Monday September 3rd – Gray / Palmer / Pope Ballroom
Immunology Vaccines 2
Moderator – Alex Primus (University of Minnesota)

3:15 PM	Immunology Vaccines 2	Karsi - Pathological And Immunological Assessment of Live Attenuated Vaccines Against Enteric Septicemia Of Channel Catfish
3:30 PM		**Kitiyodom - Mucoadhesive Nanoparticles As An Effective Delivery System For Fish Immersion Vaccination**
3:45 PM		**Sebastião - Evaluation Of Live Attenuated And Recombinant Subunit Vaccines Against Piscine Francisellosis**
4:00 PM		Sommerset - Comparative Analysis Of Performance In Vaccinated And Unvaccinated Atlantic Salmon Under Different O2 And Temperature Regimes
4:15 PM		Midtlyng - On The Way To New Batch Potency Tests For *Moritella viscosa* Vaccines: Antibody Response And Protective Immunity Correlate In A Dose–Response Manner
4:30 PM		Sandtro-Lunheim - Vaccination Against Yersiniosis In Atlantic Salmon - Experiences And Challenges
4:45 PM		Menanteau-Ledouble - Effect Of Immunostimulatory Feed Additives On The Response Of Rainbow Trout *Oncorhynchus mykiss* To A Commercial Vaccine Against *Yersinia ruckeri*
5:00 PM		Tingbo - Development Of Yersiniosis Vaccines For Atlantic Salmon

Pathological and Immunological Assessment of Live Attenuated Vaccines Against Enteric Septicemia of Channel Catfish

Iman Ibrahim[1], Hossam Abdelhamed[1], Wes Baumgartner[2], Mark L. Lawrence[1], Attila Karsi[1]*

[1] Department of Basic Sciences, College of Veterinary Medicine, Mississippi State, MS 39762 USA ii42@msstate.edu, abdelhamed@cvm.msstate.edu, lawrence@cvm.msstate.edu, karsi@cvm.msstate.edu
[2] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State, MS 39762 USA baumgartner@cvm.msstate.edu

Enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* is one of the most important bacterial diseases of farmed catfish in the United States. Use of live attenuated vaccines (LAVs) is an effective strategy for combating ESC mortalities in catfish farms. Our research group has developed two safe and efficacious live attenuated *E. ictaluri* vaccine strains (*Ei*Δ*evpB* and *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA*) against ESC. In this study, we present mucosal uptake and pathology of LAVs in catfish fry. We also provide LAVs' effects on expression of innate and adaptive immune genes as well as pronephros lymphomyeloid cells in catfish fry. Results indicated that there were significant differences between the *E. ictaluri* wild-type (*Ei*WT) and vaccinated groups during vaccination and following *Ei*WT challenge of vaccinated catfish. Pathologically, LAVs were safer and showed no (Aquavac-ESC and *Ei*Δ*evpB*) or minor (*Ei*Δ*gcvP*Δ*sdhC*Δ*frdA*) pathological lesions during vaccination. However, *Ei*WT challenge of the vaccinated catfish fry indicated that *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA* had less pathological lesions with fewer bacteria than Aquavac-ESC, *Ei*Δ*evpB*, and sham groups. Immunologically, in contrast to the vaccinated groups, a significant increase in expression of immune genes was observed in the *Ei*WT exposed control fry during vaccination, and the number of lymphomyeloid cells was reduced. Following *Ei*WT challenge of vaccinated catfish fry, a significant increase in expression of immune genes was observed, and the number of lymphomyeloid cells was increased. These findings support that *Ei*Δ*evpB* and *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA* have improved vaccine properties compared to the commercial vaccine Aquavac-ESC.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)

Mucoadhesive Nanoparticles as an Effective Delivery System for Fish Immersion Vaccination

