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BERLIN, GERMANY OCTOBER 7-10









# PATHOGENS, DISEASES AND TREATMENT

Thursday, October 10 09.00 - 16.50 Room 1 Chairs: Giorgos Rigos, Egdar Brun

- **09.00 Gabriel Arriagada** THE ROLE OF EPIDEMIOLOGY IN THE SURVEILLANCE AND CONTROL OF PREVALENT FISH DISEASES – THE CASE OF SEA LICE IN FARMED SALMONIDS IN CHILE
- **09.20** Tülay Akayli, Ozgur Canak, Remziye Eda Yardimci, Cigdem Urku, Dilek Okmen ENTEROBACTER INFECTIONS OF CULTURED RAINBOW TROUT *Oncorhynchus mykiss*
- 09.40 Trina Galloway, Jacob Seilø Torgersen, Jørgen Ødegård, Tim Martin Knudsen ROBUST SALMON SKIN – IS THERE A GENETIC COMPONENT?
- **10.00 Carolina Barroso**, J.V. Neves, P.N.S. Rodrigues THE MULTIPLE FUNCTIONS OF PISCIDINS IN THE EUROPEAN SEA BASS *Dicentrarchus labrax*
- 10.20 Mikolaj Adamek, Marek Matras, Andy Dowson, Verena Jung-Schroers, Veronika Piackova, David Gela, Martin Kocour, Jerzy Adamek, Rafal Kaminski, Michal Reichert, Dieter Steinhagen COMPREHENSIVE APPROACH TO DEVELOPMENT OF COMMON CARP STRAINS RESISTANT TO DISEASES CAUSED BY INFECTIONS WITH CYHV-3, CEV AND SVCV
- 10.40 Jose Gallardo, Carolina Figueroa, Pamela Veloso, Lenin Espin, Debora Torrealba, Juan Manuel Afonso, Carlos Soto, Drian Dixon, Pablo Conejeros FAMILIAR VARIATION EXPLAINS REDUCED PROTECTION OF COMMERCIAL VACCINES AGAINST BACTERIAL PATHOGENS IN ATLANTIC SALMON
- **11.00** Lluis Tort, Felipe E. Reyes-López, Xiaohong Liu, Ali R. Khansari, Mariana Teles, Gonzalo Martínez, Juan M. Mancera VACCINES INDUCE A SIGNIFICANT IMMUNE RESPONSE BUT NOT A ROBUST NEUROENDOCRINE REACTION IN BRAIN AND PITUITARY TISSUES OF SEABREAM Sparus aurata
- 11:20 BREAK
- 11:45 PLENARY 3 POSTER AWARDS LUNCH
- **14.30** Paul J. Midtlyng NUCLEIC ACID VACCINES ARE EFFECTIVE FOR CONTROL OF VIRAL INFECTIONS AND DISEASE IN SALMONID AQUACULTURE
- 14.50 Hans Petter Kleppen, Eirik Baardsen, Juan J.J. Martinez, Ingrid S. Larsen, Cyril Frantzen USE OF BACTERIOPHAGES TO CONTROL *Yersinia ruckeri* IN SALMON FARMING
- **15.10 Hector Abelardo Ocampo**, Andres Vasavilbazo-Saucedo, Norma Alamaraz-Abarca ANTIVIRAL EFFECTS IN *L. vannamei* OF FEED ADDITIVE OF MICROENCAPSULATED PHENOLIC EXTRACTS OF *M. umbellata*

# ENTEROBACTER INFECTIONS OF CULTURED RAINBOW TROUT (Oncorhynchus myk

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## Introduction

Rainbow trout (*Onchorhynchus mykiss*) culture was first begun in Turkey in 1970's and showed a great increase. The total production amount of Turkey has reached to 101 761 tons in 2017. Enterobacteriaceae is a large family of Gram-negative bacteria which includes the fish pathogenic genera, *Yersinia, Hafnia, Citrobacter, Klebsiella* and *Escherichia*. Upto date, various bacterial infections caused by Gram-negative pathogens were observed, but there is a limited information on the Enterobacteriaceae infections of rainbow trout except *Y. ruckeri* infections. The aim of this study is the determination Enterobacteriaceae infections of rainbow trout cultured in freshwater cage farms by using conventional bacteriologic, molecular and histopathological methods.

