze on sperm viability, motility and membrane integrity. In this study were used one ejaculate of 25 sheep of two different breeds (10 Santa Ines and 15 Texel) aging three to five years old, from a farm located in Iaras, São Paulo, Brazil. Rams were remained in similar management and nutritional conditions and selected based in yours sperm quality evaluation. Semen samples were obtained using electroejaculation, during the breeding season (July) 2014 and immediately after collection, ejaculates were evaluated in Center for Biotechnology and Diagnosis in Animal Reproduction (CERAN), College of Veterinary Medicine and Animal Science, State University of São Paulo "Júlio de Mesquita Filho" – Botucatu – SP. The rams were only considered suitable for the experiment if their ejaculates had at least: volume ranging between 0.75 to 2 ml, sperm concentration greater than 2.5x109 sperm/ml, sperm motility evaluated for computer-assisted sperm analysis (CASA) (HTM-IVOS 12, Hamilton Thorne Research, Beverly, MA, USA) greater than 70% and abnormal morphological cells analyzed using differential interference contrast microscopy less than 10%. Each ejaculate was split into four aliquots corresponding with 1) Control group using Botu-Bov extender (BB; Botupharma Ltda., Botucatu, São Paulo, Brazil) which according to producers does not contain BHT; 2) BB with 0.5 mM BHT; 3) BB with 1.0 Mm BHT; 4) BB with 1.5 Mm BHT. For all the groups, the final sperm concentration was fixed at 50 x106 sperm/mL, and the samples were packed into 0.25 mL straws. Samples were frozen using a previously described and validated methodology with the extender (Crespilho et al., 2012, Livest Sci). The samples were thawing at 37ºC for 30 seconds and then evaluated sperm kinetics by CASA and plasma membrane integrity by epifluorescence microscopy (Leica Microsystems, DMLB, Germany). Kolmogorov-Smirnov normality test (KS), ANOVA and Kruskal-Wallis were performed and considered significant when $p<0.05$. Differences were not observed between groups in total motility (%, 1 – 68,76±13,90; 2 - 69,80±11,46; 3 - 68,88±13,82; 4 - 70,36±12,27), progressive motility (%, 1 – 36,84±10,22; 2 - 35,60±9,39; 3 - 36,16±9,58; 4 - 37,20±9,83), percentage of rapid sperm (%, 1 – 47,76±14,01; 2 - 47,88±12,92; 3 - 47,48±13,58; 4 - 49,12±14,43) and plasma membrane integrity (%, 1 – 33,16±16,99; 2 - 32,64±16,30; 3 - 28,48±14,33; 4 - 29,4±15,43). Supplementing with BHT did not improve sperm kinetics and plasma membrane integrity in freeze sheep semen. (Financial support conducted by the Foundation for Research of the State of São Paulo, FAPESP- Process 2012/50277-6. The authors thank the Botupharma for providing the semen extenders).

Keywords: Antioxidant, freeze semen, rams

Development of a Multiplex PCR Assay for the Identification of *Staphylococcus spp* as a Cause of Caprine Mastitis

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Mastitis has a negative impact on hygienic milk quality and it is responsible for economic losses. Mastitis in goats can be caused by different number of pathogens, but the most important bacterial genus is*Staphylococcus*, usually divided into coagulase-negative *Staphylococcus* and *Staphylococcus aureus*. The aim was to develop a multiplex polymerase chain reaction (PCR) test to diagnose *Staphylococcus* agents involved in caprine mastitis. Thirty milk samples from animals in intensive and semi-intensive production systems were taken of different farms located at Tequisquiapan, Querétaro, México. The samples were obtained from healthy goats. The extraction of DNA was made with guanidine isothyocyanate. The multiplex PCR assay was developed to identify coagulase negative *Staphylococcus* and *S. aureus*. To achieve the identification of*Staphylococcus spp*, primers for the 16s rRNA region were used, giving a product of 791 bp. Another two pairs of primers were used for *S. aureus*, the first pair corresponded to the *clfA* gene (638 bp) and the second pair corresponded to the *S. aureus coa* gene (1200 bp). Strain references of *Staphylococcus aureus* ATCC 29737 and *Staphylococcus xylosus* ATCC 700404 were used for the standardization on the assay Results. The genus-specific primer set based on the 16s RNA gene provided PCR products for 36.67% (11/30) of samples. 10% (3/30) samples were positive to 16s RNAr region and to the *clfA* gene, only one sample 3.33% (1/30) was positive to *clfA* gene. Fifteen samples were negative (50%). Conclusion. Multiplex PCR was performed and it was possible to detect *S. aureus* and coagulase negative *Staphylococcus*. The multiplex PCR assay is a rapid, simple and practical tool for identification of *Staphylococcus*associated with caprine mastitis, and could be an effective alternative to conventional biochemical-based assays.

Keywords: Caprine, mastitis, PCR, staphylococcus

Leukocyte Profile of Anglo Nubian Goats Fed with Different Fat Sources and Submitted to Laparoscopic Ovum Pick-up

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The aim of the present study was to evaluate the leukocyte profile changes in goats fed with different fat sources and submitted to laparoscopic ovum pick-up (LOPU). Eighteen Anglo-Nubian goats were randomly divided into 3 groups based on their diets with different fat sources [soy oil (S, n=6), linseed (L, n=6) and Megalac® (M, n=6)], formulated with 40% concentrate, 60% corn silage and 4% ether extract (in dry matter) from the fat source. The animals were submitted to an adaptation period of 15 days and then, an experimental period of 77 days. LOPU was performed after 36 hours of ovarian superstimulation protocol [FSH (80mg) + eCG (300IU)], on Day 42 (LOPU1) and Day 70 (LOPU2) of the trial. Twenty blood samples were taken at the beginning (D-15), at the end of the adaptation period (D0), at 30 days of diet (D30) and in order to evaluate the surgery response, the blood samples were taken immediately before each LOPU (D42 and D70), 24 hours (D43 and D71), 72 hours (D45 and D73), five days (D47 and D75) and seven days (D49 and D77) after LOPU. Total leukocyte (TL) was counted on an automatized counter. Data were analyzed by ANOVA with post-hoc Tukey test ($p <= 0.05$) using software R. There were no interaction between groups and time and the results are expressed by principal effects (means ± sd; x103/µL). The results by groups are: 15.2 ± 0.94 (S), 15.7 ± 0.90 (L), 14.9 ± 0.75 (M). The results by moments are summarized on Table 1. There was no difference between groups; the moments with the highest values were D71, D42 and D43, and the lowest was D47 followed by D73 and D75. There was no influence from the source dietary fat on TL profile and seven days are necessary to reestablish the same TL count as before the surgery. Probably TL was not sensible enough to show the anti-inflammatory effect of the L diet, and further studies are necessary.

Acknowledgements: Financial support: CNPq and FAPESP

Keywords: Caprine, leukocyte, PUFA, LOPU

Table1- Values of Total Leukocyte(TL, x103/µL) by moments of Anglo-Nubian goats submmited to LOPU.

D	D-15	D0	D30	D42	D43	D45	D47	D49	D70	D71	D73	D75	D77
TL, x10³/µl	14.9 ± 0.83 B	14.9 ± 0.98 B	14.9 ± 0.56 B	17.4 ± 0.93 A	17.2 ± 0.97 A	14.5 ± 0.75 B	13.5 ± 0.81 C	15.8 ± 0.64 B	14.6 ± 0.94B	19.8 ± 0.89 A	14.1± 0.94 C	14.2 ± 0.95 C	15.3 ± 0.87 B

**Different letters shows difference between moments.*

Production of Corynebacterium Pseudotuberculosis Antigen Samples Isolated from Abscesses in Sheeps

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Caseous Lymphadenitis (CL) causes significant economic losses in the sheep raising industry and its presumptive diagnosis is based on examining superficial lymph nodes; however, this can be of little worth in detecting early infections or its visceral manifestation. The most affected animals show excessive weight loss, also known as cachexia; these animals can eat normally without recover lost weight. The disease is almost always detected at the slaughterhouse, through the presence of swollen lymph nodes showing a purulent, caseous exudate. The purpose of this study was to obtain a somatic antigen from Corynebacterium pseudotuberculosis, which was tested using a Western blot and indirect ELISA using sheep serum. The C. pseudotuberculosis strain was isolated and inoculated in blood agar from samples taken from hair or half-hair sheep in livestock and stock breeding units that tested clinically positive for CL, which was biochemically characterized. Strain C-11 was cultivated in an agar and PPLO culture medium. The protein amount of the antigens was 847.59 mg/ml and 5814.34 mg/ml for the antigen obtained from PPLO agar and PPLO culture medium respectively. The antigens were subsequently evaluated using a Western blot to ascertain their antigenic parts, the results being bands of 11.66, 16.84, 31.06, 42.19, 50.69, 60.91, 68.89, 77.81, 93.5 and 126.98 kDa for the antigen obtained from PPLO agar and a band of 31.06 kDa from the antigen obtained from the PPLO culture medium.* Subsequently an indirect ELISA test was carried out using 102 sheep serums. In the antigen obtained from PPLO agar, 48.07% were found to be positive, 42.15% negative and 9.8% suspect, whereas in the antigen obtained from PPLO culture medium 44.11% were found to be positive, 41.17% negative and 14.7% suspect. In conclusion, the somatic antigens obtained were recognized by clinically diseased sheep serums, and five bands reported previously by other authors were found. The molecular weight of four bands suggest that it is PLD.

Keywords: Caseous lymphadenitiss, cutaneous presentation, indirect ELISA, somatic antigen, western bloot

Bluetongue Serotype 1 Epidemic in the District of Asl 1 Sassari (Sardinia): Use of Gis for Planning Control Measures and Vaccination Strategies for the 2014-2015 Years

Francesco Sgarangella[1], *Daniela Marongiu*[1], *Sergio Masala*[1], *Vincenzo Floris*[1], *Bastianina Mossa*[1], *Giuseppe Bitti*[1], *Pietro Desini*[1]
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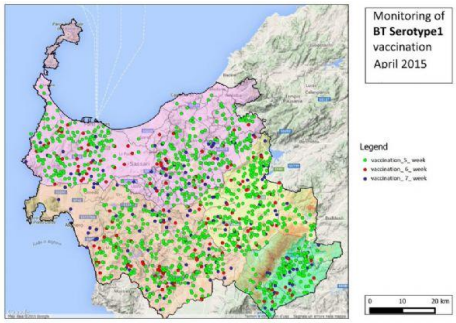
The epidemic of Bluetongue virus serotype 1 that occurred in the ASL of Sassari district in the 2013 involved 1344 sheep and goat farms with a mortality rate of 5.94%. GIS has been a useful tool that, via the analysis of disease related data integrated with other sets of multidisciplinary information, allowed identification of requirements and priorities for Health Care Workers during the vaccination campaign for the 2014-2015 years. The implementation of GIS, open source software, required the design of a database that contains complete and updated information about animal census, farms location, geographic and epidemiologic data. The data about the 2013 outbreaks were retrieved from the ministerial website (SIMAN), those regarding the farms census from the National DataBase (BDN) and Local DataBase (BDL). Geographic and other informational layers were downloaded from the "Sardinia Geoportal" website. We implemented a custom-designed database that integrates all these data to be managed and analyzed by the GIS application. This operation generated output maps firstly for the monitoring of the epidemic and subsequently as a basis for the planning and management of the vaccination campaign by the working group. In January 2014 the Animal Health Service of ASL 1 started a campaign of vaccination against Bluetongue. A working group was set up for the implementation of an operational timetable and the management of human and material resources. The first phase of the operation began in January in the south-western end of the district of Alghero. In this context the GIS application represented a suitable tool for planning risk-based interventions.The number of sheep in the district, as reported on Teramo BDN, is about 829.860. By 31 october 2014, there were 621.980 vaccinated sheep inside farms (1.225.579 doses of vaccine were used). The vaccination coverage was therefore about 74,94%. In January 2015 the Animal Health Service of ASL 1 started a campaign of vaccination against Bluetongue serotype 1. By April 2015, there were 495.694 vaccinated sheep inside 1.603 farms. The use of GIS allowed the continuous monitoring of the activities, proper management of animal movements after seroconversion of sentinel sheep, the detection of critical points and the possibility to modulate interventions and to optimize of human resources. The applied system allowed a precise monitoring of the epidemic in 2013 and the planning of vaccinations based on territorial risk factors and prevalence observed during the 2013 epidemic. The results of the vaccination campaign of the year 2014 allowed a risk reprogramming in 2015.

Keywords: Bluetongue, gis, vaccine

Figure 1



map of seventh week of BT vaccination

Evaluation of Non-Stick Solutions in Sheep Subjected to Laparoscopic Ovum Pick-Up (LapOPU)

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LapOPU is a technique that has been widely used as reproductive biotechnology in small ruminants because it is minimally invasive, provides fast recovery and it can be performed several times in a same female. However, the occurrence of adhesions in the reproductive tract is one of the complications of this technique. Thus, the aim of this study was to evaluate a method that minimizes the formation of visceral adhesions caused by manipulation during LapOPU, without causing changes in the procedure. A study was conducted using 24 multiparous Santa Ines sheep, aged 3.1 ± 1.1 years and body weight of 35.1 ± 6.2 kg. The sheep were divided into two groups of 12 animals each, comparing the solutions of 0.9% NaCl (GS) and lidocaine HCL 1% (GL) - Ethics Committee 009761/13. Ewes were submitted to LapOPU using three portals and pneumoperitoneum induced by CO2. Observed follicles were aspirated and two punctures were made on the right horn mimicking laparoscopic artificial insemination procedure. The uterus and both ovaries were washed with 0.6 mL/kg of 0.9% NaCl solution in the GS group, and 6 mg/kg of lidocaine HCL 1% in the GL group in order to remove the surface and prevent the formation of clots and adhesions. The animals were submitted to a second laparoscopy 21 days after the first procedure to macroscopically examine the presence, location and number of uterine and ovarian adhesions. No adhesions were observed in utero. Mild adhesions were observed involving the ovaries of four animals from GL (33.3%) and three from GS (25.0%). All observed adhesions were similar between groups. The adhesions surrounded the ovary with involvement of regions of the uterine tubes and mesovarium. In conclusion, both NaCl 0.9% or lidocaine HCL 1% solutions were effective in preventing the formation of adhesions. Even with the occurrence of ovarian adhesions, the proportion and severity were limited. Financial support: FAPESP–2013/09053-0

Keywords: Adhesions, laparoscopic surgery, ovary, sheep, uterus

Effects of Region and Invidualism on Sperm Freezeability of Angora Goat

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It's well accepted that freezability of the buck semen depends on many different factors, such as breed, age, season, region even with invidual differences. Therefore, we aim to compare freezability of Angora goat semen (Capra hircus ancryrensis) from different region and inviduals. Six goats from 3 different regions (Lalahan, Ayaş, Nallıhan) in Ankara were used at this study. Semen samples were collected with artificial vagina three times a week with minumum 7 replication. Volume, pH, motility, mass activity were determined immediately. Then after, fresh semen was extended with tris based extender, equilibrated (+5 °C/2h), loaded into 0.25 french straws, frozen in liquid nitrogen vapour (-120 °C/15 minutes) and stored in liquid nitrogen (-196 °C). Frozen straws were thawed in water bath (37 °C/30 seconds) and percentages of progressive motility, total motility, sperm motility kinetic parameters (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and hyperactivity) were assesed with computer assisted sperm analyzer (CASA). For fresh semen parameters, bucks were seperated into regional groups, highest volume, ph, motility and mass activity were recorded as 1,25±0,28; 6,42 ± 0,36; 72,48±7,52; 4,39±0,61 respectively from the goats in Ayaş region but there was no statistical differences for cryopreservation process (p>0,05). Frozen thawed samples, highest progressive and total motility was recorded as 9,11 ± 5,61; 56,60 ± 17,35 respectively in Ayaş region (p>0,05). Differences were not statically significance on motility or kinetic parameters in both regional groups and individuals (p>0,05). In conclusion, regional and invidual differences gives valuable information on sperm quality before and after freezing. We believe that these in vitro study results need further support with the position that invidualism is a key option for sperm cryopreservation in those instances in which genetically valuable or other are not young or in appropriate for that are susceptible to freezing process.

Keywords: Angora goat, CASA, cryopreservation, kinetic parameters, motility

Mean (±SE) regional sperm values in frozen thawed goat semen

		REGION		
Analysis	NALLIHAN	LALAHAN	AYAS	P
Progressive Motility (%)	9,97±4,05	7,83±5,95	9,11±5,61	-
Casa Motility (%)	58,42±9,40	49,22±20,05	56,60±17,35	-
VCL (µm/s)	97,97±7,00	94,11±7,93	97,76±8,53	-
VSL (µm/s)	40,08±7,30	37,63±7,37	37,26±9,19	-
VAP (µm/s)	62,33±7,00	58,92±7,63	59,57±8,83	-
LIN (µm/s)	40,73±5,67	39,86±6,32	38,02±8,73	-
STR (µm/s)	63,86±4,81	63,37±5,11	61,78±7,44	-
WOB (µm/s)	63,51±4,19	62,54±5,41	60,82±6,62	-
ALH (µm/s)	4,07±0,30	4,03±0,46	4,36±0,58	-
BCF (Hz)	8,04±0,64	7,98±0,80	8,08±0,83	-
Hyperactive (%)	21,38±4,34	18,24±6,42	20,25±6,25	-
		- No significant difference (p>0,05)		

Mean (±SE) individual sperm values in frozen thawed goat semen

Region	Individual	Progressive Motility (%)	Casa Motility (%)	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	LIN(µm/s)	STR(µm/s)	WOB(µm/s)	ALH(µm/s)	BCF(µm/s)	Hyperactive (%)
NALLIHAN	1	9,05±4,28	57,17±7,95	96,80±7,15	38,93±8,06	60,87±7,51	40,03±6,66	63,45±5,38	62,80±5,32	4,07±0,40	8,27±0,78	20,75±2,50
	2	10,40±4,04	59,00±10,25	98,51±7,16	40,61±7,20	63,00±6,97	41,05±5,42	64,05±4,75	63,84±3,77	4,07±0,40	7,94±0,58	21,68±5,03
LALAHAN	1	9,08±6,67	48,19±17,12	96,17±8,35	39,44±6,40	60,58±6,65	41,03±5,69	64,79±4,23	63,05±4,99	4,04±0,52	8,44±0,65	20,05±6,36
	2	6,58±5,10	50,24±23,34	92,05±7,25	35,83±8,09	57,27±8,45	38,70±6,94	61,95±5,68	62,03±5,97	4,03±0,40	7,53±0,68	16,43±6,21
AYAS	1	11,59±5,82	60,11±10,23	100,90±5,33	38,06±10,41	61,25±9,36	37,55±9,54	61,01±7,32	60,76±7,76	4,43±0,51	7,91±0,80	22,49±4,78
	2	7,13±4,83	48,99±18,51	95,24±9,98	36,61±8,63	58,01±8,56	38,39±8,54	62,40±7,87	60,87±6,00	4,31±0,66	8,21±0,87	18,46±6,92
P	-	-	-	-	-	-	-	-	-	-	-	-
					- No significant difference (p>0,05)							

Evaluation of the Antimicrobial Activity of Propolis on Strains of Corynebacterium Pseudotuberculosis

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Caseous Lymphadenitis is a widespread chronic disease in Mexico and affects sheep and goats. It is caused by Corynebacterium pseudotuberculosis bacteria, and leads to the formation of abcesses in the submandibular and prescapular lymph nodes, which fill up with purulent exudate; other lymph nodes can also be affected. The most affected animals show excessive weight loss, also known as cachexia; these animals can eat normally without recover lost weight. Bacteria are surrounded by purulent exudate and their facultative intracellular nature make antimicrobial treatments largely ineffective: the disease is only treated locally by removing dead tissue. Propolis is produced by bees and is made up of numerous substances, among them flavonoids, which account for its antimicrobial properties. The purpose of this study was to evaluate the antimicrobial activity of propolis on Corynebacterium pseudotuberculosis strains, by diluting them in agar. The strains were obtained from sheep with abscesses on the submandibular lymph nodes, which were debrided and sent to the bacteriology laboratory for isolation and identification; 10 of the strains isolated were then selected. The ethanol propolis extract was obtained from the FES-Cuautitlán UNAM apiary, from which a 200 mg/0.5 ml stock solution was extracted: dilutions at concentrations of 6, 3, 1, 0.75, 0.50 and 0.25 mg/ml were made, and after preparing PPLO agar the propolis solution was added to it and the agar subjected to a sterility test. Once the media had been prepared, the strains were inoculated at a concentration of 1x108 UFC/ml. Boxes without propolis inoculated with strains at the same concentration were used for the control group and incubated at 37º C for 48 hours. After that time lapse, it was observed that the growth of all the strains was inhibited with the exception of strain 7, which was able to grow at a concentration of 0.25 mg/ml. In conclusion, it was observed that Corynebacterium pseudotuberculosis is susceptible to the action of propolis, with the minimum inhibitory concentration being 0.50 mg/ml, in which no colony development took place. Further testing is required to determine the most appropriate way of administering propolis as a treatment for Caseous Lymphadenitis.

Keywords: Corynebacterium pseudotuberculosis, flavonoids, goats, linfonodes, propolis, sheeps

Comparison of the Effectiveness and Cost of Two Treatments for Four Caseous Lymphadenitis in Hair Sheep Flock in Guanajuato, Mexico

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The goal of the research work here presented was to realize a cost-effectiveness analysis between two ovine caseous lymphadenitis local treatments. Fieldwork was undertaken in the county of Irapuato in Guanajuato, Mexico, where four different flocks of hair sheep were examined. A physical examination, particularly of superficial lymphnodes, was performed to a total of 1547 animals, among which 73 cases of superficial caseous lymphadenitis were found; this animals were in turn separated into two randomly divided groups. A surgical local treatment was then performed in all animals belonging to group 1, which consisted of abscess debridement (by means of a surgical incision through the abscess fibrous tissue capsule), draining of the purulent exudate, posterior application of a 50% iodine solution within the incision to remove the remaining exudate, and, finally, application of granular 100% copper sulfate in the interior, which was not removed. In group 2, a non-surgical treatment was implemented, and consisted in the administration of two 37% formol injections directly into the abscesses by means of insulin syringes, each three days apart. Once applied, the evolution of both treatments was followed for the next 90 days, keeping records and making comparisons between the clinical evolution of the animals in each group, discharging them once wound healing was observed. At the end, the results obtained showed that the employed surgical treatment has variable recovery patterns, similar to those observed in wounds caused by both, surgically and non surgically mechanisms. On the other hand, animals that underwent non-surgical treatment, showed a local inflammatory process as well as pain between formol injections, followed by hardening, alopecia and abscess dry out, with progressive detachment of the necrotic tissue and cicatrization of the underlying area. The average final cost of the treatment used in group 1 was of $3.60 (Mexican currency) per animal, which was higher than that of group 2, namely $1.39 per animal; however, animals belonging to group 2 presented a higher complication rate during and after the treatment, as well as more serious sequels, regardless of recovery time, which is why it was concluded that the election of a specific treatment regime in a given sheep flock, where the presence of animals infected with ovine superficial caseous lymphadenitis has been established, will depend on the stage of the disease in each animal (which in turn depends on the size, maturation degree and localization of the abscess), as well as on the end purpose of the flock. Both of this aspects are very important since the quality and time of recovery are different for each treatment.

Keywords: Abscesses, caseous, cost, lymphadenitis, sheep, treatments

Prevalence of Sheep Pulmonary Parasites in Ardebil, Northwest Iran

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Pulmonary parasites are from the most important and widespread parasites of small ruminants in Iran. These parasites cause chronic pulmonary diseases and reduce animal products which cause enormous economic losses in the sector economy. Clearly, Epidemiological data are the most essential tools to control of any pathogenic agent, so this study was conducted to investigate the prevalence of sheep pulmonary parasites in Ardebil abattoir, Northwest Iran. A total of 5099 sheep lungs were inspected postmortemly to find any parasite in the airways and parenchyma from early October 2011 to late March 2012. Initially, every lung was inspected for superficial evidences of parenchyma nematodes then all of macroscopic airways were dissected by a minute scissor to find airways inhabiting nematodes. All of collected nematodes and pentastomes were clarified by lactophenol solution and recognized in accordance to standard taxonomic keys. In overall, 1582 out of 5099(31.02%)sheep lungs were condemned because of different reasons in Ardebil abattoir during study period where the rate of infection with different pulmonary parasites were observed as following; Cystocaulus.ocreatus: % 2.84 ± 0.83, Dictyocaulus. filaria: % 1.45 ± 0.55, Protostrongylus.rufescens: % 0.24 ± 0.18, Hydatid.cyst: % 21.34 ± 7.82 and Linguatula serrata: % 0.79 ± 0.22 (Mean± SD). According to the results, Hydatid.cyst was the most prevalent pulmonary parasite of sheep in Ardebil, northwest Iran, whereas in comparison, different strongylid nematodes and Linguatula serrata had a low prevalence rate in similarity to the most countries of the region.