S. Kitiyodom[1], S. Surassmo[2], K. Namdee[2], C. Rodkhum[3], N. Pirarat[1]* and T. Yata[2]*

[1] Wildlife Exotic and Aquatic Pathology-Special Task Force for Activating Research Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand nopadonpirarat@gmail.com
[2] National Nanotechnology Center, Pathum Thani 12120, Thailand teerapong@nanotec.or.th
[3] Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University Bangkok 10330, Thailand

It is well established that vaccination is the most effective approach for prevention of infectious diseases in fish. In fact, fish vaccines are mostly administered through major three routes of administration as bath or immersion, second through in-feed or oral and the third by injection. While immersion vaccination is more applicable, but this method suffers from low potency as the efficiency of uptake of antigens through the gills and skin are limited. In this study, we have successfully developed a mucoadhesive vaccine delivery system to circumvent this problem. We chose *Flavobacterium columnare*, the causative agent of columnaris disease, as a representative model antigen for a proof-of-concept study. The sonicated bacterial suspension was used to prepare nanovaccines through emulsification and homogenization techniques followed by coating with mucoadhesive polymer chitosan. The analysis of hydrodynamic diameter and zeta-potential also suggested the successful modification of nanovaccines by chitosan. The chitosan modified nanovaccines were positively charged and the overall diameter also increased. The prepared vaccines were nano-sized and spherical as confirmed by transmission electron microscopy (TEM). The *ex vivo* bioluminescence imaging showed excellent mucoadhesive property of nanovaccines coated with chitosan. Tilapia fishes were vaccinated with the prepared nanovaccines by brief immersion. The challenge test was then carried out 60 days post-vaccination and resulted in 90% mortalities in the control. Interestingly, the relative percent survival (RPS) of vaccinated fish was calculated at 89 for mucosal nanovaccine. In conclusion, we could use this system to deliver antigen preparation to the mucosal membrane of tilapia fishes and induce appropriate immune responses, resulting in a significant increase in survival compared to controls. Therefore, targeting mucoadhesive nanovaccines to the mucosal surface could be exploited as an effective method for immersion vaccination.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)
Student Presentation: (Yes)

Evaluation of Live Attenuated and Recombinant Subunit Vaccines Against Piscine Francisellosis

Fernanda de Alexandre Sebastião[1], Matt Rogge[2], Alvin Camus[3], John D. Hansen[4], Esteban Soto[1]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, CA 95616, USA dealexandresebastiao@ucdavis.edu sotomartinez@ucdavis.edu
[2] Department of Biology, University of Wisconsin-Stevens Point, Stevens Point, WI 54481, USA matt.rogge@uwsp.edu
[3] Department of Pathology, College of Veterinary Medicine, University of Georgia Athens, Athens, GA USA camus@uga.edu
[4] U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA 98115, USA jhansen@usgs.gov