## Materials and methods

With this aim, a cage facility located in a dam lake in our country was visited for a one-year period in April, June, July, September and December in 2017 and a total of 50 diseased rainbow trout samples were investigated. Bacteriologic inoculations from the visceral organs of moribund fish were streaked onto TSA (Tryptic Soy Agar) and bacteria were identified by conventional morphologic and phenotypic tests (Brenner and Farmer, 2009). Biochemical identification was confirmed with the PCR amplification of 16S/23S rRNA with the universal primers 27F and 907R (Lane, 1999). For histopathological examination, tissue samples were fixed in 10% formaldehyde, processed with routine methods and embedded in paraffin (Roberts, 2012). Histological sections were stained with Haemotoxylin – Eosin and analyzed under light microscope.



## Figure 1.

a) Sloughing in the rainbow trout infected with *H. alvei*b) Darkening of the skin and edema in the rainbow trout infected with *C. freundii*

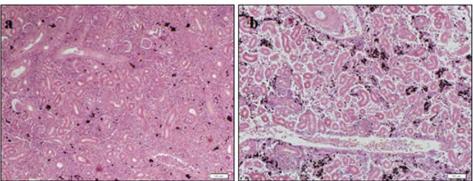


Fig 2. Pathologic differences in the kidney of rainbow trouts infected with *H. alvei* (a) and *C. freundii* (b)

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## Results

In this study, of the members of Enterobacteriaceae, *Hafnia alvei* and *Citrobacter freundii* were recovered and identified from the infected fish samples. Biochemical identification was confirmed with 16S gene sequencing and blasting. Fish samples infected with *H. alvei* showed sloughing of the skin, swollen abdomen (Fig 1.a) and hyperemia in the visceral organs clinically. Histopathologically, they showed small melanomacrophage centers, hemorrhages and hyperemia in the visceral organs, and slight degenerations in the liver hepatocytes and kidney tubules (Fig 2.a). Fish samples infected with *C. freundii* showed exophthalmos, darkening of the skin, edema and accumulation of a transparent liquid in the peritoneal cavity (Fig 1.b) and splenomegaly clinically. Histopathologically, these samples showed slight necrosis in the hepatocytes but severe necrosis in the interrenal haemopoietic tissue, severe degenerations in the hepatocytes and kidney tubules and generalised melanomachrophage centers and hemosiderin accumualtions in the kidney and spleen (Fig 2.b).

#### **Discussion and conclusion**

*Hafnia alvei* and *C. freundii* are the members of Enterobacteriaceae, which can be mis-identified by biochemical profiling and may be confused with *Y. ruckeri*. Molecular studies and gene sequencing gives more reliable results for the identification (Austin and Austin, 2016). In this study, biochemical profiling was also misidentified our isolates but molecular studies provided the final identification of these bacteria.

The members of the Enterobacteriaceae previously caused infections in freshwater fishes after transportation, under stress or during cultivation in inappropriate conditions. Generally these bacteria cause hemmorhagic septicemia in fishes, but swollen abdomen is seen in *H. alvei* infections while *C. freundii* causes exhophthalmus and surface hemorrhages (Austin and Austin, 2016). In this study infected fish samples showed similar symptoms.

Despite these bacteria were recovered from rainbow trout previously by other researchers, there is a limited information on the histopathology of these infections. The results of our study showed that *C. freundii* infections cause more severe clinical and histopathological symptoms while *H. alvei* causes more mild pathologies in rainbow trout.

In conclusion, the results of this study revealed the need for the use of molecular methods in the identification of fish pathogenic Enterobacteriaceae species and the detailed differences of pathologies these emerging pathogens in rainbow trout were demonstrated by using histopathological methods.

#### Acknowledgements

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#### References

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- Brenner, D.J., Farmer, J.J., 2009, Family I Enterobacteriaceae, Bergey's Manual of Systemic Bacteriology 2<sup>nd</sup> edition, volume II "The Proteobacteria" Part B "The Gammaproteobacteria, Springer, 587-850 pp.
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