Keywords: Ardebil, Iran, pulmonary parasites, sheep

Effects of Bluetongue Vaccination on Milk Yield in Sardinian Sheep in the District A.S.L. 1 of Sassari (Sardinia)

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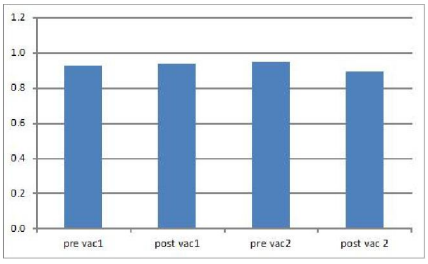
Bluetongue is an arthropod-borne viral disease to which all species of ruminants are susceptible, although sheep are most severely affected by this disease. To date, 26 distinct Blue Tongue Virus (BTV) serotypes have been identified. The first outbreak of serotype 2 bluetongue virus in Sardinia occurred in summer of 2000. More of 6200 outbreaks occurred during 2000 epidemics and 246.908 sheep died. In the following years, several outbreaks caused by different serotypes spread through Mediterranean countries were observed. To control disease a massive vector control and a vaccination program was performed in Italy. The first vaccination program started in 2002. All the ewes and goats and 90% of bovine were immunized using a modified live virus (MLV). During 2004 vaccination using serotypes 2,4 and 16 MLV vaccine caused adverse effects respectively in 25,83% and 49.22% of Sardinian and A.S.L. 1 sheep farms. Typical symptoms and lesions of the disease as well as a decrease in milk production were recorded. In consequence during the following years sheep farmers refused vaccination. In the year 2006, 2008, serotypes 1 and 8 appeared (the latter just only in A.S.L. 2). From 2010 onward more secure inactivated vaccine were exclusively used. In spite of this fact many farmers continue to adverse immunization. The aim of this work is to evaluate the presence of adverse effects on milk production due to inactivated vaccine. During the 2014 vaccination campaign a random sample of 30164 sheep from 65 flocks was investigated to evaluate the variations of pre-post vaccination mean daily milk production/head. The sheep were inoculated twice, the second injection after 21-28 days from the first (36 flocks were vaccinated against both BTV-1 and BTV-8, 29 flocks only against BTV-1). A random sample of 12073 sheep from 25 flocks, not yet vaccinated, has been chosen as a control during the same period of vaccination program. Data were collected in a database and submitted to statistical analysis. After the first inoculation the mean milk production increased in both groups, vaccinated and unvaccinated (+1.18÷+1.86%), while same parameter decreased in both groups after the second vaccination (-5.86÷-2.03%) Statistical analysis shows not significant variations between groups. There were only small differences of milk production between vaccinated and control groups after first and second injection period. The vaccinated group appears to shows a moderate tendency to more pronounced variations in milk production of not relevant statistical significance. It most likely depends on the transitory disturbance induced on the flock by the vaccination procedures rather than the effect of the inactivated vaccine employed. The decrease of milk yield after the second vaccination in both groups appears associated to the seasonal decrease of lactation curve in sardinian sheep breed.

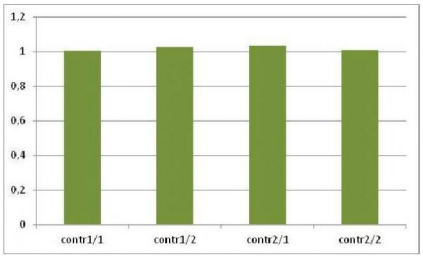
Keywords: Bluetongue, milk, vaccination, yield

Figure 1



Mean milk yield before (pre vac) and after (post vac) the first (1) and the second (2) immunization

Figure 2



Mean milk yield during the immunization periods in the control group

An Approach for the Diagnosis of Oestrosis

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Oestrosis is a parasitic disease caused by larvae of Oestrus ovis located in nasal cavities and the adjoing sinus of goats and sheeps. Oestrosis is a worldwide myiasis problem, especially Mediterranean areas that severely impairs health of animals and gives rise to serious economic losses, mainly in herds reared for meat and dairy production. Definitive diagnosis of oestrosis is confirmed with determining the presence of larvae on the post mortem head examination. The aim of present study to investigate the availability of rhinoscopy and molecular methods for diagnose of oestrosis in live animals. In our study, 18 goat and 82 sheep heads that have been just slaughtered for rhinoscopic examinations were obtained from a slaughterhouse. Additionally, 8 goat and 76 sheep heads were obtained for molecular studies. In rhinoscopic examination; how 100 heads were entered into the nasal cavity using a flexible endoscope with a 5 mm diameter tip and the visible sections of nasal turbinates, caudal nasal cavities and nasopharynxs were examined. Mucus samples were collected from nasal cavity of 84 heads by sterile cotton swaps for molecular analysis. The both method's results were confirmed by presence of O.ovis larvae in heads opened. Compared to rhinoscopy and autopsy method rhinoscopic findings were found to be identical to the autopsy findings of the 84 heads. Oestrus ovis larvae were determined in 71 of 84 heads that were investigated for molecular diagnosis examined. Semi-nested PCR was performed by using DNA of 84 swap samples. Of the 71 DNA samples extracted from the swap positive for oestrosis, 71 (%100) generated amplicons detectable on the agarose gels. No amplicons were generated from any of the negative samples. The sequences obtained were 99 % similar to the respective O. ovis sequences in GenBankTM. As a results, this study has demonstrated the availability of rhinoscopic and molecular analysis for diagnosis of oestrosis in live animals.

Keywords: Molecular diagnosis, oestrosis, rhinoscopy

Economic Impact of Coccidiosis in Small Ruminants

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Coccidiosis is an economically disease caused by protozoan parasites belonging to the Eimeria genus and which most commonly affects young animals. In goats, 17 species of Eimeria have been described, of which E. christenseni, E. arloingi, E. caprina and E.ninakohlyakimovae are serious pathogens. Clinical coccidiosis may affect 100 % of kids within the age range of 4-10 week, and cause severe economic losses by affecting animal health and profitability of the goat industry. In sheep, 11-15 species of Eimeria have been recorded, of which E.ovinoidalis and E.crandallis produce severe lesions. Lambs are more susceptible to the clinical form of coccidiosis at 4 to 8 weeks of age and also when animals of any age are kept in unhygienic and overcrowded houses and under stressor factors such as weanning, dietary changes and cold or heat weather. Older animals are usually subclinically infected and serve as a source of infection to new born. Coccidiosis leads to economic losses because of the high mortality rates and lowered productivity due to poor growth, together with costs of anticoccidials, drug administration and disinfection. Subclinical coccidiosis is the most common form of the disease and may have greater economic impact than losses due to death or clinical manifestations. Impairment of growth is the main sign of subclinical coccidiosis. Little is known about the economic impact of coccidiosis in small ruminants. No accurate estimation of losses has been made either in intensive or extensive breeding. It was estimated that yearly losses of $ 140 million in sheep production attributed to coccidiosis. The production system seems to play important role in the development of subclinical and clinical form of the diseases. The pathogenic effects of coccidiosis on sheep and goat production are stronger among animals raised in intensive systems, in which animal concentrations are much higher than in extensive systems. It is concluded that reduced environmental contamination by special measures such as high quality of bedding, proper cleaning of lambing or feding areas and improved flock management can be effective in preventing and delaying initial infections and enable farmers to minimize the use of chemicals.

Keywords: Coccidiosis, economic impact, small ruminant

Investigation of Milk Samples in Different Volume for RNA Isolation in Goat Milk

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The aim of this study was to develop a new method for RNA isolation from goat milk with different volume (7,5 and100 mL) samples. Until now, all literatures related to cell isolation from milk samples has shown that there is much milk volume (at least 150 mL) required. It was a big problem, because the transport of milk samples from farm to laboratory has a risk of sterility and degradation for RNA studies. In this study, 8 goats were used and two different groups, which was 7,5 mL and 100 mL milk volume were formed. Samples of the first group had 7,5 mL milk volume whereas the second group had 100mL volume milk samples. In the first group, each milk sample was decanted into nuclease-free 1,5 mL tubes (5 tubes per sample) and centrifugated at 8000 rpm, for 15 minutes at 4 C°. The fat layer on the top of the supernatant was removed with a spatula and the skim milk was discarded. The remaining cell pellet was washed in ice-cold PBS (PhosphateBufferedSaline) (pH 7,2), the presence of a final concentration of 0,5 mM EDTA (Ethylene D-AmineTetraAceticAcid) to eliminate casein micelles and fat globules. Then centrifugated at 8000 rpm, for 10minutes at 4 C° and supernatant was discarded.Five cell pellets from each sample were transferred to a sterile 15 mL conical tube and were added PBS upto a limit of the tube. Then centrifugated at 8000 rpm, for 10minutes at 4 C°. The upper phase was discarded and the somatic cell pellet was used for total RNA extraction. In the second group, each milk sample was decanted into sterile 50 mL conical tubes (2 tubes per sample) and centrifugated at 3150 rpm, for 15 minutes at 4 C°. The fat layer on the top of the supernatant was removed with a spatula. All the supernatant was discarded, apart from 5 mL which was used to resuspend the pellet. Two cell pellet suspension from each sample were transferred to a sterile 50 mL conical tube and were added ice-cold PBS-EDTA up to a limit of the tube.Then centrifugated at 3150 rpm,for 10minutes at 4 C°. Supernatant was discarded. The remaining cell pellet was washed in PBS and again centrifugated at 3150 rpm, for 10 minutes at 4 C°. After the last centrifugation supernatant was discarded and cell pellet was used for RNA extraction. Total RNA was extracted from isolated somatic cells using TRIzol reagent (Sigma Aldrich) according to the manufacturer's instructions. Total RNA pellets were resuspended in 20 µL of DEPC (DiethylPyrocarbonate) treated water. The concentration of isolated nucleic acid was determined by Merinton SMA 1000 UV Spectrophometer. A260/A280 was>1,70 in all samples. In the 7,5 mL and 100 mL milk samples, purity and concentrations of RNAs were 1,87±0,20 and 1,77±0,04; 149,37±122,76 ng/µL and 309,03±220,05 ng/µL, respectively. The quality of total RNA was performed agarose gel electrophoresis (5 µg of total RNA seperated on 1 % wt/volagarose gel stained with ethidium bromide) and RNA integrity was assessed according to 28 S and 18 S rRNA subunits. The integrity of RNA in gel electrophoresis has been showed that 7,5 mL milk volume should be enough for RNA isolation in milk cells.

Keywords: Goat milk, isolation, somatic cell, RNA

Evaluation of Milk Production and Milk Composition in Three Different Sheep Breeds

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Milk is considered one of the major sources of nutrients for human consumption. In Jordan, the dairy sheep industry is growing and developing as the demand for sheep milk is increasing due to the increase in population. For this reason some farmers in Jordan are looking for sheep breeds that are known for their high milk yield as well as good milk quality. The objective of this study was to evaluate milk yield and milk composition in Awassi, Assaf, and Chios sheep breeds. Data was collected from 10 randomly chosen 3 years-old-ewes of each breed from three different sheep farms in the northern part of Jordan. Milk samples were collected twice daily. Chemical analysis of the milk samples was conducted in the laboratories on Jordan University of Science and Technology at the department of Animal Science. Data was then analyzed using the Proc Mixed Procedure of SAS with ewe breed being treated as fixed effect while milking time and ewe ID were all treated as random effects. Breed was found to have a significant effect on milk production as well as milk components. Milk production has ranged from 0.86 kg in Awassi, to 4.18 kg in Assaf ewes, while Chios breed was intermediate. Awassi had higher ($p < 0.05$) milk protein and lactose compared to both Assaf and Chios. Milk fat was the highest ($P < 0.05$) in Chios milk. Milk PH was 6.96, 6.68, and 6.60 for Chios, Assaf, and Awassi milk, respectively. SNF was the highest ($p < 0.05$) in Awassi milk. Milk density was higher ($P < 0.05$) in Awassi milk compared to Assaf while no difference was detected between Assaf and Chios milk density. In brief, improving milk production and milk characteristics in Awassi breed can be done effectively through the utilization of crossbreeding between Awassi and Chios, or by backcrossing Awassi with Assaf breed.

Keywords: Breed, milk, sheep

The Lipid Peroxidation and Antioxidative Status in Sheep Naturally Infected with Endoparasites (*D. Dentriticum, Cysts Hydatid*)

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The aim of the study was to investigate the lipid peroxidation and to determine antioxidant status in sheep naturally infected with *D.dentriticum* and *cysts hydatid*. The blood and the visceral organs of the sheep that brought to the slaughterhouse were checked for *D. dendriticum, cysts hydatid*. The study was carried on 30 Akkaraman sheep which were infected with D.dendriticum, cysts hydatid, and healthy sheep. The levels of malondialdehyde (MDA), the activities of catalase (CAT) and superoxide dismutase (SOD) in erythrocyte, the concentrations of vitamins A, C, E and β-carotene in plasma were detected. The erythrocyte MDA levels were determined according to the Buege and Aust [1]. Catalase and SOD activities were measured in hemolysates by the method of Aebi [2] and Sun et al. [3] respectively. The concentration of blood hemoglobin was determined according to Fairbanks and Klee [4] 's method. The plasma concentration of vitamin A and β-carotene were determined by the method of Suzuki and Katoh [5], vitamin C and E were analyzed spectrophotometrically according to the methods of Haag [6], and Martinek [7], respectively. Statistical analysis was performed by SPSS 15.0 version for Windows. The data were expressed as arithmetic means ± standard error. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan's Multiple Range Test was performed. A probability value of $p < 0.05$ were considered as significant difference for all statistical calculations. As the levels of MDA were increased ($p<0.05$), vitamin A concentrations were decreased ($p<0.001$) in sheep infected with parasites as compared to the control group. As the erythrocyte SOD activities were decreased in *D. dendriticum* group ($p<0.05$), both SOD and CAT activities were decreased in *cysts hydatid* group as compared to control group ($p<0.001$). Vitamin C, E and β-caroten concentrations were not affected parasitic infections ($p>0.05$). These results suggest that the endoparasitic infections such as *D. dendriticum* and *cysts hydatid* caused oxidative stress in sheep.

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Keywords: Antioxidant system, cyst hydatid, d. dendriticum, lipid peroxidation, sheep

The Effect of Zataria multiflora Boiss Essential Oil and Nisin Against on Salmonella Typhimurium in Minced Sheep

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Meat microbial control from human's health and high quality of life are chiefly important. Zataria multiflora Boiss. is Native to Iran with the Persian name of Avishane Shirazi. Antibacterial properties are known. In this study, the antimicrobial effect of Avishan-e shirazi (Zataria multiflora) Essential Oil (EO) at different levels of 0, 0.3%, 0.6%, 0.9%, Nisin at 500, 1000 or 1500IU/g and Middle levels and Their combination against Salmonella typhimurium ATCC 14028 in minced sheep during refrigerated (0-4 ˚C) storage times (14 days) were used. After determining the lowest and the highest inhibitory effect on S. typhimurium growth. EO at 0.3% possessed a weak antibacterial activity against the pathogen. The combination of EO at 0.3% and nisin at 500 IU/g had the highest inhibitory effect against the pathogen. The results using combination of Essential Oil and Nisin antibacterial properties increased at minced sheep. We recommended to utilize essential oils as antimicrobial components along with other preservation techniques e.g. reduced temperature and pH or other natural preservatives, such as nisin.

Keywords: Minced sheep, nisin, salmonella typhimurium, zataria multiflora

Capsid Protein of Porcine Circovirus Type II without Nuclear Localization Signal Binds DNA Non-Specifically

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Porcine circovirus (PCV) belongs to the genus Circovirus of the family Circoviridae. Circoviruses have small (~2Kb), covalently closed, circular, single-stranded DNA (ssDNA) genomes encapsidated within nonenveloped icosahedral virions. Two genotypes of PCV have been identified: PCV type Ⅰ (PCV1) and PCV type Ⅱ (PCV2). PCV2 is the etiologic agent of postweaning multisystemic wasting syndrome (PMWS). PCV2 contains two main open reading frames (ORF): ORF1 encodes Rep protein, which involved in viral DNA replication, and ORF2 encodes viral capsid protein (Cap protein). In order to gain insight into the role of the Cap protein in the life cycle of PCV2, we analyzed the interaction between Cap protein and viral genome DNA. PCV2 strain HZ0201 (GenBank No. AY188355) with a genome of 1767 nucleotides was isolated from pig farms with naturally occurring PMWS. The viral genome DNA (vDNA) was extracted with A UNIQ-10 Column Virus Genomic DNA Isolation Kit (Sangon Biotech), followed by polymerase chain reaction (PCR) amplification. Using specific primers, full-length ORF1 and ORF2 were cloned from the PCV2 genome. Finally, the products were recovered cleanly using an AxyPrep PCR Cleaning Kit (CORNING). In addition, the plasmid used in this study was pcDNA3.0 (Invitrogen). We expressed the Cap protein gene defecting nuclear localization signal (NLS) of PCV2 in Escherichia coli (E.coli) as a fusion protein with glutathione S-transferase (rGST-dCap protein). We also expressed and purified GST-tagged protein. The purified proteins were diluted to 1μg/ml with ddH2O. Electromobility shift assay (EMSA) is a simple, efficient and rapid method for the study of the specific PCV2 vDNA-Cap interactions. The EMSA was carried out by the Electrophoretic Mobility-Shift Assay Kit (Life Technologies) according to the manufacturer's instructions. The ability of GST-tagged protein to bind viral genome DNA was assessed as a negative control. The significant retardation of the genomic DNA fragment was observed when rGST-dCap protein in binding buffer was added instead of the GST-tagged protein. A faint smear was seen when 60ng vDNA associated with 0.5μg dCap, which showed a weak DNA-protein interaction. With the increased in the amount of dCap protein added (1μg), one major band of the complexes was seen. While PCV2 dCap bound ORF1 or ORF2, it also retarded the electrophoretic movement of DNA within a polyacrylamide gel. Furthermore, when the bound DNA was replaced by intact pcDNA3.0 plasmid (60ng) that do not contain the full-length PCV2 genome, the same results occurred (Fig. 1). These results suggested that PCV2 dCap bound genomic DNA with no or little sequence specificity. We have shown that Cap protein of porcine circovirus type II without nuclear localization signal is capable of binding double-stranded DNA non-specifically. The ability of PCV2 Cap to bind DNA is independent of its karyophilic nature. We hypothesized that the dCap of PCV2 bind to bend or distort DNA. How the complexes form and the exact structure are, however, still unknown.

Keywords: PCV2, capside protein, Electrophoretic Mobility-Shift Assay, non-specifity

Figure 1

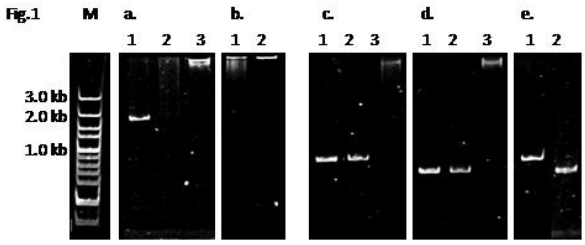

FIG. 1. Electrophoretic mobility shift analysis of the interaction of PCV2 dCap with various DNA samples. (a) vDNA, (b) pcDNA3.0, (c) PCV2 ORF1 and (d) PCV2 ORF2 were 60ng only (lane 1), incubated with purified dCap 0.5μg (lane 2) and 1.0μg (lane 3). (e) As a negative control, GST-tagged protein were incubated with PCV2 ORF1 (lane 1) and ORF2 (lane 2). Lanes M. molecular size markers.

Effects of Dietary Iron Chelate Supplementation in Swine Nutrition on Growth Performance and Meat Quality Characteristics

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Consumer awareness for the quality of animal products, as well as the environmental impact of farm animals has given a major boost on the research for alternative feed additives. Such ingredients are the chelated macro- and micronutrients, as iron chelate, which can possibly improve swine performance and the nutritional and organoleptic components of swine meat. In this trial, 64 pigs with average body weight 54.0 kg and average age 112 days were randomly allocated to 4 groups (8 males and 8 females per group). All animals were fed with a standard commercial ration with the extra addition per ton of either 200 g iron sulfate (Group S200), or 200 g iron chelate (Group C200), or 800 g iron sulfate (Group S800), or 800 g iron chelate (Group C800). All animals were reared in standrard husbandry conditions (slatted plastic floors, density, humidity, temperature, ventilation), while feed and water were offered ad libitum. Animals were slaughtered at 165 days of age. The results showed that iron chelate significantly ($P<0.001$) increased body weight compared to the iron sulfate (table 1). The subcutaneous fat (point P2) that was measured by Minitube® Backfat Measuring Device did not differ ($P>0.05$) between the groups. The meat composition was determined by FoodScan Pro/Lab, Foss showed that ferrous chelate significantly decreased ($P<0.001$) total fat in the steak, shoulder and ham parts, as well as increased protein content in the steak and the ham parts (table 2). Supplementation of iron chelate in swine nutrition improves slaughter weight, average daily growth at the fattening stage and could be used as a dietary manipulation to produce pork meat with improved chemical composition and consumer's desirable quality characteristics.

Acknowledgements: This research was funded from EEC and national Funds, under the research program Bilateral R&T Cooperation Greece-China, 2012–2014 (EPAN II-EII--12CHN91-GreenPork).

Keywords: Growth performance, iron chelate, meat quality, swine

Table 1 - Effect of dietary supplementation of ferrous sulfate and chelate in fattening pigs, in the final body weight (age 165 days)

Exp. groups	Iron addition	Bodyweight (kg)	S.D.
S200	iron sulfate, 200 g/tn	100,3 a	3,3
S800	iron sulfate, 800 g/tn	104,2 b	3,9
C200	iron chelate, 200 g/tn	106,4 b	3,7
C800	iron chelate, 800 g/tn	106,2 b	3,8
	P	<0,001	

ab: values in the same row with different superscript differ significantly (P<0,001)

Table 2 - Effect of dietary supplementation of ferrous sulfate and chelate in fattening pigs, in the chemical composition of pork meat (age 165 days)

		Fat	Fat	Fat
Exp. groups	Iron addition	rib chops %	Shoulder %	Ham %
S200	iron sulfate, 200 g/tn	8,4 c	6,9 b	5,5 c
S800	iron sulfate, 800 g/tn	8,1 c	6,4 b	3,8 a
C200	iron chelate, 200 g/tn	7,1 b	6,8 b	4,5 b
C800	iron chelate, 800 g/tn	5,6 a	5,4 a	3,6 a
	S.D.	0,055	0,068	0,068
	P	<0,001	<0,001	<0,001
		Total proteins	Total proteins	Total proteins
Exp. groups	Iron addition	rib chops %	Shoulder %	Ham %
S200	iron sulfate, 200 g/tn	19,0 a	19,9 b	21,1 a
S800	iron sulfate, 800 g/tn	21,4 c	19,7 b	21,5 b
C200	iron chelate, 200 g/tn	19,6 b	19,3 a	22,1 c
C800	iron chelate, 800 g/tn	21,7 c	19,9 b	22,6 d
	S.D.	0,054	0,034	0,039
	P	<0,001	<0,001	<0,001

abcd values in the same row with different superscript differ significantly (P<0,001)

Comparison of Mechanical and Pharmacological Properties of Longitudinal and Circular Smooth Muscle of the Pig Cervix

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For transcervical artificial insemination (AI) to be successful it is necessary to overcome the anatomical arrangement of circular (CM) and longitudinal smooth muscle (LM) that contract either spontaneously or in response to activation of intrinsic nerves. To date, the majority of pharmacological approaches to assist transcervical AI has focused activation of prostaglandin receptors to induced relaxation (Candappa & Bartlewski 2014). In the present study we have examined the properties of LM and CM from the pig cervix to better understand the factors that oppose transcervical AI. Experiment was conducted on 215 cervical strips (LM, n=87; CM, n= 128). Each preparation was submerged in an organ bath immersed in Krebs–Henseleit solution (pH 7.4, gassed 95% O2/5% CO2) at 37°C. The tissues were left to equilibrate for 30 min without any initial tension applied. Then, 15g tension was applied and allowed to stabilize to 2-5g. LM (75%) and CM (55%) preparations of cervix developed spontaneous contraction, 5.7±1.0 g wt. and 5.9±0.6 g wt., respectively, with a frequency of 1.3±0.2/5min and 2.8±0.2/5min. Greater than 90% of preparations responded to 10Hz, 0.3ms, 30s duration electrical field stimulation (EFS) with a contraction 8.5±0.9 g wt. (LM) and 9.0±0.5 g wt. (CM), respectively. EFS (2Hz-32Hz) produced frequency-dependent contractions in both CM and LM preparations that were abolished by 0.3µM tetrodotoxin. In CM atropine (0.3µM) abolished EFS contractions below 8Hz 30s and reduced by > 60% contractions to 16Hz and 32Hz. In contrast, 0.3µM atropine did not affect EFS-induced contractions to 2Hz, 4Hz, or 8Hz in LM preparations, but significantly reduced by 41.5±14.7% (n=10, Student's t-test, $p<0.05$) contractions to 32Hz 30s. The β-adrenoceptor agonist isoprenaline (30nM-3µM) produced concentration-dependent inhibition of the neurogenic response to 32Hz, 30s EFS that was significantly greater in LM than CM, but abolished spontaneous contraction in both preparations. At 3µM isoprenaline, for example, the inhibitory effect in LM (93.5±4.5%, n=5) was greater ($p < 0.05$) than that observed in CM (60.8±10.7, n=5). In contrast, the selective PDE 4 inhibitor piclamilast (10nM-1µM) did not significantly inhibit EFS responses to 32Hz, 30s in CM preparations (n=6), but abolished spontaneous contraction in 50% of preparations. Papaverine (0.1µM-30µM) and diltiazem (0.1µM-10µM) cause abolished spontaneous contraction in both CM and LM preparations and caused a concentration-dependent inhibition of neurogenic contractions (32Hz, 30s) in both preparations. At the highest concentration examined papaverine reduced neurogenic contractions by more 50%, while diltiazem reduced responses by more than 80%. These finding shows that while 50-70% of LM and CM preparations of the pig cervix exhibits spontaneous contractions, more than 90% of preparations responded to EFS with larger neurogenic contraction. In CM preparations cholinergic nerves appear to primarily activated EFS, while in the LM preparations acetylcholine appears to be involved in neurogenic contractions at high frequencies of stimulation. Although there is evidence for inhibitory β-adrenoceptors in both preparations, this effect was more pronounced in the LM. In contrast, the non-selective PDE inhibitor papaverine and the calcium channel blocker diltiazem, produced a sustained, non-selective inhibition of CM and LM cervical muscle. Papaverine and diltiazem are potential candidate drugs to reduce contractile tone of the cervix and assist with transcervical AI in other species.