Francisella noatunensis subsp. *orientalis* (*Fno*) is a bacterial pathogen of marine and fresh water fish worldwide. To date, there is no approved vaccine for this emergent disease. To better characterize immunodominant *Fno* antigens, proteomic analyses was performed in previous studies using serum collected from laboratory challenged Nile tilapia (*Oreochromis niloticus*). We hypothesized that some of these proteins could be used as recombinant subunit vaccines. In this study, the efficacy of recombinant subunit vaccine candidates was compared to two attenuated *Fno* strains previously proposed as plausible live vaccines and compared to non-vaccinated tilapia fingerlings against piscine francisellosis. Recombinant vaccine candidates included *Fno iglA, iglB, iglC,* and *vgrG* cloned into an *Escherichia coli* expression vector (pBAD (Life Technologies) inactivated with formalin or heat. Other treatments investigated were formalin and heat inactivated Top10 cells with empty vector, purified IglA (0.2 mg/ml), live attenuated Δ*iglC* and Δ*pdpA* strains, as well as mock-vaccinated (PBS) control. Approximately 10^7 CFU inactivated Top10 cells expressing the *Fno iglA, iglB, iglC,* and *vgrG,* or non-cloned pBAD vector mixed with 70% of Montadine adjuvant were used to immunize tilapia via intracoelomic injection. Similar amounts of live attenuated mutants were used for comparison. Each treatment consisted of 45 fish divided in triplicate tanks containing flow-through fresh water at 23±2°C. Approximately 690-degree days post-immunization, fish were challenged via immersion with 10^5 CFU/ml of wild-type *Fno* at 17±1°C. Mortality was monitored daily for 4 weeks. At the end of challenge, the mean percent mortality for each treatment were as follows: non-vaccinated (65%), *iglB*-F(58%), Top10-F (53%), *iglA*-P (51%), *iglB*-H (50%), Top10-H (47%), *iglA*-F (45%), *iglC*-F (44%), *vgrG*-H (38%), *iglA*-H (36%), *vgrG*-F (35%), Δ*pdpA1* (7%) and Δ*iglC* (4%), providing a relative percent survival for the vaccinated fish of 11%, 19%, 21%, 23%, 28%, 30%, 32%, 41%, 42%, 45%, 46%, 90% and 95%, respectively. Tilapia vaccinated with the recombinant subunit vaccines tested in this study and subsequently challenged with wild type *Fno* did not present significantly lower mortality when compared to non-vaccinated controls ($p>0.05$). However, significant differences were observed in fish vaccinated with the Δ*pdpA* and *Δig1C* attenuated strains when compared to non-vaccinated controls ($p<0.05$). This information demonstrates that live attenuated vaccines have higher efficacy at inducing a protective immune response in Nile tilapia fingerlings against francisellosis when compared to recombinant subunit vaccines developed so far.

Conference Session Designation: **(** Immunology Vaccines)
Presentation Format: (Oral)
Student Presentation: (Yes)

Comparative Analysis of Performance In Vaccinated and Unvaccinated Atlantic Salmon Under Different O2 and Temperature Regimes

Vegar Heen[1], Mark Powell[2], Tom Hansen[2], Per Gunnar Fjelldal[2], Hogne Bleie[3 *], Ole Høstmark[3], Niels P. Maaseide[3] and Ingunn Sommerset[3]

[1] Department of Biological Sciences, University of Bergen, Tormöhlensgate 53, 5003 Bergen mark.powell@uib.no
[2] Institute of Marine Reserch, PO Box 1870 Nordnes, 5817 Bergen, Norway, tomh@IMR.no
[3] MSD Animal Health Norge AS, Tormöhlensgate 53, 5003 Bergen, Norway ingunn.sommerset@merck.com

Growth-impairment is a well-known adverse effect of compulsory intraperitoneal immunisation with oil-based vaccines administered to commercially farmed Atlantic salmon (*Salmo salar L*). While the vaccine protection is well documented, there have been few studies focusing on the potential negative effects of vaccination and the environmental factors influencing the degree of these. As the temperature and the oxygen saturation of the water might fluctuate at and around the time of vaccination, a controlled study aimed to explore the effects of the variation of these parameters was carried out at a research facility resembling commercial farming conditions for the fish. Both vaccinated and sham-vaccinated salmon were exposed to combinations of different temperatures (12 and 17°C) and oxygen saturation (60 and 100% O2) in the post vaccination period. In addition, the different groups were exposed to a smoltification signal by switching from a 12h light – 12h darkness period to a 24h light regime one day after vaccination. Comparative measurements of body mass and length and Speilberg scoring of local adverse reactions in the abdominal cavity were carried out. Samples were secured from the gills, the brain and from the head kidney for qPCR to evaluate responses to the environmental parameters, and blood was sampled to assess the antibody response to the immunisation by an ELISA-assay. Growth-performance during the fresh water period and after transfer to sea for the various groups of fish exposed to various combinations of environmental factors will be presented, as well as the relative expression of candidate genes for environmental stressors, the smoltification process and the immune response.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: **(** Oral)

On the Way to New Batch Potency Tests For *Moritella viscosa* Vaccines: Antibody Response and Protective Immunity Correlate in a Dose–Response Manner.