Acknowledgements: Financial support: CAPES Foundation (Brazil)–BEX: 3663/14-0. Candappa IB, Bartlewski PM (2014). Induction of cervical dilation for transcervical embryo transfer in ewes. Reprod Biol Endocrinol. 28;12:8.

Keywords: Artificial insemination, cervical, diltiazem, porcine, papaverine

The Preparation of Virus-Like Particles of Porcine Circovirus Type 2 Using Baculovirus System

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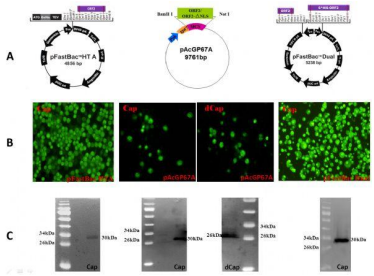
Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus–associated disease (PCVAD). PCVD is worldwide prevalent in recent years and becomes a serious threat to the domestic pig industry. Currently, vaccination is an effective way to control the diseases. The ORF2 gene of PCV2 encodes the viral capsid protein, which is the primary immunogenic protein and can self-assemble into virus-like particles (VLPs), thus it is widely used to develop PCV2 subunit vaccines. Baculovirus system expressing Cap protein, is possible to achieve the purpose of the soluble protein expression and can carry out post-translational modification and folding of proteins, close to the native protein conformation. In this study, we express capsid protein of PCV2 in insect cells using baculovirus expression system and optimize the conditions for virus amplification and protein expression, and observe whether the VLPs is formed using the electron microscope. The PCV2 ORF2 gene was inserted into multiple cloning site of the transfer vectors, PFastBac HTA, pAcGP67A and pFastBac Dual, which belong to baculovirus expression system. With the help of bacterial transposon Tn7, the recombinant transfer vector and the baculovirus genome which exist in the E.coli DH10Bac competent cells occurs the transposition reaction, form a recombinant baculovirus Bacmid plasmid, then was transfected into Sf9 cells to harvest recombinant virus (i.e. pFastBac HTA and pFastBac Dual);Or with lethal deletion Autographa californica nuclear polyhedrosis virus genome co-transfect into Sf9 cells generate recombinant baculovirus (i.e.pAcGP67A). To optimize the conditions for virus amplification and protein expression, we infected the cells with different time courses at multiplicity of infect (MOI) and analyzed the expression of Cap protein by WB at the same condition to choose the best one. We have successfully constructed four recombinant baculoviruses (Fig.1A). The expression of Cap protein gene in sf9 cell was confirmed by indirect immunofluorescence assay (Fig. 1B) and western blotting test (Fig. 1C). The analysis of Cap protein expression showed that PFastBac Dual as a donor vector has a highest expression level of Cap protein. Cap protein and dCap protein (ORF2 gene remove the highly conserved N-terminal 41 amino acid sequence) expressed in Baculovirus expression system were purfied by the sucrose density gradient centrifugation. In the observation of transmissible electron microscope, the virus-like particles can be observed in Cap protein sample but not in dCap protein sample (Fig. 2). In our study, as a donor the pFastBac Dual vector can express two target genes. The full-length Cap proteins can form virus-like particles.

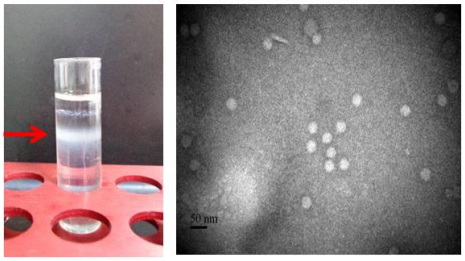
Keywords: Baculovirus system, porcine circovirus type 2, cap protein, virus like particles

Figure 1 - Construction and identification of recombinant baculovirus with the recombinant baculovirus



(A) Pattern of Recombinant transfer vector pFastBac HTA-ORF2, pAcGP67A-ORF2/ORF2-ΔNLS, pFastBac Dual-2ORF2. (B) The expression of PCV2 Cap and dCap in Sf9 cells by IFA. Recombinant baculovirus infected Sf9 cells reacted with anti-PCV2 Cap monoclonal antibody. (C) The Western blot analysis of the expressed Cap and dCap protein, Sf9 cells were harvested after infected with rBac-Cap or rBac-dCap.

Figure 2 - Virus-like particles in Sf9 cells infected



Purified recombinant Cap protein by the sucrose density gradient centrifugation can be seen a milk white band and VLPs was observed under the electron microscope.

Production and Purification of Recombinant Protein HN of the Porcine Rubulavirus as a Candidate for an Immunogen in Pigs

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In 1980, the pig farms located in La Piedad, Michoacan, México a new Paramyxoviridae was isolated and latter classified as porcine Rubulavirus (PorPV) and the disease was named Blue Eye Syndrome. There are more than 16 million pigs in at least one third of them are affected. The disease is concentrated in the central states of México. In newborn pigs 19% of the animals are born dead or mummified and of those born alive, up to 50% are killed by the PorPV. The virus is an RNA one that codifies six structural proteins (NP, L, M, F HN and P) The most immunogenic of the proteins is HN which is capable of eliciting neutralizing antibodies. In SDS-PAGE it has a MW of 60 kDa. Our aim was to purify a high amount of a previously developed bacterial clone with the recombinant protein and evaluate it as a candidate immunogen for pigs. To detect the HN gen in the pDual GC Expression Vector PCR was conducted in order to identify the *E. coli* KRX colonies that contained the insertion. These colonies were separated, purified and their DNA purified to transfect the BL21 strain of *E. coli*. We used Calcium and Magnesium Chloride. An evaluation was conducted in order to determine the expression of the protein within the bacterial cells. Once determined, the protein was quantified by a modified Bradford Method. The *E. coli* strain that that was found the highest producer, it was grown to a large volume, the cells lysed and the protein purified by affinity chromatography using the resin Chelating Sepharose resin, Fast Flow from GE Health Care". The protein was identified by indirect peroxidase reaction using as a primary antibody anti-myc labeled with horse radish peroxidase and Pig anti PorPV and was developed by chimioluminescence. Finally the neuraminidase activity of the protein was evaluated. With western blot and protein quantification, the highest expression of of the recombinant HN protein was detected in intracellular inclusion bodies. By the Student T test it was determined that the highest producer of intracellular inclusion bodies was the *E. coli* BL21 strain (P= <0.05) than the KRX. The purification process was standardized where we do not loss the recombinant HN protein, this determined by purification chromatography and western blot for the purified protein. The antibodies of the PorPV were capable of recognizing a 66kDa recombinant protein. The use of vaccines is an important tool in the control and possible eradication of diseases. We think that the recombinant HN protein from PorPV is a candidate as a good immunogen in pigs.

Acknowledgements: Grant´s: PIAPIC 12, CONACYT scholarship No. 300988. Programa de Doctorado y Maestría en Ciencias de la Producción y Salud Animal UNAM

Keywords: HN protein, recombinant protein, porcine rubulvirus

Diagnostic of Porcine Rubulavirus in Peripherical Blood Mononuclear Cells from Persistently Infected Pigs by Indirect Immunofluorescence

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In 1980 the pigs on the farms of the central México experienced the first symptoms of a new disease, specially in new born pigs, with encephalitis and corneal opacity from which a haemoagglutinating virus was isolated. The virus was latter characterized as a paramyxovirus belonging to the Paramyxoviridae Family and Porcine Rubulavirus Genus (PorPV). The disease was and remains endemic in the central states of México. It has been considered the fourth most important pig disease of the Mexican pig industry. The viruses belonging to the Paramyxoviridae Family are known to establish persistent infections both *in vitro* and *in vivo*. This raises the possibility of having persistently infected pigs shedding virus occasionally in the farm. Our aim in this study was to look at the peripheral mononuclear cells and detect the PorPV or its antigens by indirect immunofluorescence test in cell cultures. The study was conducted in two locations in México (CENID-MA INIFAP and FMVZ-UNAM). Two farms were selected for this study: the first in Pénjamo Guanajuato, where four sows were bled. This farm was selected on the basis of being positive by virus isolation. The second farm, with no previous history of PorPV as located in Toluca, Estado de México and eight sows were sampled in it. The blood was collected in heparinized tubes, centrifuged in Ficoll-Hypaque PLUS and the PBMC were placed in PK-15 Cell cultures. If no CPE was observed after a few days, a second or even a third blind passage were carried out. The Pénjamo farm had two out of four positive pigs and four out of eight were found in the Toluca farm. In both cases the positive results were found at the third blind passage. It is of importance that only after the third passage the PorPV antigens were observed, in both farms regardless of the previous history on this disease. Both farms had 50% positive samples, which is surprising considering the small samples taken. The persistently infected pigs are abundant in farms in the central states of México, but with the help of this technique, we expect that such animals may be eliminated.

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Keywords: Indirect immunofluorescence, porcine rubulavirus, persistently infected pigs

Detection of PorPV in Circulating Leukocytes Cuantified by Real Time RT-PCR to Detect Persistently Infected Pigs from a Natural Outbreak

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An outbreak of an unknown disease occurred in La Piedad, Mexico in 1980, in pig farms. This disease was characterized by encephalitis and corneal opacity in newborn piglets, also characterized by a high mortality rate. From this outbreak a hemagglutinating virus was isolated. The virus was eventually classified as a Paramixoviridae, Genus Rubulavirus (Rubulavirus porcino, PorPV). The clinical signs are dependent upon the age of the affected animals, with a nervous and respiratory picture in young animals and mainly reproductive problems in adults. The PorPV can establish persistent infections in adult pigs. This disease is endemic in the central states of Mexico. Our aim in conducting this study is to determine the presence of viral RNA in naturally convalescent pigs from an outbreak, since the persistent infection had been previously demonstrated in experimentally infected pigs. The technique chosen for this study was the real time RT-PCR from the circulating leukocytes. The study was conducted at the CENID-MA INIFAP México and at the FMVZ, UNAM, México. We selected three farms, two that had undergone an outbreak (Zumpango and Guanajuato), confirmed by viral isolation and one without having undergone a previous infection, as confirmed by serology (Toluca). We also included 8 SPF pigs as negative controls. The blood collected from all animals at the start of the experiment was centrifuged to separate serum for serology and White cells for RNA extraction. The RNA was quantified by real time RT-PCR, the antibodies were quantified by ELISA. The oligonucleotides used for the real time RT-PCR amplified a 71 BP fragment. The standard curve was obtained by ten-fold dilution of standard RNA. In the Zumpango farm (n=18) in the state of Mexico, 88.89% of the sampled saws sampled were positive with an estimated concentration of 47.67 ng/µl of viral RNA. From the farm located in Pénjamo Guanajuato, we sampled saws from 0 to 6 parturitions (n=30), where 96.67% were positive with 2.45 ng/µl of viral RNA and sementals (n=23), with a 69.57% boars positive with a concentration of 1.31 ng/µl viral RNA. In the farm located in Toluca, (n=21), Estado de México, an 80.95% of the animals were positive at 0.35ng/µl of viral ARN for the P gene. The ELISA test showed that in the three farms studies significant amounts of IgG for HN protein of the RVP. The use of the real time RT-PCR in leukocytes of peripheral blood resulted in a rapid method (90 min), quantitative and highly sensitive method for the detection of persistently infected with the Blue eye virus, since it allows the detection of minimal amounts of viral RNA, which represent an important method for the epidemiology of the Blue Eye Disease in Mexico.

Acknowledgements: Grant´s: PIAPIC 12, CONACYT scholarship No. 300988. Programa de Doctorado y Maestría en Ciencias de la Producción y Salud Animal UNAM

Keywords: Porcine rubulavirus, PBMC, persistently infected pigs, real time RT-PCR

Study of the Proteins in the Supernatant of Mycoplasma Hyopneumoniae Cultures with Biological Activity

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Mycoplasma hyopneumoniae (M.hyop.) is the etiologic agent of enzootic pneumonia and is considered primary pathogen of the respiratory complex pigs; complex multifactorial chronic respiratory diseases with many still unknown, associated with significant economic pig producers worldwide (1,5) losses phases. In recent times it has been reported numerous proteins of M. hyop. with varying molecular weight (p7413, p7048, p6523, p4026, P467, P159, P102, p97, p89, p74, p72, p70, p65, p60, p46 p54, p42, p41, p36 kDa.), in the cell membrane, cytosol and has been shown to be capable of secreting some proteins to liquid culture medium. These proteins have different biological activities, some are adhesins, able to cause cytopathic effect and / or immunological activity (1,7,9). Different biological activities of the membrane proteins and secretion is unknown, for that reason it is of great importance to continue conducting research on these proteins to understand this etiologic agent. The reference strain M. hyop. (J strain, NCTC 10110) was used in this study and cultured in broth Friis (3) The number of viable organisms was determined by a series of dilutions in broth decuple Friis and the title is expressed in color changing units (CCU).500 ml medium was inoculated with 105 Friis CCU / 5 ml. and 8 ml were collected. medium to 3,5,7,9,11,13,15,17,19 and 21 days of growth. Each sample was centrifuged at 30,000 g for 30 minutes and underwent SDS-PAGE and Western blotting (4.8) to detect proteins of interest is performed by one Semipurification centrifuge tubes with filter pair, concentrating proteins less than 100 kDa. with which MDCK cell cultures were challenged and mononuclear cells isolated from peripheral blood by Ficoll-Hypaque were stimulated. By SDS-PAGE and Western blot secretion two proteins were detected 80 and 42 kDa with which MDCK cell cultures were challenged showing cytotoxic effect, mononuclear cells fail to elicit a stimulation clonal mitogenic activity which is discarded. These results are similar to those reported by Assuncao in 2005, only that they worked with whole cells of M. hyop. Okada (6) shown by SDS-PAGE and Western blotting that this protein a secret agent using a similar molecular weight. The cytopathic effect demonstrated with secretory proteins is consistent with that reported by Geary (4). Although it has been reported that M. hyop. presents a nonspecific lymphocyte stimulator factor failed to demonstrate in this work

Keywords: Mycoplasma hyopneumoniae, SDS-PAGE, western blotting

Study of Streptococcus Suis in Swine Farms in Mexico: Isolation and Characterization

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Streptococcus suis (S. suis) is an important pathogen of swine. It is a well-known fact that most pigs are carrier of multiple serotypes of S. suis in the upper respiratory tract. It can also be a cause meningitis, arthritis and septicemia. It affects the production parameters of pigs causing important economic losses in farms. It is also a zoonotic disease affecting people that work in close contact with pigs. We characterized the strains of S. suis isolated from farm employees and pigs by means of molecular techniques (PCR, and serotyping) in order to determine which of the 35 serotypes of the existing 35 affected animals and were present in personnel in Mexico. We established three groups, from the first one the tonsils of clinically healthy animals were obtained (n=85); the samples of the second group consisted of internal organs from clinically ill animals (n=77) and those of the third group were from pharyngeal secretions taken from farm personnel (n=60). The samples were cultured in 5 % blood-agar. The α-hemolytic colonies were selected. A PCR for the gdh gene was conducted to determine that we were dealing with S. suis, and the positive colonies were genotyped to identify the serotype. In the group of healthy pigs, all samples were negative for S. suis. Similar results were obtained with the samples from the farm workers. In the group of ill pigs, 6 samples were found positive for S. suis. From these samples, one beloged to the serotype 1/2, two samples to serotype 2, one sample to serotype 3 and two samples were positive to serotype 7. The fact that none of the workers in the study group resulted positive seems to indicate that either the workers are not carrier of this pathogen or that the method used lacks sensitivity. Since it is known that S. suis is zoonotic, the failure to detect it, seems to indicate that a single sample from each person may not be sufficient to detect occasional presence of bacteria. We may need to use repeated sampling of each person, or more sensitive techniques. The human samples represented both clinically healthy and S. suis affected farms. Concerning the 85 samples in the clinically healthy farms that were negative, suggest that the prevalence of S. suis in healthy carriers is low. Again, this may also be the consequence of a low sensitive technique used. Most publications indicate a high level of infection in clinically healthy pigs, since S. suis is a normal inhabitant of the upper respiratory tract of pigs. S. suis could be isolated, on the other hand, from sick animals, with the presence of serotypes 1/2, 2, 3, and 7. This is the first time that serotypes other than serotype 2 are reported in Mexico.

Keywords: farms, genotyped, streptoccocus suis, s. suis, serotypes, PCR gdh

Loss of Weight Gain Caused by *Clostridium perfringens* of Piglets

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This study objective to examine the interference between the decreases of the weight gain an infection by *C. perfringens*. 54 samples were collected from piglet without signs of infection in the confinement of UNESP São Paulo State University – College of Agricultural and Veterinarian Sciences, Campus of Jaboticabal. All of the samples were inoculated into Brain Heart Infusion (BHI) broth and subjected to thermal shock (Oliveira et al, 2006) to active spore germination and elimination of possible contaminants. After thermal shock, were incubated anaerobically at 37ºC for 48h. DNA extraction of *C. perfringens* was done by Marmur technique (1961), and multiplex PCR reaction mixture, was prepared in a volume of 20 µL. contained 2 µL 10x PCR buffer, 0,8 µL MgCl2, 0.4 µL NTP mixture, 1.5U of Taq DNA polymerase, 1 µL each of 50pmol *cpa*, *cpb*, *etx*, *cpe*, *cpb2* and *iA*(Baums et al, 2009), 0.4 µL target DNA and 9.9 µL distilled water. There were used positive controls for each strains also. The PCR mixture were placed in ABI Veriti Thermal Cycler (Applied Biosystems®), programmed with annealing temperature of 55°C for 1 min. The resulting PCR product was run agarose gel 1% (p/v) with SYBR® Green 1x (Invitrogen) and molecular size standard 100 pb DNA Ladder (MBI Fermentas). Among the 54 samples of healthy piglets, *C. perfringens* was identified in 26 samples (48%), and all the C. perfringens culture were confirmed as been type A, which produce *alpha* toxin. The relation among the presence of *C. perfringens*and weight gain were commonly observed in animals with slaughter ages, wherever this factor become more evident. In the present study, the animals that were positive for *C. perfringens* had a weight gain lower, even not having diseases signals, when compared with the negatives animal for this pathogen. The performance of weight gain media were high in the negatives animals, where the mean was 38.88 kg, while positives animals the mean was 27.58kg. Showing a difference of 9.15%. It is known that intestinal lesions with massive *C. erfringens* infections lead to the diseases complex diarrheal fecal, and all those pathogens mainly affect the villi of the jejunum portion, and preclude the absorption of essential nutrients for a healthy body, causing decreased weight gain and economic losses for the industry. Besides studies reported that *C. perfringens* is present also in foods of animals origin, meat and meat products specially been as an important vehicle for foodborne diseases. The difference between presence of *C. perfringens* and the weight gain of the animals was statistically significant, where the correlation significance was negative -0.25. This result indicates that the positive group for pathogen showed the lowest weight gain, therefore were slaughtered underweight.

Keywords: Confinement, microbiology, porcine, PCR

Costs of Tools and Accessories Used to Compost Swine Mortality

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Disposal of mortalities and organic residues to be composted offer an environmental friendly alternative but first must demonstrate its financial viability to adopt it as a part of the biosecurity plan. The composting system generate variability in the costs, for example, in developed countries, the mayor cost involved in carcass composting is the acquisition and handling of machinery but in developing countries, the main costs are: man power and consumed water, however, the additional costs must be investigated. We obtained the cost of the accessories needed for the development of the process of composting mortality in a One-site operation of 135 breeding sows, where the process was established since 2008. Additionally, the prices for the accessories were investigated and multiplied by the number of units needed to perform the activities. The total cost of each set of accessories was divided by their lifetime. The mean rate of exchange (14.75) between Mexican Peso / American Dollar was used. Finally, to obtain the cost (tools and accessories) per metric ton of compost produced, the annual output was employed. The list of accessories, equipment and the total and annual costs is showed in Table 1. The highest percentage of the costs obtained were 66.60% for the analytical equipment in comparison with the tools and protective clothing (21.35 and 12.04 %, respectively). Dividing the annual cost of the entire equipment: US$139.44, between the compost output: 18 metric tonnes, generate the cost per metric ton: US$7.75. The investment in tools and accessories is reduced in comparison with the man power cost (without machinery) obtained of US$43.66 per metric ton, and with the obtained cost for composting weaners and suckling pigs of US$8.54, and US$4.88, respectively. The costs of the equipment to monitor the degrading process is the highest, but, the use of it allows us to monitor properly the physicochemical changes that take place inside the piles, which is necessary to perform management changes. The precise knowledge of the costs allows the investment of financial resources to adopt the composting process in-site in order to strengthen the biosecurity rules and so avoid the spread of pathogens into the environment.

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Keywords: Composting costs, swine mortality

Table 1.- Total and annual costs for equipment

Accessories	Total cost (US$)	Annual cost (US$)
Plastic container (200 litres)	19,1	1,91
Plastic hose with spray gun	30,5	6,11
Brushes	9,2	9,20
Garden rake (metalic)	7,6	1,53
Stainless steel shovels	22,9	4,58
Trolley	64,9	6,49
Protective mask	6,1	3,05
Protective gloves (plastic)	13,7	13,74
Thermometers	114,5	22,90
Hygrometer	26,7	8,91
pH meter	152,7	30,53
Electrical conductivity meter	152,7	30,53
TOTAL	620,61	139,44

Degradation of Swine Residues by Composting: Use of Bioassays

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Swine farms generate organic residues which impact the environment if they are not managed properly. Composting transform them in forms with high bioavailability to plantas and crops, however, during the initial phase of the process, intermediate products, as ammonium salts, are generated. These products are toxic for animals living in soil if the immature compost is added as an amendment. Measuring direct or indirectly the presence of nitrogen forms is the key strategy to know the maturity of a heap of compost. One compost pile of 116 kilograms was built with sawdust, wastewater solids of a pig farm and mature compost in a rate of 1:1:1 (weight:weight:weight). Was added tap water to adjust the relative humidity (RH) in 60%. Additionally, were co-composted, two pig heads. A composite sample was conformed from samples obtained (n=16) in specific zones inside the pile in days 0, 14, 28, 42 and 56 of the initial mixture. Additionally, were monitored in these samples, the pH (CONDUCTRONIC pH10;USA) and the electrical conductivity (EC; HANNA;USA). To demonstrate the toxicity of compost during the early phase of the process, were used 6 mature Eisenia andreii earthworms, which were randomly assigned to one of two treatments. Treatment 1 (T1) consisted in addition in plastic boxes of 50 grams (g) of mature compost (MC) plus 200 g of composite sample. T2 or control received just 250 g of MC (45 days of composting plus 45 days of vermicomposting). The earthworms were counted and weighted in days 7 and 14. The results showed a high mortality of the earthworms living in the 14 days compost sample. After this time, the mortality was not seen in the next assays (Table 1). The pH trend along the time showed an increase just in the compost with 14 days of process, after this time, the pH decrease to a neutral level. The EC begin to increase in the 14 days sample, afterwards, the level was maintained to the final of the trial. The results indicate the presence of ammonium salts as factors affecting the viability of the worms during the early stage of the degrading process. After this period, during the nitrite formation, and ammonia assimilation by microorganisms, the survival increased, due to the presence of N forms free of risk to them. The apparently better survival of worms of T1 in 42 and 56 days samples was a natural process due to de depletion of nutrients in MC. So, the above results induce to continuing with additional research to recognize the precise forms of N evolved inside a compost heap.

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Keywords: Ammonium salts, composting, degradation

Table 1.- Survival test and characteristic of composite samples

			Days of composting		
	0	14	28	42	56
Worm survival (%)					
Pig residues compost	100 a	0 a	100 a	100 a	100 a
SD	0	0	0	0	0
Control compost	100 a	100 b	100 a	66.6 b	66.6 b
SD	0	0	0	16.66	16.66
Physicochemical characteristics					
pH	7.2	8.0	6.9	6.8	6.6
SD	0.02	0.00	0.01	0.00	0.01
EC (Us/cm)	441.3	590.3	568.0	658	630.6
SD	9.01	21.45	27.87	31.09	7.09

a, b. Superscripts indicate statistically significant differences in columns. ($p<0.05$) t-test. SD = Standard deviation.

Study of Isolates of *Mycoplasma Hyopneumoniae* Lesions in Lungs and Bronchial Explants

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Porcine enzootic pneumonia caused by *Mycoplasma hyopneumoniae* continues to produce significant economic losses to swine producers. *Mycoplasma hyopneumonia*e is also considered to play a primary role in the porcine respiratory disease complex (PRCD).3,4 The gold standard for detection of Mycoplasma hyopneumoniae has been culture of the organism., isolation of the organism is difficult due to its requirement specialized media (Friis medium), slow growth properties and lack of experience and capabilities to perform culture diagnosis of swine tissue samples (Kurth et al., 2002; Thacker, 2006). Polymerase chain reaction has an inherent advantage over other diagnostic methods because it is ideally suited for detection of fastidious organisms such as Mycoplasma. The purpose of this work was to grow *M. hyopneumoniae* by the "classical" method and compare them with bronquial explants by Polymerase Chain Reaction (PCR). Thirty pneumonnic lungs were collected at a TIF (Type Inspection Federal) abattoir, based on typical lesions of Mycoplasmal infection in its acute phase. The four anterior lobules were examined and tissue samples were collected for culture. The Friis Medium was prepared in liquid and solid forms.2 Each sample was processed in the classical way and 0.5 ml of tissue was inoculated into 4.5 ml of Friis medium and identified as 102, further dilutions were made to 104. The alternate method was to inoculate a section of bronchus from each lobule, which had been previously dissected into 4.5 ml of liquid medium identified as a 102 dilution and each inoculated tube was carefully starred. All inoculated samples were incubated at 37°C for 7 days. Afterwards all of the previouly inoculated lung samples were inoculated into solid Friis médium, incubated at 37°C, with 5% CO_2 for 5 to a 6 days.1 The suspect colonies were prepared for PCR identification using the specific primers for M. *hyopneumoniae.* The growth of these microorganisms is extremely difficult. For this reason it is usual to add inhibitors such as Penicillin and Thallium Acetate. Since the biochemistry of these bacteria is irregular, they are identified by serologic techniques. The most common are the growth inhibition test and immunofluoresence. The PCR is a more recent development for a rapid identification of micoplasma colonies.

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Keywords: Bronchial explants, lungs, mycoplasma hyopneumoniae, porcine enzootic pneumonia

Table 1. Relation of isolations of Mycoplasma hyopneumoniae from the 30 samples of lung tissue compared to the bronchial explants.

Method	Apical right lobe	Apical left lobe	Cardiac right lobe	Cardiac left lobe	Accesory lobe
Lung tissue	30/19	30/17	30/15	30/19	30/8
Bronchial explant	30/23	30/22	30/21	30/24	30/11

Samples tested/positive cultures.

A Potential Nanoparticle Formulation as Inhibitors of PRRSv: Glycyrrhizinic Acid in Aqueous Solutions and its Effect on Replication on MARC 145 Cell Cultures

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Since the late 80´s, the porcine reproductive and respiratory syndrome (PRRS) has been the cause of important economic lost all over the world due to the lack of an efficient treatment. Nanoparticles are structures that can carry drugs so that they can reach organs or even cells of interest. Glycyrrhizinic acid (GA), a saponin from licorice root has been used as anti-inflamatory, anti-ulcer, anti-tumor, etc., for many years1. It has also antiviral activity against several viruses. The aim of the research was to study the effect of GA solutions on uninfected and PRRS virus-infected cells in culture. An attempt of testing loaded nanoparticles with GA on cells was also explored. MARC cells were maintained in RPMI medium at 37 ºC and 5% CO2. PRRS strain VR 2332 was a gift from Laboratorios Avi-Mex, S.A. de C.V. For cytotoxicity determination, when confluence was reached 100 µl of the sterile solutions of GA (1-30 mg/ml and 0.1-0.9 mg/ml) were added. Daily observation for 144h was done. At the end, trypan blue staining (TB) and MTT assay were performed. Inhibition of the cytophatic effect was evaluated as follows: 100 µl of virus (106 TCID50/ml) in RPMI were added to confluent cells. The plate was kept at 37 ºC for 1 h. After this time, the cells were treated with GA solutions at concentrations 0.1-0.9 mg/ml and maintained for 144 h. Viability and selectivity index were calculated. The simultaneous assay of virus-infected cells treated with GA was performed on cells that previously were in contact with virus (101-108 TCID50/ml); GA solutions (0.1-0.9 mg/ml) were added to the cells. Virus titer was calculated daily using the Reed & Muench method. Nanoparticles were obtained by the microemulsion method5. Once sterilized, they were tested on cells previously infected with virus 101-108 TCID50/ml, at a GA concentration of 0.54 mg/ml. Observation of the culture was performed daily during 72 h and TB staining was applied at the end of the assay. Viral titer decreased two logarithms compared to that obtained in the control viral titration (without GA treatment). EC50 was determined at 0.5 mg/ml. Although the selectivity index (CC50/EC50) was relatively low (1.73), GA showed reduction of PRRS replication in vitro. After testing nanoparticles, TB dye exclusion results indicated an inhibition of virus replication (viability of 98-99%) compared to control infected cells (60% viability) after 72 h. Although GA exhibited a low selectivity index, it showed reduction of PRRS replication in vitro. It can be concluded that GA is active, though not selective against PRRSv. The higher viability observed in cells treated with GA loaded nanoparticles; suggest that the drug exerted its effect and that these carriers reached the inner space of the cell. Formation of needle like structures was observed at 24 h of the assay, they could be the result of precipitation of the drug. Nanoparticles can be used as potential carriers of GA with the probably advantage of reaching anatomic organs as lungs. We are conducting further assays to have more evidence at this respect.

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Keywords: Glycyrrhizinic acid, nanoparticle, PRRS

Susceptibility of Rat and Human Neuronal and Glial Cell Lines to Porcine Hemagglutinatig Encephalomyelitis Virus Infection

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Porcine Hemagglutinating Encephalomyelitis Virus (PHEV) is a member of the betacoronavirus, which causes encephalomyelitis or vomiting and wasting disease (VWD) in suckling piglets. It was reported that PHEV 67N, rodents adapted strain, infected to rodent's nerve cells and the virus spreaded from peripheral nerve cells to the CNS by neural routes. In the field of neuroscience, neurotropic viruses have been used as neurotracer for single neuron-tracing studies. In this study, we examined the susceptibility of the neuronal and glial cell lines derived from rat and human to PHEV infection in order to evaluate the utility of PHEV as a neuronal tracer. U251-MG (human glioblastoma), C6 (rat glioblastoma, differentiate to astrocytes), NB-1 (human neuroblastoma), PC12 (rat adrenal pheochromocytoma, differentiate to sympathetic neurons), and FS-L3 (porcine kidney cell line) cells were inoculated with PHEV strain 67N or ONS204 (non rodent adapted strain). Morphological change of these cells were observed and the supernatants were sampled over time. The growth curves of PHEV in each cell were made from the results of titration of collected samples. To detect the viral antigens in PHEV inoculated cells, indirect fluorescent antibody technique (IFA) was also done. S glycoprotein gene, that bind with a cellar virus-receptor, of 67N and ONS204 strains were sequenced. There were not apparent CPE on the glial cell lines, U251-MG and C6, and the human neuronal cell lines NB-1 after PHEV inoculation. Similarly, PHEV propagations were not observed and viral antigen in these cells were not detected by IFA. On the other hand, 67N strain propagated in PC12 cells and the virus titer of culture supernatants increased. Viral antigens were detected in PC12 cells inoculated with 67N strain by IFA, although obvious CPE was not observed. However, we could not obtain the evidences of infection and propagation of ONS204 on PC12 cells. There were 21 amino acid differences in S glycoprotein (1349 amino asids) between 67N and ONS204 strain. Our results indicated that PHEV could not infect to the glial cells or human neuronal cells. These results supported the utility of PHEV 67N strain as a neuronal tracer because 67N strain could not infect to the glial cell lines and could infect only rodent's neuronal cells selectively. Additionally, it was suggested that 21 amino acid differences observed in S glycoprotein affected the viral adsorption to rodent neural cells.

Keywords: Glial cell, neural cell, porcine hemagglutinating encephalomyelitis virus, susuceptibility

Comparison of Three Diagnostics Methods of *Mycoplasma Gallisepticum* in Batna Governorate (Algeria)

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Mycoplasma gallisepticum (MG) is the most pathogenic avian Mycoplasma; however, strains may differ markedly in virulence. Because of the multiplicity of pathways of transmission of the pathogen, early detection of new infections is essential. For a long time, control programs were based on use of techniques with internationally accepted standardization: Serum plate agglutination (SPA), hemagglutination inhibition (HI) and ELISA are the most common serological techniques. Direct diagnosis requests isolation and identification of the agent in selective culture media or the demonstration of the DNA of the pathogen in the host using the polymerase chain reaction (PCR). The aim of this study is to compare the effectiveness of three methods for detection of MG by the PCR, culture and serology (SPA)for the detection and differentiation of MG infection in order to highlight the best techniques from those below. In this study, the technical performance of culture, a commercially available polymerase chain reaction (PCR) test and rapid plate agglutination (SPA) test were compared for the detection of *Mycoplasma gallisepticum* infections from 18 birds. Results showed a high percentage of positive samples of both culture and PCR tests (72.22% and 63.63% respectively). SPA showed a less positive rate (61.11%). the utilization of SPA towards MG diagnosis is limited by its reduced specificity and the high incidence of false positives Contradictory to other studies, bacteriology was more sensitive than PCR. Several studies support strongly the use of the PCR as a technique for the diagnosis of Mycoplasma infections since this technique is more sensitive than serology and culture. This study showed that it is not advisable to rely completely on one test (system) only.

Keywords: Culture, diagnostic, mycoplasma gallisepticum, PCR, SPA

Influence of the Dietary Oregano Essential Oil and Attapulgite on Microflora Composition in Broiler Chickens

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Attapulgite is a layered magnesium aluminum silicate with high absorption properties of pathogenic bacteria and toxins, used in animal nutrition. *Origanum vulgare L. subsp hirtum* extract is widely used in broilers for its antibacterial activity as natural growth promoting substance. The aim of the study was to investigate the effects of oregano essential oil and attapulgite supplementation on performance and intestinal microflora of chickens. Thirty thousand broiler chickens (Ross-308) were divided into 2 groups (Control vs Blend) and reared for 42 days in a commercial farm (APFCA, Arta). Control diet was further supplemented with oregano essential oil 5% (Ecodiar® powder at 300 g/tn) and attapulgite 80% (Ultrafed® at 3kg/tn) blend. The composition of the microflora was determined at the day of slaughter. Intestinal samples were collected from the ileum and caecum of 18 broilers per diet. Total anaerobes, total aerobes, *Clostridium perfringens, Enterobacteriaceae, Enterococci, Lactobacilli spp. and Bifidobacteria spp.* were estimated by conventional microbiological techniques using selective agar media. Statistical analysis was performed by one-way analysis of variance using SPSS for Windows (Version 16, Chicago, USA). Duncan's multiple-range test was used, with differences considered to be significant at $P < 0.05$. Dietary supplementation with Ecodiar® powder and Ultrafed® improved significantly the average daily growth and the body weights (2.550g versus 2.355g) $p<0,05$ and the FCR (1,70 versus 1.81) $p <0,01$ and mortality rate (3,01% versus 3,62%) $p<0,05$. Bacterial counts proved that *Lactobacillus spp* were significantly lower ($p<0,05$) in the broilers fed the control diet in both ileum and caecum samples. Total aerobes were also significantly increased in the caecum ($p<0,05$) in the experimental Blend diet and Enterobacteriaceae were significantly higher in the caecum in the control diet (Tables 1&2) The present study showed that a dietary combination of oregano essential oil supplementation with attapulgite in broiler chicken exerted a positive effect on *Lactobacilli* populations in both ileum and caecum, with a concomitant enhancement in growth performance and feed conversion efficiency.

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Keywords: attapulgite, broiler chickens oregano essential oil, microflora composition

Table1. Influence of dietary oregano essential oil and attapulgite on ileum microflora composition in broiler chickens 42 days old.

Ileum	Control diet (n=18)	Blend diet (n=18)	SEM	P value
(cfu / g)				
Total aerobes	6.06 x 10^8	1.19 x 10^8	0.073	0.255
Total anaerobes	1.81 x 10^7	1.96 x 10^7	0.122	0.355
Enterobacteriaceae spp	2.98 x 10^7	7.14 x 10^6	0.107	0.216
Clostridium perfringens	1.50 x 10^2	4.33 x 10^1	0.082	0.850
Enterococci	7.50 x 10^6	1.55 x 10^7	0.062	0.760
Lactobacilli spp	3.86 x 10^6 a	2.88 x 10^7 b	0.102	0.005
Bifidobacteria spp	3.17 x 10^6	1.16 x 10^6	0.191	0.231

a,b: values in the same row with different superscript) differ significantly p<0.05

Table 2. Influence of dietary oregano essential oil and attapulgite on caecum microflora composition in broiler chickens 42 days old.

Caecum	Control diet (n=18)	Blend diet (n=18)	SEM	P value
(cfu / g)				
Total aerobes	1.02 x 10^6 a	1.82 x 10^7 b	0.123	0.005
Total anaerobes	5.20 x 10^7	4.37 x 10^7	0.129	0.314
Enterobacteriaceae spp	2.07 x 10^5 a	9.67 x 10^3 b	0.109	0.001
Clostridium perfringens	5.78 x 10^2	8.35 x 10^2	0.083	0.540
Enterococci	9.35 x 10^5	2.84 x 10^6	0.115	0.432
Lactobacilli spp	5.55 x 10^6 a	4.07 x 10^7 b	0.086	0.005
Bifidobacteria spp	1.09 x 10^6	1.50 x 10^6	0.134	0.121

a,b: values in the same row with different superscript differ significantly p<0.05

The Effect of Vit E and Thyme Oil on Meat Quality in Japanese Quails

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The objective of this study is to determine the effect of Vitamin E and Thyme Oil which is extracted from Thymbra spicata l. Var. Spicata on the quality of quail meat. Four different test groups were formed for the study. Animal material composed of 120 quails in total, each group including 15 male and 15 female quails. Thirty-five-day ad libitum feeding was applied in the test unit using feed including 22.1% crude protein and 12.6 Mj/kg metabolizable energy. No additive was added to the feed of one of the test groups, while 200 mg/kg Vit E (VE) and 400 mg/kg Thyme Oil (TO) and 100 mg/kg Vit E + 200 mg/kg Thyme Oil (VE+TO) was added to the feed of the other groups. Four male and 4 female chicks were slaughtered at 35th days of feeding and carcass weights and some meat quality characteristics, including pH, water-holding capacity, cooking loss, color, malondialdehyde, dry matter, crude ash, ether extract, crude protein, and fatty acids were determined. Although no differences among the treatment groups were defined carcass weight, pH, water-holding capacity, cooking loss, dry matter, crude ash, ether extract, crude protein, TBARS and color (except for a* values in 7th day) in the brisket, there were differences hot carcass weight ($P<0.01$), water holding capacity ($P<0.05$), color for L* at 1st day and b* 7th day ($P<0.05$) in sex. Between the 1st - 4th days, while the malondialdehyde level of the meats of the treatment groups were similar, same parameter in control groups at 4th day were detected significantly higher than 1st day ($P<0.001$).On the other hand, treatment had influence on MUFA ($P<0.01$), PUFA ($P<0.05$) and AI ($P<0.01$), but sex had only effect on NV ($P<0.05$). As a result of the experiment showed that the efficacy of VE and TO on oxidative stability was depended on passing time after slaughter. On the other hand, it was determined that carcass weight, pH, WHC, cooking loss and chemical composition of meat were not affected from VE and TO supplementation to feed. The diet supplemented with VE significantly affected long change fatty acids especially 18, 20 and 22 carbon fatty acids. Relationship with this change, MUFA, PUFA and AI values of breast fillets altered to control group.

Keywords: Japanese quail, meat quality, thyme oil, vit E

Massive Application in Matanzas of the Inactivated Vaccine PARAMIX in Pigeons

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The PARAMIX vaccine was developed by LABIOFAM Entrepreneurial Group for the prevention of Paramyxovirus in Pigeons. Given the satisfactory results of quality control of the vaccine according to international regulations, mass vaccination was conducted in the province of Matanzas. The aim of this study was to evaluate the results of vaccination applied, over 4 weeks old, applying 0.5 mL subcutaneously in the thigh fold of pigeon from the Cuban Pigeon Association of Matanzas. A random samples of vaccinated birds prior to vaccination, one month and four months post-vaccination was realized. Also clinical examination was performed pre- and post-vaccination for the presence of clinical signs, reaction at the site of inoculation and serological survey was conducted by IHA test to determine the value of antibodies. As a result there were no clinical signs indicative of an adverse process by the application of the vaccine, or local damage at the site of inoculation. Antibody titers increased by an average of 5 Log2 with a significant increase at 4 months post vaccination, demonstrating the effectiveness of the vaccination, after one year of the competition schedule with no reports of the disease.

Keywords: Hemagglutination inhibition antibodies, paramyxovirus

Microsatellite Typing of *Aspergillus Flavus* from Clinical and Environmental Avian Isolates

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Aspergillosis is one of the most common causes of death in captive birds. *Aspergillus flavus* is the second most frequent organism associated with avian infections. In the present study, the fungi were grown from avian clinical samples (post-mortem lung material) and environmental samples (eggs, food and litter). Microsatellite markers were used to type seven clinical avian isolates and 22 environmental isolates of *A. flavus*. *A. flavus* was the only species (28 %) detected in the avian clinical isolates, whereas this species ranked third (19 %) after members of the genera *Penicillium* (39 %) and *Cladosporium* (21 %) in the environmental samples. Upon microsatellite analysis, five to eight distinct alleles were detected for each marker. The marker with the highest discriminatory power had eight alleles and a 0.852 D value. The combination of all six markers yielded a 0.991 D value with 25 distinct genotypes. One clinical avian isolate (lung biopsy) and one environmental isolate (egg) shared the same genotype. Microsatellite typing of *A. flavus* grown from avian and environmental samples displayed an excellent discriminatory power and 100 % reproducibility. This study showed a clustering of clinical and environmental isolates, which were clearly separated. Based upon these results, aspergillosis in birds may be induced by a great diversity of isolates.

Keywords: Aspergillus flavus, avian isolates, microsatellite typing

Effects of Oregano and Laurel Essential Oils on Growth Performance of Chickens, Intestinal Microflora Composition and Intestinal Morphology

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In this experimental study, a mixture of essential oils (EO) containing oregano EO 5% and laurel EO 0,5% was dietary supplemented to broiler chickens at the level of 500 mg/kg for 44 days to investigate the effects of this combination on growth performance, intestinal microbiota and intestinal morphology, as replacers of antibiotic growth promoters. Day-old broiler chickens were randomized into two groups of twelve thousand six hundred birds each. Control group received a basal diet only whereas the experimental group received the basal diet plus the mixture of oregano and laurel EOs. A typical wheat – corn – soybean meal diet was provided to meet nutrient requirements for starter (1-12), grower (13-24), prefinisher (25-36) and finisher (37-44) periods. Chickens had free access to water and feed. Body weight gain and feed to gain ratio was calculated for each fattening period and mortality was daily recorded. Total counts of aerobes, anaerobes, coliforms, *Enterococci*,*Enterobacteriaceae*, *Lactobacilli*, *Bifidobacteria* and *Clostridiumperfringens* w re enumerated by conventional microbiological standard techniques with selective agar media at the end of the trial at both jejunum and caecum. Also, evaluation of intestinal morphology was carried out in small intestine and caecum. Statistical analysis was performed by one-way analysis of variance using the IBM SPSS Statistics 20 statistical package. Tukey's range test was used, with differences considered to be significant at $P < 0.05$. The results of the trial showed that the group that received the mixture of the essential oils, although had better BWG and FCR at both starter and grower periods ($P<0.05$) this difference was only numerical at prefinisher and finisher period ($P>=0.05$) [281g *vs* 242g, 1495g *vs* 1405 g, 2001g *vs* 1968g, 2689g *vs* 2678 g and 1.01 *vs* 1.12, 1.44 *vs* 1.49, 1.64 *vs* 1.67 and 1.81 *vs* 1.83 for experimental vs control group, respectively). Total mortality was lower ($P<0.05$) in the experimental group compared to control group (2,71 *vs* 4,99). Bacterial counts were similar among the two groups except for the *Lactobacilli* counts that were higher ($P<0.05$) in the experimental group compared to the control group at both jejunum and caecum. Evaluation of intestinal morphology revealed no significant differences among the experimental groups at both small intestine and caecum and no clinical signs and macroscopical lesions were noted at both groups; however the histopathological evaluation revealed mild inflammatory reaction in the control group at the ileal lamina propria. In conclusion, the results showed that the mixture of oregano and laurel EOs could enhance growth performance and improve intestinal microflora composition. Acknowledgements: This research has been co-financed by the European Union (European Social Fund – ESF) and Greek National Funds through the action Green Poultry "COOPERATION 2011" of the NSRF 2007-2013 Operational Programme "Competitiveness and Entrepreneurship" General Secretariat for Research and Technology, Ministry of Education.

Keywords: Broilers, intestinal microflora, intestinal morphology, laurel, oregano

Effects of Oregano Essential Oil, Laurel Essential Oil and Attapulgite on Chemical Composition and Lipid Oxidative Stability of Chicken Breast and Thigh Meat

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The objective of this experimental study was to investigate the effects of a mixture of oregano essential oil and attapulgite and a mixture of oregano and laurel essential oils on the lipid oxidative stability of breast and thigh meat under refrigerated storage. Attapulgite is a layered magnesium aluminum silicate with high absorption properties of pathogenic bacteria and toxins, used in animal nutrition. *Origanum vulgare L.*subsp *hirtum* essential oil is widely used in broilers as a source of antibacterial and antioxidant compounds, while laurel essential oil of *Laurus nobilis* plant is a source of potent antimicrobial compounds. One day old broiler chickens (Ross-308) were divided into three groups of twelve thousand six hundred birds each (3 replications of 4.200 birds per group). The control group was fed the commercial diets (starter, grower, prefinisher, finisher) based on corn and soya bean meal. The second group (OR-AT) received the control diets further supplemented with a Ecodiar® powder (containing 5% oregano essential oil) at 300 g/tn and Ultrafed® (containing attapulgite 80%) at 3kg/tn. The third group (OR-LA) received the control diets further supplemented with Panaroma® powder (containing 5% oregano essential oil and 0,5% laurel essential oil) at 500 g/tn. All birds were reared for 44 days in a commercial farm (Pindos, Ioannina, Greece). Feed and water were offered to birds ad libitum. At the end of the trial, all birds were slaughtered under commercial conditions, their carcasses were processed, samples were taken from each replication and stored at -20ºC for further analysis. From each sample, breast and thigh meat were analyzed (FoodScanTM Lab, FOSS Denmark) for moisture, fat and protein content. Moreover, lipid oxidation determined as malondialdehyde (MDA) was measured in breast and thigh meat stored at 4^{o}C for 1, 5 and 8 days. Statistical analysis was performed by one-way analysis of variance using using the IBM SPSS Statistics 20 statistical package. Tukey's range test was used, with differences considered to be significant at $P < 0.05$. The results of this investigation revealed no differences ($P>0.05$) on moisture, fat and protein content of breast or thigh meat from the three experimental groups. The measured MDA levels after 1 day of refrigeration showed that the control groups had significantly ($P<0.05$) lower MDA in thigh meat compared to the other experimental groups, but not on the breast meat. Nevertheless, after 5 and 8 days of refrigerated storage no significant ($P>0.05$) difference were noted between the three groups for both breast and thigh meat. According to the results, the use of oregano essential oil, laurel essential oil and attapulgite did not affect the chemical composition and the oxidative stability of breast and thigh chicken meat. It was noticed that MDA levels were reduced at the 8th day compared to the previous measurements in all groups. Further studies could elucidate the potential effects of the examined substances, as well as the underlying synergistic or antagonistic mechanisms.

Acknowledgements: This research has been co-financed by the European Union (European Social Fund – ESF) and Greek National Funds through the action Green Poultry "COOPERATION 2011" of the NSRF 2007-2013 Operational Programme "Competitiveness and Entrepreneurship" General Secretariat for Research and Technology, Ministry of Education.

Keywords: Attapulgite, chicken breast, laurel essential oil, lipid oxidative stability, oregano essential oil, thigh meat

Evaluation of the Protective Efficacy of a Commercial Vaccine against Circulating H9N2 Influenza Viruses in Chickens in Korea

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Since 2007, Korea has used inactivated H9N2 low pathogenic avian influenza vaccine to control the disease. Despite the long-term vaccination programs, H9N2 avian influenza viruses (AIVs) continue to persist in chicken populations, mainly in Korean native chickens of unvaccinated flocks and live bird markets. We evaluated the protective efficacy of a commercial vaccine against recently circulating H9N2 AIVs in chickens. Challenge experiments using 7 vaccinated chicken groups indicated a commercial vaccine did not prevent completely viral shedding in both oropharynx and cloaca. Unlike the other viruses, A/Korean native chicken/Kr/LBM074/2014(H9N2) replicated in cecal tonsils of two among eight chickens. Our study indicated that commercial vaccine has low protection efficacy against circulating H9N2 AIVs in Korea. Therefore, we should regularly evaluate the vaccine protection efficacy and consider updating the vaccine strains.