Paul J. Midtlyng[1*], Anne Ramstad[2], Bjørn Krossøy[3] and Liv Jorun Reitan[4]

[1] School of Veterinary Medicine, Norwegian University of Life Sciences, Ullevalsveien 72, N-0104 Oslo, Norway paul.midtlyng@nmbu.no
[2] VESO Vikan, N-7810 Namsos, Norway anne.ramstad@veso.no
[3] Vaxxinova Norway AS, N-5006 Bergen, Norway bkrossoy@vaxxinova.no
[4] Norwegian Veterinary Institute, N-0454 Oslo, Norway liv-jorun.reitan@vetinst.no

The studies reported here were part of a project to lay the foundation for development of antibody-based tests that can replace experimental inoculation methods for routine batch potency testing of multivalent, adjuvanted salmon vaccines. Atlantic salmon pre-smolts were immunized with multivalent salmon vaccines that are commercially available in Norway or with experimental formulations, followed by splitting study groups for subsequent blood sampling or a waterborne challenge experiment that were carried out in parallel. Antibody activity against the *M. viscosa* antigen measured in an ELISA was clearly above the pre-vaccination level from 4 weeks of immunization. When being held at 15^{o}C, fish that had received experimental vaccine formulations with reduced content or completely lacking the *M. viscosa* antigen, formulations with reduced antigen content could be revealed by analyzing blood samples taken 6 and 9 weeks post vaccination. In the parallel waterborne challenge experiment, clinical protection induced by the same formulations was reduced correspondingly. The results suggest that antibody-based assay protocols for the *Moritella viscosa* antigen of multivalent salmon vaccines can replace current challenge tests for assuring batch quality, at the same time shortening the time to batch release by one month or more.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: **(** Oral)

Vaccination Against Yersiniosis in Atlantic Salmon - Experiences and Challenges

Ane Sandro-Lunheim[1]*, Ragnhild Hanche-Olsen[2], Børge N Fredriksen[3], Arne Guttvik[4], Monica G Tingbø[5]

[1-3,5] PHARMAQ part of Zoetis, Harbitzalleen 2A, 0275 Oslo, Norway ane.sandtro@zoetis.com ragnhild.hancheolsen@zoetis.com borge.nilsen-fredriksen@zoetis.com monica.gausdal-tingbo@zoetis.com

[4] SalMar, Industriveien 51, 7266 Kverva, Norway arne.guttvik@salmar.no

In recent years, there has been a drastic increase of yersinosis outbreaks in Norwegian salmon farming. The disease, caused by *Yersinia ruckeri*, has mainly been reported as a problem in juveniles and in smolts in the period after transfer to sea, but since 2015 an increase of outbreaks on larger fish (>1kg) at sea has been observed. In fact, more than 90 % of the reported outbreaks in 2017 were months or even years after transfer to sea. The outbreaks cause massive and acute mortality, up to 80% in some cages.

Today more than 100 million smolts are vaccinated annually against yersinosis in mid and northern Norway. The vaccine used, ALPHA DIP ERM Salar, is a water based inactivated vaccine authorized for bath or immersion use. Due to the emerging situation of outbreaks in the sea phase, the farming industry has rapidly implemented vaccination by intraperitoneal injection. The injection is done as co-injection with a multivalent basis vaccine. The presentation will show how the vaccinations are carried out, both manually and by machines.

Efficacy laboratory data from the various regimes of vaccination against yersinosis will also be presented along with results from field observations. Our studies have demonstrated good protection against *Yersinia ruckeri*. No yersinosis outbreaks have been registered in vaccinated fish and the injection of the water based vaccine did not show negative effect on the co-administrated multivalent vaccine.