Keywords: Chicken, H9N2, influenza, Korea, protective efficacy, vaccine

Effects of Dietary Supplementation of Bromelain on Antioxidant Status in Laying Hens

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Bromelain is a protein extract derived from pineapple (Ananas comosus) and contains various components such as proteinases, peroxidises, phosphatises, and protease inhibitors. It is organically bound calcium (1). In vitro and in vivo experiments revealed its therapeutic properties including suppression of malignant cell growth, thrombus formation, inflammation, and dermatological debridement (2,3). The objective of the present study was to investigate the effect of bromelain on antioxidant capacity in laying hens. Ninety-six 28-wk-old laying hens were assigned randomly to diets supplemented with 0, 0.15, 0.30, or 0.45 g per kg diet. The experiment lasted 8 weeks. At the end of treatment period, plasma samples were collected to determine levels of glutathione (GSH), malondialdehyde (MDA) and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-s-transfrease (GST) using the colorimetric assay. Results: All hens fed the bromelain-supplemented diet had higher antioxidant activity. Increasing bromelain level in the diet lineraly decreased level of MDA level and increased activity of SOD, GSH-Px, and GR ($p < 0.05$). Responses of GSH level and GST activity were in quadratic manner (Table 1). Conclusion: Our data showed that dietary supplementation of bromelain improved the antioxidant activity in laying hens. Bromelain has the potential as functional feed additive in birds subjected to stressors.

Keywords: Antioxidant activity, bromelain, lipid peroxidation

Table 1.Levels of MDA and GSH and activities of SOD, GSH-Px, GST and GR in laying hens fed diets supplemented with bromelain.*

Bromelain (g/kg)	MDA nmol/mg protein	SOD U/gr protein	GSH µmol/mg protein	GSH-Px µmol/mg protein	GR µmol/mg protein	GST µmol/mg protein
0	133±4a	209±4c	0.80±0.08d	9.28±0.39d	14.09±0.68c	2.70±0.16c
0.15	120±4b	255±3b	3.50±0.10a	13.76±0.43c	14.90±0.65c	5.17±0.20a
0.30	81.9±2.5c	257±3b	2.20±0.07b	15.39±0.50b	17.49±0.56b	5.13±0.20a
0.45	57.3±1.6d	284±5a	1.87±0.08c	19.78±0.75a	25.02±0.72a	3.80±0.29b

**Different superscripts within the same columns differ ($p < 0.05$).*

Effects of Dietary Supplementation of Hesperidin, Quercetin and Naringin on Antioxidant Status in Laying Hens

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Flavonoids are phenolic substances isolated from a wide range of vascular plants (1). They are found in the body of the plant, the leaves, bark, flowers, and root (2). Flavonoids have many positive effects on human and animal health and act as antioxidant, antiviral, antimutagenic, antiinflammatory, anticancer, antibacterial and antiallergenic agents (3). Free radicals are produced excessively in the state of stress or aging, which results in structural abnormalities and dysfunction of the cell and mitochondrial membranes. This in turn affects performance of animals, even leading to the occurrence of the disease (4,5). The purpose of this study was to evaluate the effects of supplementation of diet with hesperidin, naringin, and quercetin on antioxidant status in laying hens. A total of 96, 28-week old Lohmann White strain laying hens were fed a basal diet and the basal diet added with quercetin, hesperidin and naringin at a rate of 0.5 gr/kg. Experimental groups were replicated in 6 cages, each containing 4 hens. The study period was 8 weeks. At the end of treatment period, plasma samples were collected to determine the levels of glutathione (GSH) and malondialdehyde (MDA) and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-s-transfrease (GST) using the colorimetric assays. Results: The flavonoids decreased MDA level and increased GSH level and GSH-Px, GR and GST activities ($p< 0.05$) as compared to the control group. It appeared that quercetin was the most effective flavonoid. Laying hens were responsive to flavonoids as reflected to improvement in antioxidant capacity. Among flavonoids, quercetin was superior to naringin and hesperidin. Flavonoids can be considered as feed addtives in hens exposed to stressors.

Keywords: Antioxidant activity, Hesperidin, laying hens, naringin, quercetin

Table 1. The effect of flavonoids on oxidative status parameters.*

Group	MDA nmol/mg protein	GSH µmol/mg protein	GSH-Px µmol/mg protein	GR µmol/mg protein	GST µmol/mg protein	SOD U/gr protein
Control	119.07 ± 2.88a	1.39 ± 0.04d	29.60 ± 2.12c	5.99 ± 0.38c	2.22 ± 0.14b	247.43±7.30b
Hesperidin	96.87 ± 2.68b	2.51 ± 0.13a	122.18 ± 4.37b	14.07 ± 0.60b	4.63 ± 0.16a	263.47±7.58b
Naringin	80.93 ± 2.07c	2.03 ± 0.07b	136.17 ± 6.78b	15.69 ± 0.55a	4.54 ± 0.24aa	253.52±5.67b
Quercetin	75.13 ± 1.70c	1.66 ± 0.09c	156.67 ± 6.86a	13.63 ± 0.62b	4.24 ± 0.22a	294.56±6.13a

**Different superscripts within the same columns differ ($p < 0.05$).*

Can Dietary Cumin Supplementation Alleviate Heat Effects on Growth Traits and Some Physiological Responses of Broiler Chickens Reared Under Algerian Summer Conditions?

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This study was conducted to determine the effect of a dietary supplementation with cumin (Cuminum cyminum) on growth performances and some physiological parameters (respiration rate, body temperature and blood haematology) of broiler chickens subjected to natural fluctuation of Algerian summer ambient temperatures between 28 and 49 days of age. A total number of 440 28-d old chickens (mixed sexes) were divided into 2 groups (5 replicates of 44 birds) with similar body weight (971g±48): a “Control” group fed with a standard diet adapted to the age and a “Cumin” group receiving the same basal diet supplemented with 0.2% of cumin. From 28 to 49 d of age, the 2 groups were reared under an average diurnal ambient temperature of 30°C±1 and an average relative humidity of 58%±5. Feed and water were provided ad libitum. In our conditions, dietary cumin supplementation did not significantly modify the growth rate and final body weights of heat-exposed chickens (2303g±62 vs. 2264g±69 at 49 d of age) but it slightly reduced feed intake (-12%; $p=0.07$), thus significantly improved feed conversion ratio (-12%; $p<0.05$). Furthermore, heat-exposed chickens supplemented with cumin exhibited a significantly ($p<0.01$) lower values of serum hematocrit and hemoglobin concentration than those of control group. Also, this additive significantly increased level of panting (+9%; $p<0.001$) at 49d old of broilers and modify the cloacal temperature of the same broilers.

Keywords: Broiler, cumin, dietary supplementation, heat stress

Effect of Purslane Ethanolic Extract on Blood Lipid Parameters and Antioxidant Status of Broiler Chickens

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Domestic animals are frequently exposed to oxidative stress, especially in intensive breeding systems. Excessive production of reactive oxygen species (ROS) can be stimulated by stressful conditions, causing oxidative damage to lipids, proteins, and nucleic acids. In general, ROS are produced continually by aerobic cells and are instantly removed by antioxidant scavengers such as natural and synthetic antioxidants and the antioxidant enzymes present in the biological system. Oxidative stress refers to a lack of balance between production of ROS and the level of antioxidants, which results in oxidative alteration of biological macromolecules. Food plays a vital role in upholding the oxidative system, and most of the antioxidants come from either food or the gut microbiota. Portulaca oleracea L. (commonly called purslane) is an herbaceous weed widely distributed throughout the world. It was reported that purslane is an excellent source of melatonin and other bioactive substance. Melatonin has a variety of important functions including direct free radical scavenging and indirect antioxidative actions via its stimulation of antioxidant enzymes. Therefore, the aim of the present study was to evaluate the effect of purslane extract on plasma lipid concentration and blood antioxidant status of broiler chickens. One hundred and ninety two 1–day old broiler chicks (Ross 308) were allocated randomly in 4 groups with 4 replicates and 12 chicks per replicates to receive diets supplemented with 0 (control), 100, 200 and 300 ppm of purslane extract for 42 days. All diets were isocaleric and isonitrogenous and offered ad libitum to all groups. Four chicks from each treatment were bled to determine plasma lipids. Eight chicks from each group were bled via brachial vein on day 42 for measuring blood antioxidant status such as total antioxidant activity (TAA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA). The results of this experiment showed that, purslane extract did not influence plasma lipids concentration such as Triglyceride (TG), HDL-c and LDL-c when compared with those in the control group (P>0.05). Total cholesterol was affected by inclusion purslane extract to the diets (P<=0.05). Dietary purslane extracts had no significant effect on TAA and SOD activity compared with the control diet (P>0.05). MDA concentration and GPx activity significantly reduced by inclusion of purslane extract in diet when compared with the control group (P <= 0.05). On the basis of the above results, it can be concluded that purslane is a promising natural product, which could be useful for the prevention of oxidative stress in broilers.

Keywords: Antioxidant status, broiler chickens, purslane extract, plasma lipid

Table 1 - Effects of different levels of purslane extract on blood parameters concentration of broilers at 42 d (mg dl-1)

Treatments	Glucose	Triglyceride(TG)	Cholesterol	HDL-c	LDL-c
Control	200.56	22.326	94.228ab	60.422	29.341
Pur1 (100ppm)	209.29	24.344	96.527a	71.882	19.776
Pur2(200ppm)	208.94	27.008	86.814b	62.478	18.934
Pur3(300ppm)	201.27	24.752	90.544ab	65.448	20.106
SEM	3.691	1.098	1.606	3.320	2.863

HDL-c: high density lipoprotein- cholesterol; LDL-c: low density lipoprotein- cholesterol Means in the same column with different superscripts differ significantly (p<=0.05). SEM: Standard error of means

Table 2 - Effects of different levels of purslane extract on antioxidant status of broilers at 42 d

Treatments	TAA(mmol g-1 Hb)	GPx(U g-1 Hb)	SOD(U g-1 Hb)	MDA(µmol g-1 Hb)
Control	0.092	102.84a	271.70	0.137a
Pur1(100ppm)	0.109	70.85 b	319.48	0.076b
Pur2(200ppm)	0.092	68.66 b	291.19	0.069b
Pur3(300ppm)	0.095	65.20 b	286.67	0.066b
SEM	0.007	5.301	12.390	0.008

TAA: Total Antioxidant Activity,GPx: Glutathione Peroxidase, SOD: Superoxid dismutase, and MDA: Malondialdehyde. Means in the same column with different superscripts differ significantly (p<=0.05). SEM: Standard error of means

Evaluation of Protective Immune Response against Fowl Typhoid in Chickens Vaccinated with an Attenuated Strain of *Salmonella* Gallinarum

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Salmonella enterica subsp. *enterica* serovar Gallinarum biovar Gallinarum is an avian host-specific biovar that causes fowl typhoid in wild birds and poultry. The outcome of the infection is marked by high morbidity and up to 80% mortality of naturally infected birds. The onset of the disease in experimentally infected chickens starts at 4 days post infection (dpi). The prevention of this disease in commercial poultry flocks worldwide is based on biosafety measures and vaccination. Despite many efforts to control, outbreaks of this disease are reported to World Organisation for Animal Health (OIE) every year. Live vaccines against *Salmonella* Gallinarum are commercially available and frequently used in commercial layer hens. However, the role of each immune mechanism involved in the protective immune response against *Salmonella* Gallinarum still needs better elucidation. The cell mediated immune response (CMI), especially by CD4 and CD8 T cells is important and may eliminate intracellular bacterial infection. The humoral immune response (IgY and IgM) in chickens may inhibit high burden of bacteria in the bloodstream after systemic invasion. In the present work we aimed to evaluate the CMI and humoral immune response of commercial brown layer-hens vaccinated with a live attenuated*Salmonella* Gallinarum strain in comparison with unvaccinated chickens, before and after challenge with wild *Salmonella* Gallinarum 287/91 strain. Chickens were vaccinated at 25 days of age and challenged at 45 days of age. The sampling was done at 15 and 1 days before challenge and 1, 3 and 7 dpi. Mortality rates were recorded until 28 dpi. After challenge mortality in unvaccinated group started after 6 dpi and reached 86.7%, whilst vaccinated animals showed no clinical symptoms or mortality. The bacterial numbers in cecal tonsils reached 5.17 cfu/g and 5,47 cfu/g in the liver (log10) at the 7th dpi in unvaccinated chickens. The highest bacterial numbers of the challenge strain in vaccinated hens was 2.83 cfu/g in liver samples at the 7th dpi. The quantification of effector CD4 T cells (CD4+CD44+CD28+) and memory CD8 T cells (CD8+CD44+CD28+) cells in spleen and cecal tonsils by flow cytometry showed a depletion of effector CD8+ T cells in the spleen of unvaccinated layer-hens in the 3rd and 5th dpi. Meanwhile, vaccinated birds showed increasingly higher numbers of both population of effector lymphocytes (CD4 and CD8). A fast humoral immune response marked by constantly high IgM and increasing IgY (ortholog to IgG in mammals) titer after challenge was noticed in vaccinated layer-hens before and after challenge, whilst unvaccinated chickens showed a slow and weak humoral immune response, with low IgM titer after challenge. No mortality was recorded in the vaccinated group and the bacterial numbers of the challenge strain decreased faster in the infected tissues (cecal tonsils, spleen and liver), demonstrating a protective immune response conferred by the live vaccine. Overall, the attenuated strain of*Salmonella* Gallinarum protected chickens against fowl typhoid, reducing the bacterial numbers and the mortality caused by the pathogenic challenge strain. The flow cytometry revealed that a depletion of CD8 T cells in the unvaccinated group may be responsible for the uncontrolled bacterial multiplication in the tissues, which did not occur in vaccinated chickens.

Keywords: ELISA, FACS, immunoglobulin, lymphocytes, poultry, vaccine

Clostridium Perfringens in Poultry Slaughterhouse

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Brazilian poultry industry is the world's third largest producer, behind only USA and China, with 12.31 million tons produced, according to 2014 data. The consumption and production of chicken meat was increased in last decades, this cause a concern with human health due the transmission of harmful pathogens, such as *Clostridium* spp., *Escherichia coli*, *Salmonella* spp., etc. Nevertheless, proper hygiene in the slaughterhouse could inhibit this transmission. The aim of this study was to identify the presence of *Clostridium perfringens*, in the slaughter line and poultry slaughterhouses equipment's by PCR. The microbiological analyses of 144 samples collected on the slaughter line were processed by opening intestines, removed its contents and with a disposable loop, was done scraping of the mucosa. For the equipment, were collected 70 samples using sterile swabs that were placed in tubes containing BHI broth. After both collects in slaughterhouse, the samples were transported on ice until the laboratory. Then, these samples were plated by pour plate method on RCA agar and incubated at 37 for 48 hours under anaerobic conditions with GasPak® system. All samples were submitted to DNA extraction by boiling method and subsequently subjected to PCR with specific primers. *Clostridium perfringens* was detected by the amplification of specific band for cpa gene responsible by toxin. Then, the positive samples for cpa gene, were subjected to multiplex PCR amplification for *cpa*,*cpb*, *etx*, *iap* and *cpe* genes to detected the types of *Clostridium perfringens*. From an amount of 214 samples, only two were positive for *cpa* gene in a bacterial identification by PCR, one in slaughter line and one on equipment. The Clostridia positive samples were subjected to multiplex PCR for detecting type. The multiplex PCR showed that the *C. perfringens* were type A. This microorganism is part of the natural intestinal poultry microbiota, but becomes a problem if it occurs in carcass contamination by inadequate evisceration. If the hygiene during slaughter and meat processing is performed following the standards established by the Brazilian legislation, there is a greater chance of security of inhibition of this pathogen and its transmission to humans.

Keywords: Aviculture, molecular biology, pathogens

The Dietary Use of Botanicals as Natural Anthelmintics in Poultry

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Parasitism presents a main threat to the poultry production worldwide, inflicting heavy production losses in livestock. Gastrointestinal helminthes are the most devastating parasites in the different poultry production systems, with the nematode *Ascaridia galli* being the most prevalent species. This parasite can cause subclinical or clinical parasitism. In heavily parasitized birds the clinical signs include droopiness, hemorrhages and diarrhea, decreased weight gain, retarded growth, and decreased egg laying. Accordingly, these infections result in serious economic losses, associated with the treatment cost, the decreased feed efficiency, and the poor egg and meat production. Also, during the past few decades the indiscriminate use of some antiparasitic drugs has generated several cases of resistance in helminthes. The severity of the associated risks of chemical residues in poultry products and the high cost of treatment compliance in endemic regions, necessitate further efforts into the discovery of novel ways to combat helminthes. Moreover, nowadays consumer demands have focused on natural and ecofriendly approaches in livestock production. Thus, attention has been drawn to botanicals with possible anthelmintic activity both *in vivo* and *in vitro*. Botanicals have been traditionally used throughout history to treat human and animal diseases. Such plants can synthesize a wide variety of phytochemicals, i.e. secondary plant metabolites, with bioactive substances such as alkaloids, polyphenols, glycosides and terpenes. Many of these plants such as aloe, citrus, pomegranate, and ginger have substances with direct anthelmintic effects. They can disrupt the life cycle of helminthes through various possible mechanisms: interfering with cell glycoproteins and binding free proteins (phenolics, tannins); paralyzing the central nervous system, interfering with local homeostasis and glucose metabolism (alkaloids); others not adequately identified. Furthermore, the phytochemicals can offer various supportive beneficial effects for the parasitized animal: digestive, antimicrobial, anti-inflammatory, stimulating and immunomodulating. Botanicals can be incorporated in animal diets, usually dried and ground, although more often their active substances are separated and used in the form steam distilled essential oils or non-aqueous solvent extracts. Several up-to-date studies have demonstrated the anthelminthic efficacy of different botanicals and the use of phytogenic bioactive compounds for poultry nematode control is increasing in different commercial production systems. Additional extensive studies are needed to identify the actual active components, to clarify the underlying mechanisms of action and their possible interactions, and to quantify the optimal doses. In conclusion, the anthelmintic properties of plants represent a very promising natural alternative solution to overcome current treatment inadequacies and drug resistance, and to maintain high levels of poultry health and productivity, under commercial or domestic conditions.

Keywords: Ascaridia galli, botanicals, bioactive compounds, helminthes, poultry

Development and Evaluation of Tetra-PCR for Differential Diagnosis of *Mycoplasma gallisepticum* and *M. synoviae*

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Avian mycoplasmosis, caused by *Mycoplasma gallisepticum* and *M. synoviae*, is one of the economically significant diseases of the commercial poultry flocks. So far, even in developed countries, the poultry farmers could not eliminate the disease completely from the commercial flock because of its chronic mode of infection. There is always a need of quick and authentic method of diagnosis and confirmation. Among the available methods, PCR remains one of the most reliable and sensitive tool as compared to culture and serology. In this study we have developed Tetra primer PCR assay for the simultaneous detection of *M.gallisepticum* and *M.synoviae* along with the identification of any other mycoplasmas. We targeted the 16s rDNA for primer designing and evaluation. The PCR was optimized by incorporation of variable concentrations of each of the primer designed as Outer Forward & Outer Reverse and Inner Forward and Inner Reverse. The outer pair specifically amplified the 16s rDNA universal region of mycoplasmas while the Inner pair primer targeted the specie specific region. The optimum MgCl2 concentration was 2.5 mM, dNTPs 200 µM and the optimum annealing temperature 56°C. The sensitivity of outer pair of primer was comparable to other universal mycoplasmas primers which could detect the DNA up to pico grams while the Tetra PCR (using all four primers) sensitivity was up-to 100 cfu/mL. The specificity of the designed primers as assessed by the sequencing of the amplified product confirmed that the primers specifically anneals to the target species sequence. The mycoplasmas was detected after 10-12 hours of inoculation of clinical specimen in broth which made possible the positivity of culture in a very short span of time. Analysis of clinical samples submitted for diagnosis indicated sensitivity and specificity of the developed PCR test were 90% and 100% respectively.

Keywords: TETRA-PCR, M.gallisepticum, M.synoviae

The Branching of the Aortic Arch in the Great Cormorant (Phalacrocorax Carbo)

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The Great cormorant Phalacrocorax carbo is one of the five species belonging to the Phalacrocoracidae. This study was aimed at determining the vascular architecture of the aortic arch in the great cormorant. For this purpose, the heart arteries of one great cormorant were evaluated. The latex injection method was used to observe the branching of the aortic arch. Two brachiocephalic trunks were arising separately from the base of aortic and these arteries were giving to the common carotid and subclavian arteries. the right common carotid stated the ventral of cervical while the left common carotid placed the dorsal of cervical. the axillary arteries were originating from the subclavian arteries firstly and then divided into two branches. One of the thin branches arising from the subclavian artery was the sternoclavicular artery, which was in turn dividing into a sternal and a clavicular artery supplying the thoracic inlet and pectoral muscles. The thickest branch of the subclavian artery was the thoracic artery, which was dividing into internal and external thoracic arteries. It is hoped that this study will enhance morphological data on exotic birds since the reports on species-specific vascular morphology in wild birds are insufficient and lacking in detail.

Keywords: Branching of aortic arch, cormorant, great cormorant, phalacrocorax carbo

The Arteries Originating from the Aortic Arch and the Branches of These Arteries in White-Headed Duck (Oxyura Leucocephala)

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White-headed ducks (Oxyura leucocephala) are the only stiff tail Oxyurinii native to the Palearctic. White-headed ducks can also be found in parts of the Oriental region and the Ethiopian region. The largest populations of white-headed ducks are found in Russia, Kazakhstan, Turkey, Iran, Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Armenia, and Mongolia. Central and east-Asian populations tend to be migratory while populations in Spain and North Africa are non-migratory. This study was aimed at determining the vascular architecture of the aortic arch in the White-headed ducks. For this purpose, the heart arteries of one White-headed ducks were evaluated. The latex injection method was used to observe the branching of the aortic arch. The brachiocephalic trunks branch off separately the ascending aorta almost immediately above the aortic valve. The first major arteries that run anteriorly from the brachiocephalic artery are the paired common carotid arteries that run anteriorly along each side of the cervical spine then two brachiocephalic arteries pass under the clavicular region and are then called subclavian arteries. subclavian arteries divided into two branches. One of the thin branches arising from the subclavian artery was the axillary arteries supplying the thoracic limb. The thickest branch of the subclavian artery was the pectoral artery. It is hoped that this study will enhance morphological data on exotic birds since the reports on species-specific vascular morphology in wild birds are insufficient and lacking in detail.

Keywords: Branching of aortic arch, white-headed duck, oxyura leucocephala

Food Poisoning (Salmonellosis) as a Public Health Problem through Consuming the Meat and Eggs of the Carrier Birds

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The present research endevour was made to investigate the Public Health impact of Salmonellosis through consuming the meat and eggs of the carrier's birds and to see the prevalence of Salmonella enteritidis and Salmonella typhimurium from poultry feed, poultry meat and poultry eggs and their role in the chain of transmission of salmonellae to human beings and causing food poisoning. The ultimate objective was to generate data to improve the quality of poultry products and human health awareness. Salmonellosis is one of the most wide spread food borne zoonoses in all the continents of the world. The etiological agents Salmonella enteritidis and Salmonella typhimurium not only produce the disease but during the convalescent phase (after the recovery of disease) remain carriers for indefinite period of time. The carrier state was not only the source of spread of disease with in the poultry but also caused typhoid fever in humans. The chain of transmission started from poultry feed to poultry meat and ultimately to humans as dead end hosts. In this experiment a total number of 200 samples of human stool and blood were collected randomly (100 samples of human stool and 100 samples of human blood) of 100 patients suspected from food poisoning patients from different hospitals of Lahore area for the identification of Salmonella enteritidis and Salmonella typhimurium through PCR method in order to see the public health impact of Salmonellosis through consuming the meat and eggs of the carrier birds. On the average 14 and 10 stool samples were found positive against Salmonella enteritidis and Salmonella typhimurium from each of the 25 patients fo each hospital respectively in case of suspected food poisoning patients. Similarly on an average 5% and 6% blood samples were found positive from 25 patients of each hospital respectively. There was a significant difference ($P< 0.05$) in the sero positivity of stool and blood samples of suspected food poisoning patients as far as Salmonella enteritidis and Salmonella typhimurium was concerned. However there was no significant difference ($P<0.05$) between the hospitals.

Keywords: Food borne disease, salmonell enteritidis, salmonella typhimurium, poultry meat, poultry eggs

The Effect of Mannan Oligosaccharide and Chitosan Oligosaccharide on Performance in Broiler Rations

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This study was conducted to investigate the effects of prebiotics, mannan oligosaccharide and chitosan oligosaccharide on body weight (BW), body weight gain (BWG), feed consumption (FC), feed consumption ratio (FCR) and carcass yield. A total of 120, one day old Ross 308 male broiler chicks were used in this study. The chick were fed with broiler starter (days 1-14) diet and grower (days 15-42) diet. Poults were assigned into three groups, with 4 replicates of 10 birds each. Treatment for each group consisted of: first group (control group) received basal diet without supplementation; second group received 100 ppm Mannan oligosaccharide (MOS); third group received 100 ppm Chitosan oligosaccharide (COS). The experiment lasted 42 days. The chickens were individually weighed at 0, 7, 14, 21, 28, 35 and 42 days. The end of experiment, the average body weights were measured as 2340.45; 2458.11 and 2325.38 g, respectively. Average body weights of chicks received MOS groups was 5.03% higher than the control groups as numerically. During the experiment (0-6 weeks) FCR were determined as 1.72; 1.65 and 1.72 kg, respectively. There were no statically significant differences among trial groups (P>0.05). At the end of experiment, the supplementation of MOS and COS did not effect BWG, FC, carcass yield of the broilers among three trial groups (P>0.05). Conclusions: The effect of prebiotics on performance on broilers has been known well for years. In this study, at the end of experiment, the supplementation of MOS and KOS to the diet did not have a beneficial effect on performance. This results may be due to the dose of MOS and COS used in this study. Therefore it is suggested to conduct progressive studies on this subject.

Keywords: Broiler, chitosan oligosaccharide, mannan oligosaccharide, performance, prebiotic

Eleven Years of Surveillance of West Nile Virus in Domestic and Wildlife Animals in Brazil

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West Nile virus (WNV) is an emergent pathogen in the Americas and the transmission network involves mosquitoes and birds, and horses and humans as incidental hosts. WNV is widely distributed in North and Central America, but the recent introduction in South America has focused attention on the spread of WNV across Southern American countries, like Brazil. Brazil is a tropical country with a large territory (8,514,215 km2), and is the biggest country in Latin America. More than ⅓ of this territory is covered by tropical forests or other natural ecosystems, providing ideal conditions for the existence and establishment of many arboviruses, which are held in a variety of zoonotic cycles. Brazil also has the largest herd of horses in Latin America and the third largest in the world. Thinking about these risk factors, the aim of this study was to capture wild and domestic animals in different places and biomes of Brazil between the years 2002 until 2013, to analyze the presence of antibodies and virus by serological and molecular tests, to understand how widespread WNV is disseminated in Brazil and how important our findings are to the public health and economic policy in the country. The seroprevalance was evaluated from 678 equids and 478 birds using a WNV-specific blocking ELISA, and the positive results were confirmed by plaque reduction neutralization tests (PRNTs). Molecular analysis by RT-PCR was performed on sera from 1241 healthy equids and on 63 macerates of brains from equids that died of encephalitis and had previously tested negative for other pathogens. We also tested swabs from 3.445 birds, sera from 24 bats and 11 jaguars. We identified WNV antibodies by ELISA in thirteen equids and five birds, and PRNT90 confirmed WNV positivity in four equid samples collected in 2009 in an area between the Amazon and the Pantanal regions. None of the ELISA positive bird samples were confirmed by PRNT90. Of the 4.784 samples tested by RT-PCR, only two were positive for the detection of WNV: a resident wild bird in the Pantanal region, middle west of Brazil, and a duck in the region of Maranhão, near the Amazon forest, in Northern Brazil. Despite all efforts to isolate and characterize this virus, we couldn't confirm these findings using next generation sequencing. WNV circulation is evidenced by this large scale survey even in the absence of detection of clinical cases, and shows that the virus is not spread throughout the country and probably will be confined in these regions where detected. As there are numerous ecological barriers in Brazil that interfere with their rapid widespread dissemination, but does not discard out the possibility that it will spread and become a new major problem for the economy and Brazilian public health. So, it's clear for us that an important active surveillance system in Brazil is necessary.

Keywords: Brazil, equid, serology, molecular biology, migratory birds, West Nile virus

Figure 1 - Sampling sites

Site of sampling and major Brazilian biomes. The major biomes of Brazil are indicated by different colours. The grey lines represent the borders of the Brazilian states.

West Nile Virus seroprevalence in equids and wild birds in Brazil

Location Geographical coordinates	Date of sampling	Species	Inhibition ELISA 3.112G	PRNT WNV titre	PRNT SLEV titre	PRNT diagnosis
Monte Negro – Rondônia state S 10°17'13" W 63°14'27"	06/15/2002	Equus caballus (Horse)	43%	80	160	Flavivirus
Teodoro Sampaio - São Paulo state S 22°22'70" W 52°25'66"	03/17/2002	Equus caballus (Horse)	49%	20	160	SLEV
Teodoro Sampaio - São Paulo state	03/19/2002	Equus caballus (Horse)	35%	40	160	SLEV
Teodoro Sampaio - São Paulo state	04/12/2002	Equus caballus (Horse)	39%	10	20	Flavivirus
Teodoro Sampaio - São Paulo state	01/18/2006	Equus caballus (Horse)	44%	320	1280	SLEV
Teodoro Sampaio - São Paulo state	02/06/2006	Equus caballus (Horse)	64%	160	320	SLEV
Nova Brasilândia - Mato Grosso state S 14º57'25" W 54º57'56"	04/27/2009	Equus caballus (Horse)	51%	80	20	WNV
Nova Brasilândia - Mato Grosso state	04/27/2009	Equus caballus (Horse)	60%	160	320	Flavivirus
Nova Brasilândia - Mato Grosso state	07/06/2009	Equus caballus (Horse)	32%	80	20	WNV
Nova Brasilândia - Mato Grosso state	09/21/2009	Equus caballus (Horse)	48%	80	10	WNV
Nova Brasilândia - Mato Grosso state	10/16/2009	Equus sp. (Mule)	42%	40	10	WNV
Juruena - Mato Grosso state S 10º19'05" W 58º21'32"	11/06/2009	Equus caballus (Horse)	30%	20	160	SLEV
Juruena - Mato Grosso state	11/06/2009	Equus caballus (Horse)	26%*	1280	640	Flavivirus
Pinheiro - Maranhão state S 2°31'22,64" W 45°05'33,23"	05/14/2010	Dendrocygna autumnalis (bird)	53%	< 20	< 20	negative
Ilha de Canela - Pará state S 00º46'54,77" W 46º43'44,90"	11/25/2008	Arenaria interpres (bird)	37%	< 20	< 20	negative
Parque Nacional da Lagoa do Peixe - Rio Grande do Sul state S 31°21'17,94" W 51°02'52,37"	11/19/2009	Sterna hirundo (bird)	41%	< 20	< 20	negative
Parque Nacional da Lagoa do Peixe - Rio Grande do Sul state	11/20/2009	Sterna hirundo (bird)	37%	< 20	< 20	negative
Parque Nacional da Lagoa do Peixe - Rio Grande do Sul state	3/26/2010	Sterna hirundo (bird)	34%	< 20	< 20	negative

*Inhibition values of >=30% are considered significant. *Doubtful between 25%. A serum sample was considered to contain antibodies to WNV if 4-fold greater than the corresponding WNV titre. ELISA: enzyme-linked immunosorbent assay; PRNT: plaque reduction neutralization test; SLEV: St Louis encephalitis virus; WNV: West Nile virus.*

Infected Vectors and Reservoir hosts in an Area of Transmission of Trypanosoma Cruzi in Western Mexico

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Chagas disease is one of the most important zoonoses in Mexico, where is considered that more than two million of people are infected by Trypanosoma cruzi, affecting also many species of wild and domestic animals. Objective: In order to know infection rates of vectors and reservoir hosts, it was carried out a study in an area of Western Mexico. Material-Methods: In an area of the South of the state of Jalisco, where ten villages with 6% of infected people by T. cruzi are sited, it was carried out a study on infection by T. cruzi on triatomines, wild and domestic animals. There were manually searched more than 100 human dwellings, once a month, along a year in order to collect domestic triatomines. Peridomestic and areas were searched with the aid of modified Noireau traps. Reservoir hosts were collected by using baited Tomahawk traps and were analyzed for T. cruzi infection by direct blood test, IHA, ELISA and Western Blot analysis. Results: Infection rate for the only triatomine species (Meccus longipennis) fond in the area was of 66.7% (n = 111). Twenty four opossums (Didelphis marsupialis) (32.4%, n = 74), thirty one rats (Rattus rattus) (35.2%, n = 88) and 17 (8.1%, n = 210) dogs were positive to the presence of T. cruzi. High infection rate on triatomines is compatible with those detected on reservoir hosts. A close relationship between triatomines and rats as well as between those insects an opossums can be assumed. Apparently, even when many triatomines are infected by T. cruzi, their peridomestic habits reduces vector-human and vector-dog contact, leading to have few infected people and dogs. However, special attention has to be focused on sylvatic animals, since they usually invade human dwellings, potentially carrying different T. cruzi strains, which could represent a high risk of disease to human and dog populations in the study area.

Keywords: Chagas, reservoir host, Mexico, triatomines, trypanosoma cruzi

Epidemiological Investigation of First Outbreaks of LSD in Turkey

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The aim of this study was to investigate the first introduction time of Lumpy skin disease virus in Turkey. The first LSD clinically suspected cattle were seen in Kahraman Maras and Batman Provinces on September 2013. After that, 8 samples including, skin lesion, blood sample and nasal discharge were examined by PCR test, according to OIE Manual (2010)in LSD Reference Laboratory, Pendik Veterinary Control Institute. 4 skin lesions, 3 blood sample and 1 nasal discharge sample were LSD positive. After first determination of the disease, epidemiological investigation was conducted in 3 villages, Elbistan County, Kahraman Maras province. The method of assessment was a combination of active disease follow up, examination of collected samples from diseased and suspected animals and questionnaire data collected from 5 dairy farms involving 235 animals. A questionnaire format was included individual bio data, husbandry system, animal movement, climate and season, presence of water courses; lead to an increase in populations of blood feeding arthropods and vector population, communal grazing and presence of animal market, biosafety measures, veterinary services, vaccination history etc. Collected samples from sick and suspected animals was examined by PCR test, according to OIE Manual (2010) Only 30 out of 235 animals were found to be positive for LSDV. Number of sick, exposed and died animals and morbidity and mortality rate for different 5 dairy farms. The first entry of LSD into our Country was at 2013. LSD outbreaks and epidemics were mainly associated with climate changes. These changes caused serious outbreaks and recently outbreaks highly affected countries (Israel, Egypt) that have been existing of disease. For this reason, implementation of control and prevention measure, vaccination and screening of LSD cases on field are very important to fight against LSD. In Turkey, LSDV outbreaks was emerged at the same time in different dairy herd in different village even Province (Kahramanmaras and Batman). Although, there was no animal movement, outbreaks was emerged in some herds. In addition, according to the information collected from the owner of the herds, all herds was located near the water courses (pond, stream, swamp, river etc.) and they had different husbandry system and communal grazing. For this reasons, it was concluded that cause of the first outbreak in Kahramanmaraş was LSD viruses transmitted by vectors. And main reason of entry of the disease in Turkey like other Country is uncontrolled animal movement and global climate change.

Keywords: Cattle, climate change, epidemiology, lumpy skin disease, outbreak, vector

Bakırköy Sheep and Goat Pox Vaccine (BK(LK53)V12) Efficacy Assesment and Overview on LSDV Outbreak Impact in Dairy Cattle in Kayseri Province in Turkey

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The aim of this study was assessment of the efficacy of Bakırköy Sheep and goat vaccine for controlling of Lumpy Skin Disease in field conditions and determine the risk factors for the occurrence of the disease. The study was conducted from May 2014 to December 2014 in Yahyalı County, Kayseri Province. The method of assessment was a combination of active disease follow up, examination of collected samples from disease and suspected animals and questionnaire data collected focused on 39 dairy farms involving 739 animals in 17 villages. A questionnaire format was included individual bio data, husbandry system. Collected samples from sick and suspected animals was examined by PCR test, according to OIE Manual (2010) Only 49 out of 739 animals were found to be positive for LSDV and 20 out of 49 positive animals were not vaccinated. 29 of this positive animals were vaccinated against LSD. 13 out of 29 sick animals infected 30 days after vaccination. Therefore, sick animals after vaccination were found only 1,75%(13/739). In this study, Bakırköy Sheep and goat vaccine efficacy was revelad against LSD in cattle. Predisposing factor for LSD in study population compared of breed specific morbidity, animal age and gender.

Keywords: Cattle, efficacy, LSDV, vaccine

The Investigation of Akabane Infection in Cattle and Sheep by Serological, Virus Isolation and Real Time RT-PCR

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This study aimed at virus isolation and detection of Akabane virus-RNA by real-time RT-PCR in the blood of cattle and sheep and tissue samples of aborted fetuses and cattle as well as detection of antibodies to Akabane virus in cattle and sheep sera. It was also aimed to perform sequence and phylogenetic analyses by using primers targeted to S segment of this virus. In this study; A total of 175 cattle and 50 sheep blood were collected from farms in the Marmara region. 50 cattle and 25 sheep lung, liver, spleen and blood samples were collected from Hadımköy and Tuzla slaughter houses by random-sampling. Also clinically suspected 25 cattle and 25 sheep aborted foetuses samples (liver, spleen, brain, lung and plecanta) were collected from Pendik Veterinary Control Institute and İstanbul University Veterinary Faculty Department of Virology. All samples were kept at -80ºC until use. Blood samples analysed by using a commercial competitive ELISA antibodies kit (ID VET, innovative diagnostics, France). Total RNA from tissue samples of aborted fetuses and samples of slaughter house were extracted using Rnaesy Mini kit (Qiagen Group,USA) and virus infected cell culture supernatant and buffy coats were extracted using the QIAamp Viral RNA Mini kit (Qiagen Group,USA) according to manifacturer's instructions. Improm II (Promega) kit was used for to obtain complementary DNA (cDNA) from RNA. Vero, BHK-21 and MDBK monolayer cell cultures were used for virus isolation and the samples were passaged 3 times in cells. The inoculated cells were observed daily for the occurrence of cytopathic effect (CPE). Antibodies to Akabane virus were detected in 17 cattle while none of the sheep was found to be positive for antibodies to Akabane virus. As a result of RT-PCR and Real Time RT-PCR 2 samples were found suspected. 25 cattle and 25 sheep internal organs of aborted fetuses, 2 suspicious samples and positive control virus were used for virus isolation. As a results of virus isolation; In all the studied samples except the positive control CPE wasn't detected. The results of this study indicate that Akabane disease exist in the Marmara region of Turkey with a considerable frequency. Therefore, preventive measurements like fly control should be carried out to protect cattle, sheep and goats. However; to demonstrate the prevelance of the disease in our country a multidisciplinary projects and large scale researches should be conducted. Further investigations are necessary to determine the genetic characterisation of Akabane virus circulating in Turkey. As shown in this study ELISA as a sreening test and Real Time RT-PCR which the confirmation of positive samples can be used safely.

Keywords: Akabane, cattle, isolation, realtime-RT PCR, serology

Bluetongue Outbreaks and Studies of Etlik Veterinary Control Central Research Institute in Turkey

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Bluetongue outbreaks between 1977 and 2014 and results of the projects of Bluetongue (BT) in Turkey are summarized in this study. The first reported outbreak of BT was in the west of the country, Aydın Province in 1977. Disease spread between 1977 and 1979 and became endemic in the provinces bordering the Aegean and Mediterranean Seas. The causative agent was isolated in samples from sheep and calves and was identified as bluetongue virus (BTV) serotype 4. The disease was controlled successfully by vigorous control measures (quarantining, animal movement control, disinfection, insecticide treatment and vaccination campaigns) in sheep in the western provinces. Attenuated BTV-4 vaccine, produced in Etlik Veterinary Control Central Research Institute (VCCRI), was used in the vaccination campaigns. Unexpected BT outbreaks occurred in Edirne, Manisa, Aydın, Denizli, İzmir provinces in 1999-2000. Serotypes were reported as BTV-9 and BTV-16 by the Institute for Animal Health (IAH) in Pirbright. Diagnosis was based on clinical findings, serological surveillance and virus isolation. "Investigation of Bluetongue Diseases and Culicoides species in Mediterranean Region" Project was conducted in sentinal cattle herds in 2007, it was aimed to reveal the risk of the Culicoides born diseases in Mediterranean area in Antalya, Mersin, Adana, Osmaniye, Hatay provinces also by early detection of Bluetongue virus and providing database. During the study, 3332 blood sera collected, 3109 of them were negative and 223 of them were positive for BT antibodies. Positivity rate was 6.3 %. 20 of defibrinated blood samples taken from the antibody positive animals were found positive by Antigen ELISA and RT-PCR. 4 isolate had been recovered by ECE and cell culture inoculations. In May, 2010, BTV 16 was recorded in Antalya, dignosed in Etlik VCCRI, by RT-PCR and virus isolation, confirmed and serotyped by OIE Reference Laboratory IZS Teramo Italy. The last cases were recorded in Trace Region, Kırklareli, Çanakkale and Balıkesir Provinces after occurence of BTV4 in Greece and Bulgaria. BTV positive samples were confirmed in BT Reference Laboratory Etlik VCCRI and serotyped as BTV4 with Real Time RT PCR. These results were also confirmed by Pirbright EU Reference Laboratory. The disease was controlled successfully by the measures described above. New Project “Important Vector-borne Viral Animal Diseases in Turkey: Diagnosis, Identification of Vectors and The Creation of the Early Warning System” started in 2015. Determination of the distribution, transmission and incidence of BT, EHD, BEF and Akabane arboviruses in Turkey and also revealing their epidemiology thus creation of the Early Warning System is intended with these Project in whole country. BTV 4 in 1977-1979, BTV 9 and 16 in 1999-2000, BTV 16 in 2010, BTV4 in 2014 were detected in the outbreaks. During the epidemic that affected Balkan countries in 2014, widespread and effective application of attenuated BTV4 vaccines, produced in Etlik Veterinary Control Central Research Institute (VCCRI), resulted in the prevention of the epidemic and the cases were restricted to three provinces. Results of the studies show that BTV have been circulating in hosts, possibly subclinicaly. Therefore effective serological, clinical and entomological control programmes have to be implemented. Revealing BT, EHD, BEF and Akabane arboviruses epidemiology, thus creation of the Early Warning System is intended with the new Project in Turkey.

Keywords: Bluetongue virus, BTV-4, BTV-9, BTV-16, 1977-2014, Turkey

Presence and Prevelans of Lumpy Skin Diseases in Responsible Province's Of Erzurum Veterinary Control Institute

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Lumpy Skin Disease (LSD), or Neethling, is an acute viral infection of cattle, caused by the capripoxvirus of the poxviridae family, and characterized by skin nodules. It is known that the disease is transmitted by Rhipicephalus appendiculatus and Amblyoma hebraeum ticks, and also by biting flies of the genus Culicoides. Accordingly, the disease is frequently seen in places close to sea level, with high annual average temperatures and in places where watercourses are plentiful. In Turkey, the disease was first seen in Kahramanmaraş, and subsequently identified in many southern provinces, especially Batman, Osmaniye and Hatay. In the northeastern region of Turkey, the disease was first identified in July 2014 by our Institute's Virology Laboratory in the village of Alaca in the Aziziye District of Erzurum Province. Shortly after the disease was observed in Erzurum it was identified in the provinces of Ağrı, Artvin, Kars, Erzincan and Gümüşhane, which lie within our area of responsibility. This study was carried out to determine the presence, prevalence and possible transmission routes of LSD in the provinces within our Institute's sphere of responsibility. Between July 2014 and February 2015, 200 suspected LSD samples taken from cattle from provinces in the area of responsibility of our institute, were examined by the conventional gel-based PCR method. Positive control samples used in the PCR test were provided by the Pendik Veterinary Control Institute's Sheep and Goat Pox National Reference Laboratory. Nasal swabs, blood, lungs, liver, skin and skin nodules were used material for testing, and 108 of the samples were found to be positive. In general, the disease is most commonly observed during the summer when the vectors are active. However, our study found 42% (25/59) prevalence of LSD between October 2014 and March 2015, which suggests that this disease might have been transmitted by other vectors, such as the ticks of the family of Argasidae, known as winter ticks. In this study LSD was found for the first time in our region. Further detailed studies will make an important contribution to identyfing the source of diseases. Monitoring programs should be done to determine of the diseases prevelance and which vector responsible for transmission of the virus in cold region and high altitude such as Erzurum, Kars, Ağrı.

Keywords: Lumpy skin disease, PCR, Erzurum, Turkey

Studies on West Nile Virus Activity in Bodrum Mumcular Village, Fethiye and Bursa Karacabey in Turkey

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West Nile virus (WNV) is a zoonotic mosquito-transmitted arbovirus belonging to the genus *Flavivirus* in the family of *Flaviviridae*. Surveillance for WNV activity demonstrate that the virus range has dramatically expanded including North, Central and South America as well as Europe and Turkey. These studies were carried on in Bodrum Mumcular Village, Fethiye and Bursa Karacabey. There were suspected human cases in Mumcular Village and Fethiye, WNV Lineage 2 positive horse was detected in Karacabey. Virus circulating in animals and vectors was examined in these regions. First study was carried out in Bodrum Mumcular Village and Fethiye in June, 2014. One hundred and five serum samples were collected from different animal species and tested for the presence of WNV IgG and IgM antibodies by ELISA. 3 CDC light traps were placed in these regions and microscopic identification of collected vectors was done. In August, 2014, a 9 year old mare was euthanized after showing severe neurological signs in Karacabey, Bursa region. WNV was detected in the brain tissues with Real Time RT PCR. In an attempt to define the viral circulation area and identify the potential vectors involved, serum samples were also collected from 27 horses living in the outbreak surrounding area and 7 CDC light traps were placed close to the infected stable. WNV IgG and IgM antibody were investigated in sera samples with ELISA, vectors were tested for WNV with real time RT-PCR. In Bodrum Mumcular Village and Fethiye, a total of 69 mosquitoes were caught by 3 CDC light traps. They included *Culex pipiens* (94%) and *Anopheles plumbeus* (6%). While WNV IgM antibodies were detected in none of the tested sera, WNV IgG antibodies were detected in 8 sera samples: 3 from horses, 3 from turkeys 1 from donkey and 1 from chicken. In August 2014 WNV was detected in the brain tissues with Real Time RT PCR, confirmed as WNV Lineage 2 with Real Time RT-PCR and done Phylogenetic analyses by OIE WNV Referans Lab in IZS Teramo. Phylogenetic analyses of 290bp of NS3 coding region clustered the Turkish strain together with the 2010-2012 Greek isolates. The field study was done after the positivity detected in Bursa Karacabey, a 13 of 27 horse sera showed WNV IgG antibodies, in 2 of them detectable levels of IgM were also revealed. The mosquito catches included 290 *Culex pipiens* (58%), 154 *Ochlerotatus caspius* (31%), 50 *Culex theileri* (10%) and 5 *Anopheles hrycanus*(1%). No WNV RNA was detected in pools of *Cx pipiens* (n=2), *Oc caspius* (n=2), and *Cx theileri* (n=1). The results of the study shows that WNV has been circulating in Turkey, humans and animals will be more exposed to the virus and the WNV cases will increase in the near future. For the prevention of future deaths and economic losses; further investigation of WNV virus activity in vectors and hosts, improvement of effective vaccination, treatment studies and setting up an early warning system should be implemented.

Keywords: Lineage 2, vectors, Turkey, West Nile Virus, WNV

Prevalence of Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) Infection in Feral Cats in Seoul, Republic of Korea

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel bunyavirus and a causative agent of an emerging disease, the sever fever with thrombocytopenia syndrome (SFTS). Based on the risk analysis of SFTS infection, rural habitat and ownership of free-ranging domestic animals have been identified as important factors for SFTSV infection in addition to seasonality and age range. Recent studies showed seroprevalence of SFTSV using ELISA in various domestic animals, including cattle, goats, chickens and dogs, with goats and cattle. Currently we are lack of data about SFTSV infection in animals, including identification of host species functioning as main reservoir, potential host range and pathogenesis/clinical signs in infected animals. In this study we attempt to investigate the prevalence of SFTSV in feral cats that inhabit in Seoul, Korea. In many large and modernized cities, feral cats are known to live in high population densities, utilizing various anthropogenic resources. Considering the habitat sharing of feral cats, human and other domestic animals in urban area, identifying the potential role of feral cats in SFTSV circulation is critical to provide information for management of public health and feral cat welfare. This study tested serum samples of feral cats from highly urbanized habitat, Seoul, Korea to determine the infection to SFTSV. Serum samples were collected through animal hospitals performing Trap-Neuter-Return (TNR) within the Seoul city. Blood samples were collected in serum separation tubes. We performed one-step RT-nested PCR to amplify S segment of the SFTS viral gene using SFTSV genome-specific primer sets. 126 samples were collected from seven districts (Guro-gu, Geumcheon-gu, Seongdong-gu, Mapo-gu, Dongdaemoon-gu, Yongsan-gu, Gangnam-gu) within the Seoul city. Out of 126 specimens, we were able to detect RNA sequence of SFTSV in 22 (17.5%) samples through molecular analysis. Out of 22 positive samples, 10 samples were identified as male and 11 as female individuals. Four positive were from juveniles and rest of 17 samples was adults. Sex and age of a positive individual from Guro-district was unidentified. According to the phylogenetic tree, subset of sequences acquired from this study was clustered with sequences identified in China, whereas the others were closer to sequences from Japan. Our result provides data that SFTSV may be circulating in settings that have been suspected to have relatively low risk, such as highly urbanized habitat. Thus it warrants further study to investigate the potential impact of SFTSV in urban-dwelling host species in addition to humans.