Conference Session Designation: (Immunology Vaccines)
Presentation format: (Orai)

Effect of Immunostimulatory Feed Additives on the Response of Rainbow Trout *Oncorhynchus mykiss* to a Commercial Vaccine Against *Yersinia ruckeri*

Simon Menanteau-Ledouble[1]*, Frouke van Sorgen[1], Rui Alexandre Gonçalves[2] and Mansour El-Matbouli[1]

[1] Klinische Abteilung für Fischmedizin, Veterinärmedizinische Universität Wien (Vetmeduni Vienna), Veterinärplatz 1, 1210 Wien Austria menanteaus@staff.vetmeduni.ac.at 0807635@students.vetmeduni.ac.at matboulim@staff.vetmeduni.ac.at

[2] BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria rui.goncalves@biomin.net

Immunostimulatory feed supplements are of great interest in fish farm management not only due to having a protective effect but also they can result in improved farming performances. However, most studies of these supplements have focussed on their effects on the innate immune system and relatively little consideration has been given to their potential effects on the specific immune system. Because one of the functions of the innate immune system is to present the antigens to initiate the specific response, it is plausible that an improvement in the innate response would also result in an improvement in the specific immune system. Consequently, the present study was designed to investigate two commercial feed supplements (Biotronic® Top 3 and Levabon® Aquagrow E) with a known protective effect against bacterial infections as well as a combination of both of these supplements. Their effects on the ability of rainbow trout (*Oncorhynchus mykiss*) to generate an antibody response was analysed using vaccination with a commercial vaccine against *Yersinia ruckeri* followed by sampling of the serum and ELISA. Afterwards, an infection trial was performed using *Y. ruckeri*. Finally, the effect of the supplements on the growth parameters of the fish was also investigated. While this effect on growth was not found statistically significant, the combination of both supplements was found to have a protective effect against infection, moreover, they were associated with slightly higher titters of specific anti-*Y ruckeri* antibodies and an improved response to the vaccine compared to the fish that had only received the control feed.

Conference Session Designation: (Immunology / Vaccine)
Presentation Format: **(** Oral)

Development of Yersiniosis Vaccines for Atlantic Salmon

Monica G Tingbø[1]*, Ane Sandtrø[2], Børge N Fredriksen[3], Ragnhild Hanche-Olsen[4], Hege Hardersen[5], Bjørn E Brudeseth[6]

[1-6] PHARMAQ part of Zoetis, Harbitzalleen 2A, 0275 Oslo, Norway
monica.gausdal-tingbo@zoetis.com ane.sandtro@zoetis.com
borge.nilsen-fredriksen@zoetis.com ragnhild.hancheolsen@zoetis.com
hege.hardersen@zoetis.com bjorn.brudeseth@zoetis.com

During recent years, the Norwegian aquaculture industry has experienced increasing numbers of yersinosis outbreaks in farmed Atlantic salmon after sea transfer and even into the second year in sea. Massive and acute mortalities have been registered in certain cages. The disease occurring at such a late stage in the production cycle causes huge economic losses in addition to the obvious animal welfare challenges. Not much reviewed literature describing salmon isolates of *Yersinia ruckeri* is available, despite the fact that salmon isolates are fairly different from trout isolates.

We will show that the salmon isolates previously serotyped to O1, more specifically belong to subgroup O1b, and no evidence of subgroup O1a in Atlantic salmon has to our knowledge thus far been found in Norway. Western blot of O-antigens from serotypes O1a, O1b and O2 showed different binding patterns when comparing polyclonal antibodies raised against O1a and O1b serotypes. Moreover, dose-response results for *Y. ruckeri* serotype O1b from water-based as well as oil-adjuvanted vaccine systems will be shown. Also, a cross protection study revealed that O1a immunization of salmon did not provide any protection against O1b challenge. The results demonstrate the importance of choosing the proper isolates for vaccination, as the different serotypes may provide limited cross-protection against others.

Conference Session Designation: (Immunology Vaccines)
Presentation format: (Oral)