Keywords: feral cats tick-borne pathogens, severe fever with thrombocytopenia syndrome virus (SFTSV)

Surveillance of Tick (Acari: Ixodidae) and Severe Fever with Thrombocytopenia Syndrome Virus in Korea National Parks in 2013-2014

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Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV) which is in the Phlebovirus genus in the Bunyaviridae family, and reported in central and northeast China, southern Japan and Korea. The objective of this study was to determine prevalence of SFTSV infection in ticks from natural environments, especially National Parks in Republic of Korea (ROK). Ticks were collected from July to October 2013 and April to July 2014 in 4 national parks (Odaesan, Gyeryongsan, Naejangsan and Mudeungsan) by dragging and sweeping methods. Ticks were separately collected by on trails and non-trails (10~30 m into the forest). One-Step RT-nested PCR was performed for amplification of SFTS virus from ticks. Genomic DNA sequencing of the amplicons were performed for phylogenetic analysis. In total, 3,949 ticks (2,232 *Haemaphysalis longicornis*, 619 *Haemaphysalis flava*, 1,060 *Haemaphysalis* spp. larvae, 30 *Ixodes nipponenesis*, 4 *Ixodes* spp. larvae and 4 *Amblyomma testudinarium*) were collected from 68 sites (2013) and 64 sites (2014) chosen from 4 national parks. The mean value of minimum field infection rate (MFIR) of SFTS virus in 4 national parks was 0.84%. For each park, MFIRs were 1.65% for Odaesan, 0.66% for Gyeryongsan, 0.88% for Naejangsan and 0.46% for Mudeungsan. Total 2,909 ticks were collected on trails from 4 national parks and mean value of MFIR of SFTS virus was 1.0%. MFIR of SFTS virus on trails was 2.61% for Odaesan, 0.66% for Gyeryongsan, 1.4% for Naejangsan and 0.58% for Mudeungsan, respectively. Total 1,040 ticks were collected from non-trails in 4 national parks and mean value of MFIR of SFTS virus was 1.04%. MFIR of SFTS virus was 3.17% for Odaesan non-trails, 0.71% for Naejangsan and 0% for Mudeungsan, and no collection on non-trail in Gyeryongsan. Two genotypes of SFTS virus were found. One was similar with the strains from Chinese isolates and the other type was different from Chinese and Japanese strains. SFTS virus were detected in all 4 national parks and found two genotypes of the virus from *H. longicornis* and *H. flava* ticks.

Keywords: Ixodidae, tick-borne pathogens, severe fever with thrombocytopenia syndrome virus, national parks

Tick (Acari: Ixodidae) Surveillance on Goats, Cows, Wild Boars and Habitats Near Animal Farms

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Ticks are notorious vectors of various pathogenic protozoa, rickettsiae, bacteria, and viruses that cause serious and life-threatening illnesses in humans and animals worldwide. Recently, *Anaplasma, Ehrlichia, Borrelia, Theileria, Babesia*, tick-borne encephalitis virus, and sever fever with thrombocytopenia virus infections in ticks, wild and domestic animals and/or humans have been reported to exist in the Republic of Korea (ROK). The purpose of the present study is to provide basic information about tick species related to their reservoir animals and habitats in ROK. Ticks were collected from goat, cow and wild boars and the habitats near grazing goat and cow farms. Ticks from habitats were collected by flagging and sweeping methods at mountain areas near animal farms and picked by a fine forceps from animals. Ticks were stored in 15 ml tubes with one grass leaf to maintain the humidity until transporting to the laboratory. Ticks were identified species and developmental stages following taxonomic identification keys using the stereomicroscopy. In total, 2,215 hard ticks (*Haemaphysalis longicornis, Haemaphysalis flava*) were collected near the animal farms from 8 provinces and Jeju-island in Korea. In each 8 provinces and Jeju-island, more than 200 hard ticks were collected. Ticks were collected from cattle, goats and wild boars. 209 *H. longicornis* were collected from cattle and 569 *H. longicornis* were collected from goats. 213 *H. longicornis* were collected from wild boars, followed by *H. flava* (57), *Amblyomma testudinarium* (35), *Ixodes nipponensis* (1). More than 2,000 hard ticks were collected in 8 provinces and Jeju-island in Republic of Korea and *H. longicornis* were collected 10 times more in number than *H. flava*. Only one species (*H. longicornis*) were collected from cattle and goats, but 4 species (*H. longicornis, H. flava, I. nipponensis,* and *A. testudinarium*) were collected from wild boars.

Keywords: Cow, goat, habitat, tick species, wild boar

Major Parasitic Zoonoses in Sfax -Tunisia

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Parasitic zoonosis is an important problem of public health in Tunisia. The aim of our study was to analyze the clinical, epidemiological and biological characteristics of the main parasitic zoonoses diagnosed in our laboratory. It was a retrospective study made during a period of 5 years (January 2007- December 2011). For the diagnosis of toxoplasmosis, we collected 26031 samples from pregnant women, 575 from patients who had adenopathy and 212 from immunosuppresssed patients. For hydaditosis, the diagnosis has been searched for 2187 samples. For leishmaniasis, we collected 617 samples for cutaneous leishmaniasis (CL), and 277 for visceral leishmaniasis (VL). Diagnosis was confirmed by the usual techniques. The toxoplasmosis: 70.3 % of pregnant women were seronegatives. The prevalence of evolutive toxoplasmosis during the pregnancy was 0.8%. The diagnosis of congenital toxoplasmosis was confirmed in 12 cases. We have also diagnosed 3 cases of cerebral toxoplasmosis in HIV patients and one case of primary toxoplasmosis in renal transplant patient. The hydaditosis: 928 have hydatidosis. The average age was 42.9%. Risk factors were present in 75.52% of cases. The cyst localizations were, mainly, hepatic (77.2 %) followed by the pulmonary localizations (16.6 %). The leishmaniasis: CL was diagnosed in 43.6% of cases. The average age was 31.8 years. The lesions were single in 38.8 % of cases and were predominant in the face in 40.1% of cases followed by upper lumbs (30.9%) and lower lumbs (24.8%). The ulcerated- crusted form was the most frequent clinical presentation (61.5%). Parasitic zoonoses deserve more efforts. They require an approach of public health coordination to epidemiological monitoring for the adoption of appropriates control measures.

Keywords: Hyditidosis, leishmaniasis, sfax, toxoplasmosis, zoonosis

First Report of Anaplasma sp. in Korean Native Goat (Capra Aegagrus Hircus)

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Anaplasma species are obligate intracellular pathogens causing tick-borne diseases in mammalian hosts. Clinical manifestations of the disease include hemolytic anemia, fever, weight loss, decreased milk production, abortion, and frequently death. To date, very few incidences of its occurrence in Korean native goats (Capra aegagrus hircus) in the Republic of Korea (ROK) have been reported. The objective of this study was to determine the prevalence of the infection in Korean native goats (Capra aegagrus hircus) and to perform molecular characterization of Anaplasma species circulating in the ROK. Whole blood samples from 39 Korean native goats on Jeju Island in the ROK were collected in April, 2014. All the cattle were clinically healthy and no ticks were found. DNA was extracted from the whole blood samples using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, USA). Anaplasma infection was assessed using the AccuPower® Rickettsiales 3-Plex PCR kit to detect species belonging to Anaplasma, Ehrlichia, and Rickettsia (Bioneer, Daejeon, Korea); this system amplifies a portion of 16S ribosomal RNA. The PCR products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, USA). The nucleotide sequences were determined by direct sequencing of the PCR product and a phylogenetic tree based on the nucleotide alignments was constructed using the neighbor-joining (NJ) method. The prevalence of Anaplasma infection was determined in a population of 39 total Korean native goats; blood samples from seven animals (17.9%) tested positive for Anaplasma by 16S rRNA gene-based PCR. None of the goats exhibited any clinical signs of illness. The sequence analysis from these seven samples could belong to be a potentially new species, Anaplasma sp. The phylogenetic tree revealed that our seven sequences are very similar to the species isolated from goats in China (EU709493 and FJ389576), which also belong to Anaplasma sp., and are clustered in the same group. These results indicate that new Anaplasma sp. is prevalent in Korean native goats in the ROK. To our knowledge, this is the first study to identify Anaplasma sp. infection in Korean native goats.

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Keywords: 16S rRNA gene, anaplasma, korean native goat, phylogenetic analysis

Epidemiological Survey of Anaplasma, Ehrlichia, and Theileria Infections in Korean Water Deer (Hydropotes Inermis Argyropus)

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Tick infestations in animals are of increasing concern, mainly because the epidemiology and geographical distribution of these infestations are constantly changing, due to climate change, abundance of wildlife animals as hosts, and management of environmental biodiversity. The objective of this study was to investigate the pathogens of TBDs in Korean water deer and to evaluate their roles as potential reservoirs for TBDs circulating in the ROK. The capture of wild Korean water deer and sample collection were performed with permission from the Wildlife Rescue Center located in the Chonbuk province in the ROK. Between March and June 2014, five animals were captured and whole blood samples were collected. Five more animals were killed in the traffic accidents, found dead and transferred to the Wildlife Rescue Center by local residents and their spleens were collected. DNA was extracted from the whole blood samples and spleens using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, USA). Diagnosis of Theileria infection was performed using an AccuPower® Theileria PCR kit (Bioneer, Daejeon, Korea). AccuPower® Rickettsiales 3-Plex PCR kit (Bioneer, Daejeon, Korea) was used to detect Anaplasma, Ehrlichia, and Rickettsia. The PCR products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, USA). The nucleotide sequences were determined by direct sequencing of the PCR product and a phylogenetic tree based on the nucleotide alignments was constructed using the neighbor-joining (NJ) method. The prevalence of TBD pathogens was analyzed from blood and spleen samples of ten Korean water deer by PCR. Rickettsia was not detected, whereas Anaplasma, Ehrlichia, and Theileria infections were detected in four, two, and eight animals, respectively. The most prevalent pathogen observed in Korean water deer was Theileria. Of the eight Theileria-positive animals, two animals were mixed infected with three pathogens (Anaplasma, Ehrlichia, and Theileria) and another two animals were mixed infected with two pathogens (Anaplasma and Theileria). Sequencing analysis was used to verify the PCR results. The pathogens found in this study were identified as Anaplasma phagocytophilum, Ehrlichia canis, and Theileria sp. This is the first report identifying these three pathogens in Korean water deer. Our results suggest that Korean water deer could serve as a major reservoir for these tick-borne pathogens and may lead to spread of tick-borne diseases (TBDs) to domestic animals, livestock, and humans. Further studies are needed to investigate their role in this respect.

Acknowledgements: This work was carried out with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ010092), Rural Development Administration, Republic of Korea.

Keywords: Anaplasma, ehrlichia, Korean water deer, theileria, tick-borne pathogens, reservoir

Seroprevalence of *Coxiella Burnetii* Among Horses in Korea

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Q fever is a rickettsial disease caused by *Coxiella burnetii*. Stillbirth, abortion and neonatal death are major cause of equine mortality and cause severe economic loss to the equine industry. In Korea, there are few reports concerning the distribution of disease in horses. The aim of the present study was to determine the seroprevalence of *C. burnetii* among horses reared in Korea. A total of 748 horses (*Equus caballus*) reared throughout Korea during 2007 to 2013. Blood samples were collected from the jugular vein of horses. For statistical analysis, data on the age, gender, breed, and region of sampling were recorded for individual horses. Samples were tested for the presence of specific antibodies to *C. burnetii* by using ID Screen® Q Fever Indirect Multi-species kit (IDvet, France), according to the manufacturer's instructions. When interpret the result, doubtful results were considered as negative. For statistical analysis, Chi-square and Fisher's exact tests were used. The data of the "unknown" group were disregarded in the chi-square and Fisher's exact tests. A $P<0.05$ was considered as significant difference. Among 748 samples, 11 (1.5%) samples were seropositive to *C. burnetii*. When the results were analyzed according to age, seropositivity was detected in 5 (2.4%) of 212 horses under the age of 5 years, 4 (1.7%) of 241 horses aged 5–10 years, 2 (1.0%) of 201 horses over the age of 10, and none of 94 horses of unknown age. According to gender, 3 (2.3%) of 131 male horses, 5 (2.0%) of 254 female horses, and 3 (1.1%) of 269 castrated horses tested seropositive. According to breed, all the seropositive samples were thoroughbred, and none of the positive samples were detected in Korean native pony, warmblood, and mixed breed. By region, 7 (3.1%) of 227, 2 (1.1%) of 184, 2 (0.8%) of 243, and none of 94 horse samples tested seropositive in northern, central, southern, and Jeju-do, respectively. Statistical significant difference was not observed for all variables. To the best of our knowledge, this is the first report to show the serological detection of *C. burnetii* among horses in Korea. Considering the zoonotic potential of *C. burnetii* and previous detection in various animals in Korea, continuous monitoring on *C. burnetii* among horses and detection using molecular method are needed.

Keywords: Equine, Q fever, rickettsial disease

Presence of *Trypanosoma Cruzi* Infection in Dogs in an Area of the Brazilian Amazon

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The aim of the study was to determine the occurrence of dogs naturally infected by Trypanosoma cruzi in four communities from São Domingos do Capim municipality, located in the northeastern region of Pará state, Brazil. Blood samples were collected from 113 dogs and 85.7% (30/35) of the serologically positive dogs had their blood re-collected after three months. The diagnosis of T. cruzi infection was performed by: fresh blood examination, hemoconcentration, hemoculture, besides the serological assays Indirect Immunofluorescence Test (IIFT) and Imunoenzimatic essay (ELISA). The occurrence of positive dogs in both serologic tests (IIFT + ELISA) was 31% (35/113), distributed in four communities as follows: (12/44) Uricuriteua, (19/40) Cezaréia, (1/16) Aliança and (3/13) Catita. None of the samples was positive in the fresh blood examination or hemoconcentration, althought was possible to isolate T. cruzi, DTU TcI in one dog sample during its blood re-collection. The proportions in the different areas, age and sexual gender were compared using the chi square method considered as non-parametric data, the Kruskal-Wallis test with 95% significance level for both tests. In the area studied was shown that dogs were exposed to T. cruzi transmission cycle, are infected with the parasite, but do not exhibit a profile with high parasitemia of infection and are not involved in amplification of the parasite population. In this study, these dogs can´t be considered T. cruzi reservoirs but play an important role as markers of presence of the parasite and reinforce the idea of monitoring these animals as sentinels in identifying areas at high risk of transmission. The T. cruzi transmission chain in resident areas in the Brazilian Amazon is far from being defined, because of the characteristics peculiar to each biome and hosts involved, this way, it is essential to study the various parasite hosts responsible for maintaining the transmission in different areas. These results indicate the exposure of dogs to T. cruzi transmission cycle, revealing its importance as the movement flags of this parasite in the area.

Keywords: Brasilian amazon, chagas disease, trypanosoma cruzi

The First Female Academicians in the Turkish Higher Education of Veterinary Medicine

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The earliest forms of biographies in veterinary medicine in Turkey were produced by Bekman. These studies were later conventionalized by the works produced by Prof. Erk and Prof. Dinçer, which have been further perpetuated by the researchers delving into the history of veterinary medicine. Today, there are studies which are overwhelmingly focused on women-related issues such as enabling the manifestation of women's existence throughout the history, women roles in the process of modernization, female solidarity, and the identity of a woman as a career woman. What is more, these studies have been published in a wide range of forms including periodicals, articles, books, survey articles, biographies, autobiographies, and other literary works. Moreover, to address these issues, both national and international congresses and symposiums have been instituted and a number of innovative institutions such as women's works libraries, associations, centers for research on women, and academic departments of women's works have been established. The aim of this study is to contribute to the studies on the history of veterinary medicine and to add valuable insights to the recently increasing body of research in women's history. To this end, brief biographies of the first women academicians in the field of veterinary medicine were investigated and included into this study. The essential materials of the study were the original documents available at Ankara University Veterinary Faculty Archives. To gain further insights, relevant books, articles, and official websites were utilized, and several interviews were conducted with the academic staff. The study only included the women who worked as tenured academicians in the early years of the faculties/institutes who were graduates of the department of Veterinary Medicine. On the other hand, the study excluded the women who were working innon-tenured or part-time positions, the ones substituting for tenured academicians, and the ones who attended a postgraduate study but did not hold a job as an academician. The universe of the study was formed with the first women academicians with retrievable records who had worked in the early years of the subfields of the five divisions at the Veterinary Faculties in Turkey which were operative during the year 2015. The data regarding these women were classified and their brief biographies were cited in the study. These data were chronologically formulated based on the initial date of entry to the faculty. To enhance the intelligibility of the tables, the current names of the subfields were used and footnotes were inserted for denoting the original version of these names and for explicating the essential points and brief information about the documents cited. The socio-economic and cultural reforms that took place in the early periods of Turkish Republic paved the way for women's education, and Merver Ansel was recorded as the first woman veterinarian in the Turkish history. The presence of women manifested itself in academic settings as well; in 1938, Abide Koray took the first step into academic settings by becoming an academician at the Institute of Internal Diseases. Following this, Ertürk; Physiology (1947), Erk; Deontology and History of Veterinary Medicine (1950), Tolgay; Parasitology (1950), Özgümüş; Microbiology and Food Hygiene and Technology (1951) and the other 15 woman academicians started their academic career, respectively. The results of this study revealed that although the first women started their academic career in Veterinary Medicine within a short period after they started their education at this department, they did not sustain their career and opted for the basic or pre-clinic studies in later years and have later started an academic career in the subfields of Veterinary Medicine–particularly in clinical disciplines–only recently.

Keywords: Biography, female, history, Turkey, veterinary medicine

Determining of the Relation of Virus-Cell Adaptation to Obtain High Titer 146s Particle of FMDV

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Fully adaptation of virus to cells for the production of high titer virus and cell cultures and to be passaged until a suitable titer is obtained following adaptation of the virus in cell culture. This study was performed to determine the appropriate number of passage number for the preparation of master seed virus which is isolated from the epithelium by using monolayer and suspension cell cultures. The study was also carried out to create the basis for obtaining high yield 146 particles in the vaccine virus production. For this purpose, BHK-21 An73 cell line as the cell culture and the O Tur 14 and the A Tur 15 virus isolates were used. When the BHK-21 An73 monolayer cell culture in the 2L rolling reached 90% confluency, they were inoculated with O Tur 14 ve A Tur 15 viruses at MOI of 0.04. At the end of 18-24 hour incubation period at 37oC infected cells were frozen and thawed three times. The adopted viruses at tenth passage obtained from the monolayer cell culture passed to BHK-21 An73 suspension cell culture and 5 passages were carried out. 146S particle yield and infectious titers of Foot and Mouth Disease (FMD) viruses produced in monolayer and suspended cell cultures were determined in each passage level. Totally fifteen passages were conducted in monolayer and suspension cell culture of BHK-21: At the end of the monolayers virus culture studies conducted with the O Tur 14 strain of FMD, while TCID50 titer and 146S amounts at the first passage were 106.02 / mL and 0.3 µg/mL,respectively, they were determined as 107.82 / mL and 1.38 µg/mL at the end of 5th passage. It was also seen that titers reached the highest level at 5th passage and they had no significant changes until the 10th. In the suspension cell culture passages carried out with the same virus strain, TCID50 titer and 146S amounts at the first passage were 105/mL and 0.35 µg/mL respectively, and they were 107.60/mL and 1.10 µg/mL at the end of 5th passage. At the end of the monolayers virus culture studies conducted with the A Tur 15 strain of FMD, while TCID50 titer and 146S amounts at the first passage were 106.54 / mL and 2.83 µg/mL, respectively, they were determined as 107.35 / mL and 4.45 µg/mL at the end of 5th passage. It was also seen that titers reached the highest level at 5th passage and they had no significant changes until the 10th. In the suspension cell culture passages carried out with the same virus strain, TCID50 titer and 146S amounts at the first passage were 107.07/mL and 0.10 µg/mL, respectively, and they were 108/mL and 1.78 µg/mL at the end of 5th passage. It was also found that while the incubation time was 20-24 hours at thirth monolayer passages of the virus culture it was reduced 16-18 hours in the last passage. It showed us that increased passage number resulted in decreased incubation time. To sum up, it was found very effective that adaptation of the field isolates that will be used in the FMDV vaccine production by passaging them in the monolayer and suspension cell cultures to obtain high titer of vaccine virus.

Keywords: Adapted virus, high titer, monolayer and suspended cell culture, round glass bottle, the incubation time, the passage

Determination of the Optimal Incubation Time of Obtaining High Titer 146S Virus Particles in Suspended Cell Culture

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Foot and Mouth Disease(FMD) 146S protein affects potency value of the vaccine suspension of FMD in a correct proportional.Therefore, 146S particle value of FMD vaccine is so important that it should be obtained higher quantity and higher potency of FMD vaccine production to combat disease. Purpose of FMD vaccination study production in cell culture after the inoculation of FMD virus determining the appropriate incubation time, was to achieve the highest level of 146S virus particles. For this purpose, 30 L volume of the bioreactor with the suspended BHK 21 30 cell culture, GMEM(Glasgow Minimum Essential Medium) with 10% TPB (Tryptose Phosphate Broth) 10% BAS(Bovine Albumin Serum) in medium 2-3 × 106 cells / ml so that the cell finally was incubated. At the end of the incubation period cells were separated from the medium by precipitation method not including sera GMEM added to the vaccine seed virus MOI(Multiplicity of infection, virus / cell) ratio of 0.04. Followed by inoculated virus 16.,19.,22.,25.,28. hours microscopic CPE formation was observed. Sucrose gradient centrifugation method and continuous flow using a combination UV spectrophotometer 146S quantities particles was detected as µg/ml. Following incubation A TUR-14, OTUR-07, Asia 1/TUR 14 FMD virus to 50-60% of the average of 16 hours, 19 hours, 70-80% was observed in 100% CPU 22. hours formation. At 22. hours, 146S particle quantities with the formation of 100% CPE was observed in the following way for each type:A virus particle the amount of 0.82 which virus particles in 22.hour for virus 25.hour 1.42; O virus particle the amount of 22 hours 1.36 for virus at 25. hours, It was 1.98 Asia 1 virus particles the amount of 0.36, which virus particles in 22. hour for virus 25. hours determined to be 0.89. For 25. hour onwards value of 146S not increasing was determined that occur. In conclusion, the results of the virus inoculation of the data obtained from a viral culture after interest 100% CPE, the culture and allow to stand for three hours, a high amount 146S particles will be obtained and the dose of vaccine have potency in high doses, therefore it was concluded to be produced.

Keywords: 146S, CPE, incubation, suspended cell culture, seed virus, titer

The Legislative Regulations on Veterinary Specialization Education in Turkey

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Veterinary specialization in Turkey was first delivered by the Turkish Military Academy of Veterinary Science in 1881, and the earliest forms of contemporary level of specialization were founded in the 1940s. The earliest regulation in the field of veterinary medicine was the Law No.3203, which was enacted in 1937. Based on this law, a specialization was launched for the veterinarians working for the Ministry of Agriculture by various Ministry-run institutions, the Higher Institute of Agriculture, and later in collaboration with veterinary faculties. To disseminate the specialization to all the veterinarians working for the Ministry, the first regulation for the specialization in veterinary medicine was enacted on May 6, 1942 by the Ministry of Agriculture, which was later followed by several other regulations. The aim of this study was to evaluate the regulations enacted for the veterinary specialization in Turkey through a historical perspective. The primary materials used in the study consist of the regulations published on the Turkish Official Gazette and the archive documents retrieved from the Department of History of Veterinary Medicine and Deontology of Ankara University. In addition, relevant books, journals, and articles were also reviewed. All the documents were placed in chronological order by using document analysis method. There are basically eight regulations which enacted by the Ministry (dated 1942, 1955, 1958, 1963, 1968, 1970, 1975, 1995, respectively). In these regulations, the education period was designated as two years only in the regulation dated 1958, as opposed to three years in the others. In the first regulation which was enacted in 1942, there were only nine veterinary specialties, which were raised to 20 in 1975, following the 33-year history of veterinary education. This improvement could be regarded as the indicator of a progress parallel to the scientific advancements and the governmental policies of the time regarding animal husbandry. These issues were first mentioned in the regulation enacted in 1963. The veterinary specialization education was centralized through the establishment of “Ankara University Veterinary Faculty Graduate School of Livestock Breeding and Health Sciences of Specialization” in 1965. This school also enacted several regulations regarding academic rules and assessment, and the specialization education in this school was carried out in collaboration with the Ministry. With this education, a total of 433 veterinarians working for the government, mostly including the ones working for the Ministry, specialized in veterinary medicine. Moreover, the credit and grading system implemented in this graduate school laid the foundation for the postgraduate education designated by the Higher Education Law No. 2547. However, the school was closed in 1982 and thus the major source of specialty education was abolished, thus terminating the catering of specialized veterinarians for the institutions such as veterinary health centers and institutes as well as stud farms. Much later, the Ministry enacted another regulation for veterinary specialization in 1995, but this one was suspended by the Council of State on grounds that it contradicted the Law No. 6343 due to not covering all the veterinarians. Subsequently, the specialization education was restarted in 2014, which covered all the veterinarians and thus could be accepted as a positive development. This study reviewed the regulations that have been enacted in the field of veterinary medicine and in this way it is likely to shed light on the evaluations having been made on the specialization in veterinary medicine throughout the history.

Keywords: Education, history, specialization, Turkey, veterinary medicine

One World One Health: Academic Training in Veterinary Medicine from Southeast Region of Brazil
One World One Health: Veterinary Medicine Education from Southeast Region of Brazil

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In the context of "One World, One Health" veterinarians become important actors helping to face the health-related challenges, required to consolidate the positions won by these professionals. However, there is in Brazil a shortage of skilled professionals working in public health, as there are deficiencies in veterinary medical education with devaluation of this area, giving priority to the curative model. Therefore, the purpose of this study is to evaluate the academic background of the veterinarian to act under the concept of one world one health. The curricular matrices of undergraduate courses in Veterinary Medicine of High Education Institutions of southeastern Brazil were evaluated. To analyze them, was considered the contents that are essential for the degree course in Veterinary Medicine, established by the National Curriculum Guidelines. They are separated in required courses in Human Sciences, Biological Sciences and Veterinary Medicine Sciences. The Veterinary Medicine Sciences include theoretical and practical knowledge related to health and disease, animal production and environment, with emphasis in the areas of Animal Health and Veterinary Clinical Surgery, Preventive Veterinary Medicine, Public Health, Animal Science, Animal Production and Inspection and Technology of Animal Products. Thus, 63.22% curricular matrices of Veterinary Medicine schools in the southeast region of Brazil were analyzed. The Human Sciences have a percentage of low workload, 5.8% when compared to the Basic Sciences (42.3%) and Veterinary Medicine Sciences (51.8%). The concept of "One World, One Health" requires for professionals with humanistic characteristics, understanding the importance of their profession for the communities, but it is less emphasized area. And the contribution of the Social Sciences field of disciplines would be more effective if its contents were covered in a manner that the students could correlate their applicability to Veterinary Medicine course, suggesting the interdisciplinarity as a mean to achieve that goal. Regarding the disciplines relating to the Veterinary Medicine Sciences, the average of working hours shows that the highest percentage is from Veterinary Clinic, with 55.90%, followed by 25.20% for Animal Science and Production, 11.32% for Preventive Veterinary Medicine and 7.59% for Inspection and Animal Products Technology. This shows that the area of the clinic is still considered more important, to detriment of public health, when in fact such content should be addressed in a well balanced way, giving to the student an incentive to interdisciplinary thinking and raising awareness of the importance of all areas of expertise. This way, the education of veterinarians in the southeast region of Brazil does not address adequately the of Veterinary Public Health acting, directing more hours to veterinary clinic disciplines to detriment of disciplines focused on public health, it sets out the individual and curative training, not preparing the professionals to act under the concept of one world one health.

Keywords: Educationa, one world, profile, students

Pharmacokinetics of Meloxicam in Red-Eared Slider Turtles (Trachemys Scripta Elegans) after Single Intravenous and Intramuscular Injections

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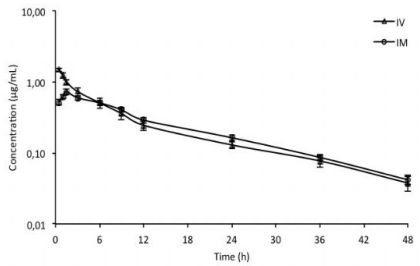
The aim of present study is to determine the pharmacokinetics of meloxicam after single intravenous (IV) and intramuscular (IM) injections at a dose of 0.2 mg/kg BW in red-eared slider turtles. Eight clinically healthy red-eared slider turtles weighing 0.417±0.057 kg were used for the study. The animals were maintained at 24 0C. In pharmacokinetic study, the crossover design was performed. The withdrawal interval between the phases of the study was 30 days. Meloxicam was administered by IV bolus and IM injections at the dosage of 0.2 mg/kg BW to each turtle. Intravenous and intramuscular doses were injected into the left jugular vein and the left deltoid muscle, respectively. Blood sampling alternated between right and left cervical sinuses at 0 (predose sample), 0.5, 1, 1.5, 3, 6, 9, 12, 24, 36 and 48 h after drug injection. Plasma concentrations of meloxicam were determined using the high-performance liquid chromatography. Pharmacokinetics were described by a two-compartment open model. Clinical examination of all turtles before and after each trial did not reveal any abnormalities. No local or adverse reactions to meloxicam occurred after IV or IM injections The mean plasma concentration-time profiles of meloxicam following a single IV and IM administrations of 0.2 mg/kg BW, were presented graphically in the Figure 1. Following IV injection, the major pharmacokinetic parameters (mean±SD) were distribution half-life (t1/2α) 1.02±0.41 h, elimination half-life (t1/2β) 9.78±2.23 h, volume of distribution at steady-state (Vdss) 215±32 mL/kg, area under the plasma concentration-time curve (AUC) 11.27±1.44 µg*h/mL, total body clearance (ClT) 18.00±2.32 mL/h/kg. After IM administration, the principal pharmacokinetic parameters (mean±SD) were absorption half-life 0.35±0.06 h, peak plasma concentration 0.72±0.06 µg/mL, time to peak concentration 1.5 h, t1/2α 3.73±2.41 h, t1/2β 13.53±1.95 h, AUC 11.33±0.92 µg*h/mL. The bioavailability after IM injection was 101±6.29%. The absence of general adverse reactions in the turtles of the study, and the favourable pharmacokinetic properties (the long half-life and the high bioavailability) of meloxicam administered IM at the single dose of 0.2 mg/kg BW suggest the possibility of its safe and effective clinical use in turtles. However, further studies are needed to establish a multiple dosage regimen and clinical efficacy.

Keywords: bioavailability, meloxicam, pharmacokinetics, red-eared slider turtle

Figure 1



Mean ± SD semilogarithmic plots of the plasma concentrations to time data ofmeloxicam in red-eared slider turtles (n=8) after single intravenous (IV) and intramuscular (IM) administrations of meloxicam at a dosage of 0.2 mg/kg of body weight.

A Case of Enterotoxemia in a Gazelle

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A culture material from a gazelle which was suspected enterotoxemia disease was sent from Bornova Veterinary Control Institute to Pendik Veterinary Control Institute Anaerobe Reference Diagnosis Laboratory. The aim of this study was to identificate *Clostridium perfringens* type. The material was plated on blood agar and incubated anaerobically for 24 h. The hemolytic colonies were chosen after examination from the blood agar plates and then inoculated in a chopped meat peptone water medium. Pure culture was obtained. Half-antitoxin plate test was done with one day culture. The cultures, which were incubated in a chopped meat peptone water medium during 6 h and 1 night for identification were santrifuged at 3500 rpm on 10 minutes. Supernatants were studied for toxin- serum neutralisation test (TNT) on mice. Verification of the test was done by Real time PCR. By the half-antitoxin plate test, the microorganizm be detected as a *Clostridium perfringens*. The consequences of toxin – serum neutralisation test was evalueted and it was determined that the strain was *Clostridium perfringens* type A. It was confirmed by Real time PCR at the moleculer genetic laboratory of Pendik Veterinary Control Institute. The culture was refresh in a chopped meat peptone water medium and was lyophilized. Alfa toxin (CPA) is produced all *Clostridium perfringens* genes, type A produces more than amount the others. Toxins produced by type A strains are lethal under experimental conditions. Type A associated enteritis is more common in beef and dairy calves, than in lambs, is also caused Necrotic Enteritis of domestic chickens. The role of *C. perfringens* type A in natural diseases of these species remains controversial and poorly documented so should be considered for Enterotoxemia in death of gazelle.

Keywords: Alfa toksin, clostridium perfringens, gazelle

Intraocular Pressure in Raptors: Preliminary Report

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In the present study, intraocular pressures (IOP) of 1 eagle (2 eyes), 23 buzzard (46 eyes), 2 falcons (4 eyes), 3 eagle-owl (6 eyes), 2 owls (4 eyes), 3 sparrow hawks (6 eyes), 1 kestrel (2 eyes) which referred to Erciyes University, School of Veterinary Medecine, Department of Surgery Clinic with traumatic wound between 2014-15 years were evaluated. In the ophthalmologic examination there was no lesion detected related to eyes. The mean body weight raptors was 3,55 kg (eagle), 1,07±0,29 kg (hawks), 0,52±0,26 kg (falcons), 0,80±0,26 kg (eagle-owl), 0,18±0,28 kg (owls), 0,19±0,04 kg (sparrow hawks) and 0,14 kg (kestrel). Rebound tonometer (Tonovet) (RBT, Icare VET, Helsinki, Finland) was used for measurement of IOP. Mean intraocular pressures of right and left eyes were in eagle 26-27, hawks 26,59±5,57-26,28±6,18, falcons 13,62±2,29-15,76±2,49, owls 13,13±1,69-12,89±2,76, night owls 9,90±1,56-11,12±1,24, sparrow hawks 12,4±2,43-12,33±3,06 and kestrel 11,48-12.As a result of the study, repeated intraocular pressure measurements of raptors can be made by using rebound tonometer. In addition, the application of rebound tonometer is very easy to application do not require local anaesthetic. Therefore, raptors may be able to tolerate the application easily. The present data are considered to contribute to the literature.

Keywords: Intraocular pressure, raptor, rebound tonometer

Echocardiographic Parameters in Spotted Pacas (*Cuniculus Paca*) Under General Anesthesia

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Cardiovascular evaluation in Neotropical rodents is limited and the normal parameters have not been defined. For this reason, the cardiac morphology of eight adult, healthy female Spotted paca (Cuniculus paca), anesthetized with isoflurane, was studied using echocardiography. The aim of the study was to establish reference values based on evaluation of cardiac morphology in B and M mode, patterns of, aortic, pulmonary, mitral and tricuspid flows, with the aid of Doppler in three different periods with an interval of 15 days. The ethics committee on animal use (CEUA-UNESP 027420/11) approved the whole procedure. Valvular abnormalities were not observed in the qualitative assessment and by color Doppler echocardiography. The technique proved to be simple to implement, and images were acquired between the second and fifth left intercostal space and right next to the point of maximum intensity to heart auscultation. The heart of the paca is located in the thoracic cavity in craniocaudal oblique position between the first and fifth intercostal space, presenting the shape of an elongated cone consisting of two atria and two ventricles (left and right). It is anatomically and topographically similar to that of domestic mammals, differing by his localization in a more cranial intercostal space and also present two cranial vena cava, just as observed in other rodents except in the agouti, one the closest species of paca. The mitral and tricuspid valves showed in the pulsed Doppler examination, E and A waves fused in approximately 80% of patients and, therefore, the parameters relating to these waves (AM cm/sec: A-wave mitral peak velocity; AM mmHg: A-wave mitral pressure gradient; EM cm/s: E-wave mitral peak velocity; EM mmHg: E-wave mitral pressure gradient) could be measured only in cases in which these waves were visible separately. The results were similar during the evaluation period. The confidence interval at 95% of significance is considered as a reference for the variables. The echocardiographic exam proved to be simple to perform, it was possible the visualization, evaluation and measurement of the heart chambers and mitral, tricuspid, aortic and pulmonary flows as well as the calculation of, ejection and shortening fractions. The reference echocardiographic parameters defined in this study can be applied to epidemiological, morphophysiological and case analysis in the target specie.

Keywords: Anesthesia, captive, cardiology, cuniculus paca, echocardiography, spotted paca

Effect of Indomethacin and Dimethylsulfoxide on the Clinical Healing of Hydrofluoric Acid Induced Corneal Burns

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The aim of this study was to investigate the effect of indomethacin and dimethylsulfoxide (DMSO) on the healing of corneal burns induced with hydrofluoric (HF) acid in 72 rabbits. Right eyes were burned by instillation of 0.05ml of 2% HF acid for 60 seconds under general anesthesia, followed by irrigation with 500 cc normal saline. Rabbits were divided into four different groups. Eyes in Group D (DMSO) and I (indomethacin) were instilled 4 drops of 40% DMSO and 0,1% indomethacin four times per day respectively; in Group DI (DMSO and indomethacin) were instilled the same amount of dose for DMSO and indomethacin as in groups D and I. No therapeutic agent was instilled in Group C which was kept as the control. The groups were divided into three subgroups for 2, 7 and 14 days. The eyes were clinically examined immediately after the chemical burning and at the follow up periods of days 2, 7 and 14. Treatment efficacies were evaluated as clinical (corneal haziness, conjunctival status, conjunctivitis, corneal erosion area and intraocular pressure). According to the clinical findings at days 7 and 14, the group D was the best among other groups. On the other hand, group C was better than groups I and DI. Although improvement was seen clinically in the groups I and DI, no complete healing was observed. As a result, 40% DMSO efficiently ensured healing the corneal burns, whereas 0,1 % indomethacin both alone and along with DMSO did not.

Keywords: Chemical burn, cornea, DMSO, healing, indhomethacin, rabbit

Electrocardiographic Parameters in Spotted Pacas (*Cuniculus Paca*, Linnaeus, 1766)

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The Spotted paca is the second largest rodent in South America and has high zootechnical value, therefore studies to obtain biological data of the species have been stimulated, seeking greater understanding of the clinical features as health indicators. Some clinical normal indicators have been established for wild rodents however, only one study on the assessment of cardiovascular function in Pacas was published involving morphophysiology of the heart. Among the forms of evaluation of this system, the electrocardiographic exam (ECG) is a cheap diagnostic test, noninvasive, able to determine the rhythm and heart rate. The reference values for this exam have not been established for this specie and may be important to standardize methods for the study of cardiac function. Considering the limited information available on cardiovascular physiology in wild animals and its importance to the pathophysiological study of these species, the objective of this study was to determine the normal electrocardiographic parameters (ECG) of healthy Paca, kept in captivity. The tests were conducted under chemical restraint with ketamine combinations and midazolam every 15 days, during 45 days (3 times). The ethics committee on animal use (CEUA-UNESP 027420/11) approved the whole procedure. For the ECG, the animals were kept in the supine recumbence, and alligator type electrodes were placed in the thoracic limbs above the olecranon and in the hind limbs above the patella. In computerized electrocardiogram were obtained bipolar derivations (DI, DII and DIII) and augmented unipolar (aVR, aVL, aVF, y) in the recording speed of 50 mm/s and voltage calibration of 1 cm/mV. 3 minute intervals were recorded for later analysis. Using the DII derivation were evaluated: rhythm characteristics, rate (HR in beats per minute - bpm), cardiac electrical axis and the values for the duration in seconds (s) and/or amplitude in millivolts (mV) of the P wave (Ps and PmV), R wave (RmV), PR interval (PRs), QRS complex (QRSs), QT interval (QT) and T wave (TmV). Descriptive statistics were calculated for each parameter and built the confidence interval (CI) at a significance level of 95% through Student test. Heart rate oscillated between 115 and 197 bpm with a mean of 150 ± 17 bpm. Sinusal rhythm was observed in 100% of the animals. A ventricular extra-systole (VPC) isolated was recorded in one animal. Cardiac electrical axis ranged widely between -19 and 82° and an average of 33.4 ± 21.9°. The ECG tracing of pacas in DII represents a QRS complex with positive polarity, preceded by a P wave of the same polarity and followed by T wave of variable polarity, similar to that described for the agouti, capybara, dogs, cats, leopards, maned wolves, cheetahs and capuchin monkey, with similar reference values to those found in most species. This study established the first reference electrocardiogram values for the Spotted Paca and the applying the ECG recording technique with chemical restraint was well tolerated, allowing the quick acquisition of reliable ECG tracings and high repeatability, which produced sufficient results to determine the heart rhythm and suggest measures of duration and amplitude of the ECG complexes.

Keywords: Electrocardiographic, spoted paca, wild rodent, veterinary cardiology

The Gross Anatomy of Male Reproductive System in the Hedgehog

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The literature available on the hedgehog (Atelerix Albiventris) is very limited. A hedgehog is any of the spiny mammals of the subfamily Erinaceinae, which is in the order Erinaceomorpha. There are seven teen species of hedgehog in five genera, found through parts of Europe, Asia, Africa and New Zealand. This study focuses on one of the many unexplored areas relating to the species, the male reproductive system. The results showed that the mean testicular length, diameter and weight was 2.67 ± 0.12 cm, 1.45 ± 0.04 cm and 4.03 ± 0.62 g respectively. The paired testes and epididymis were found in contact with the abdominal muscles within scrotal pouches, which are evaginations of the crainocaudal abdominal wall. The caput epididymis is enclosed by a fat body in the dorsal and caudal pole of testis. The ductus deferens has a mean length and diameter of 2.98 ± 0.40 cm and 0.25 ± 0.01 cm respectively. There is not ampulla in the urethral end of the ductusdeferens. The accessory sex organs of the male hedgehog (A. Albiventris) include the very large and lobulated vesicular glands in the dorsal of urethra, the lobulated prostate glands in the ventral of urethra and the bulbourethral glands. The penis of the agouti is U-shaped with a mean length of 8.21 ± 0.13 cm.

Keywords: Anatomy, hedgehog, male reproductive system

The Gross Anatomy of Male Reproductive System in Persian squirrel

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There isn't information about reproduction system of Persian squirrel. The Persian squirrel is a rodent found in iran,Iraq, Palestine, Jordan,loblan. The aim of this study was to evaluate the male reproductive system. The results provided that the mean testicular length, diameter and weight respectively was 1.81 ± 0.12 cm, 0.79 ± 0.04 cm and 2.12 ± 0.52 g respectively. The testes covered with scrotal and tonica vasinalis in ventral part of the hypogasteric region and contact with the abdominal muscles, which are evaginations of region of abdominal wall. The head of epididymis is located caudodorasl of testis. The ductus deferens binged the medial part of tail of epididymis and was very thin and has a mean length and diameter respectively 2.56 ± 0.47 cm and 0.09 ± 0.14 cm.. The accessory sex organs of the male Persian squirrel consisted of, the coagulating glands, the prostate glands and the bulbourethral glands. The penis of the Persian squirrel is U-shaped with a mean length of 5.89 ± 0.74 cm. The glans penis contains an os penis, and one hyaline structure in ventral and end of penis.

Keywords: Anatomy, male reproductive system, persian squirrel

The Incidence of TBE in Ticks from Northwestern Croatian

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Tick-borne encephalitis TBE is a growing public health problem in central and northern European countries. Although the tick-borne encephalitis occurs in Croatia, and there is a significant lack of information in relation to the identification and distribution, and the prevalence in ticks or animals. The target of our study was to identify and investigate the incidence of TBE in ticks which we sampled on the fox. Research we conducted in the period from autumn 2012 to spring 2013. The foxes are delivered to the Northwest and Continental Croatian from the regular hunting to rabies. Number of animals with which we sampled ticks was 211 with 687 ticks. After that ticks are stored at -80 ° C. The samples are comminuted and homogenized in a buffer containing guanidine thiocyanate. RNA is isolated using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. Reverse transcription was performed using GoScript Reverse Transcription System (Promega) according to manufacturer's instructions.Nested PCR (Platinum Taq DNA Polymerase, Invitrogen) specific to the tick-borne encephalitis was made according to the two protocols (Ana Saksida et al. 2005, Angelina Wójcik-FATL et al. 2011), in which they used the primers for the NS5 region of the genome TBEV FSM -1 / FSM2 (external) and FSM-1 and / FSM-2i (internal) and primers for the TBEV genome 5'NC region PP1 / PM1 (external) and PP2 / Pm2 (internal). Heminested polymerase chain reaction (Platinum Taq DNA Polymerase, Invitrogen) was performed using universal primers for the genus flavivirus according to the protocol described by Natale Scaramozzino et al. (2001) and primers for the NS5 region of the genome flavivirus cFD2 / MAMD (external) and cFD2 / FS 778 (internal). PCR products were electrophoresed on 3% agarose gel, stained with ethidium bromide and visualized by UV transilluminator. We have confirmed the presence of tick-borne encephalitis (TBE) in six ticks to six different animals. All the positive ticks in TBE belonging to the genus Ixodes hexagonus. All ticks were positive for TBE were free to coat. The results suggest that ticks are not common to the red fox are positive for TBE and our results suggest that further epidemiological research to understand and recognize the transfer of the frequency of the disease in Croatia.

Keywords: Ixodes hexagonus, red fox, TBE, ticks,

Uterine Prolapse and Cystic Papillary Hyperplasia in Two Djungarian Hamsters (Phodopus Sungorus)

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A 2 year-old multiparous (Case 1) and 2,5 year-old primiparous (Case 2) Djungarian hamsters were presented with history of a mass prolapsed from the vulva. Prolapsus uteri was diagnosed in both cases according to clinical examination. The body tempature and respiratory rate of the animals were within normal values. In case 1, the mass kept under pressure with warm sterile water to loose its edema. Then the uterus was put back into normal anatomic position and a suture around vulva was placed to avoid recurrence of prolapsus. However, the other day, it had prolapsed again and surgery was decided. In case 2, the edematous tissue protruding from the vulva had necrotic and haemorrhagic areas. Also surgery was decided in this case. Inhalation anesthesia with isoflurane was performed for the surgery. The animals were placed on dorsal recumbency and midline laparotomy was performed. The prolapsed mass was replaced in its anatomic position. Then, ovariohysterectomie was performed and tissues were sent to the laboratory in both cases. During postoperative 5 days, enrofloxacine was administered. Histopathological examination revealed ovarian cystic papillary hyperplasia in both cases. Uterine prolapse usually occurs during parturition in female dogs and cats. The possible causes are dystocia, hyperoestrogenism, polyps or cysts. But it is rarely seen in rodents and the main cause is not fully understood. To the author's knowledge, this is a rare report that uterine prolapse was seen with cystic papillary hyperplasia in hamsters.

Keywords: Cystic papillary hyperplasia, hamster, uterine prolapse

First Case of Pulmonary Bovine Tuberculosis in a Free-Living Fallow Deer in North West Italy

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Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB) and it is characterized by a wide host range, including deer. In Europe, M. bovis infection has been reported in red and fallow deer and less frequently in roe deer. Hence, deer may have an important role in the bTB epidemiology. In Piedmont Region (North-West Italy), a regional monitoring Plan to control the wildlife health status is active since 1997 and enforced at the beginning of 2012. Passive surveillance on found dead animals or euthanatized because of injured or in poor conditions and hunted game species has never detected bTB in wild ruminants. This report describes the first case of M. bovis infection in a free-living fallow deer. The animal was euthanatized for its poor body conditions and necropsied according to the guidelines of the specific wildlife monitoring regional plan. Samples from lungs and lymph nodes, with suspected lesions, were collected and split into two halves: one was fixed in10% buffered formalin for histopathology and the second for culture and identification purposes. For histopathological examination, samples were processed by standard paraffin wax techniques, cut in 4±2µ sections and stained with haematoxylin-eosin (HE) and histochemical acid-fast stain Ziehl-Neelsen (ZN). M. tuberculosis complex genome was directly detected from homogenated tissue samples using a heminested PCR-based protocol targeted on the element insertion IS6110 and performed in house. Acid-fast organisms were isolated from lesions following 4 days of incubation using automatic liquid system (Versatrek System, Thermofisher, Oxoid) and 12 days on solid media, then identified by means of multiplex PCR based on simultaneous detection of RNAr16S sequence, insertion element IS986 and mpt40 gene. Postmortem examination revealed gross lesions in the thoracic cavity, characterized by few encapsulated necrotizing nodules on lungs dorsal and caudal layers and a small single mineralized focus on peribronchial lymph node. Lesions in the lungs and lymph nodes were consistent with mycobacterial infection confused by the presence of a severe parasitic bronchopneumonia due to lungworms. At histopathological examination lungs showed severe unencapsulated granulomatous and necrotizing multifocal to coalescing inflammation with rare Langhans giant cells. Lymph node showed multifocal areas of necrosis with mineralization surrounded by epithelioid macrophages, lymphocytes, plasma cells, neutrophils, and rare Langhans giant cells enclosed partly or completely by a thin capsule. The lesions had high number of acid-fast bacilli. M. tuberculosis complex genome was directly detected from homogenated tissue samples using a heminested PCR-based protocol targeted on the element insertion IS6110 and performed in house. M. bovis strain isolated was further characterized by Spoligotyping and VNTR typing (ETR A,B,C,D,E) as SB0120 45533. SB0120 spoligotype associated with 45533 VNTR profile is one of the most common isolated since 2003 in cattle herds of our region. In 2008 a homologous strain was also isolated from a water buffalo with extensive bTB lesions in a zoo, not far from the site of finding of infected fallow deer. The poor condition of the animal was also attributable to heavy parasitic infestation and salmonellosis. Tubercular lesions were detected only in the lungs and associated lymph nodes, suggesting an airborne infection. Due to the presence of other about 20 free-living fallow deer, it is needed to improve the surveillance and avoid the infection spread out of the group.

Keywords: Automatic, bovis, liquid, system, mycobacterium

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