# Monday September 3rd – Gray / Palmer / Pope Ballroom
## Immunology Vaccines 1
## Moderator – Simon Menanteau-Ledouble ( University of Veterinary Medicine – Vienna )

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| 1:15 PM | Immunology Vaccines 1 | Wallis - Autogenous Vaccines In Principle And Practice |
| 1:30 PM | | |
| 1:45 PM | | Aarattuthodiyil - Fish Vaccination – Factors To Consider |
| 2:00 PM | | **Delphino - Economic Evaluation Of Vaccination Against *Streptoccocus agalactiae* In Nile Tilapia Farms** |
| 2:15 PM | | Powell - Immersion Vaccination Research For Aquatic Animals Guided By Computer Assisted Laser Scanning Cytometry |
| 2:30 PM | | Braden - Vaccine-Induced Protection Against Infection With *Aeromonas salmonicida* Subsp *salmonicida* In Arctic Charr *Salvelinus alpinus* Involves Pre-Emptive Priming Of Humoral Immunity |
| 2:45 PM | | Bruce - Cross-Protective Ability Of A Live Attenuated Coldwater Disease Vaccine In Juvenile Rainbow Trout |

**Autogenous Vaccines in Principle and Practice**

Timothy S. Wallis* and J. Gustavo-Ramirez Paredes

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Autogenous vaccines are farm-specific vaccines formulated with antigens derived from pathogens isolated from disease outbreaks on the farm. They can be used when fully licensed vaccines have proven to be ineffective or are unavailable. They should be used under the supervision of the responsible veterinary surgeon. The development of anti-microbial resistance is having a significant impact on antibiotic use in food producing animals. Autogenous vaccines can be a useful tool for the control of infectious diseases thereby reducing the need for undesirable antibiotic interventions. Autogenous vaccines are widely used in poultry, pig, cattle and aquaculture industries for disease control. We have developed and supplied immersion (dip & bath) and injectable mono- and multi-valent vaccines for the control of a wide range of pathogens (bacterial and viral) in farmed salmonids, sea-bass, tilapia and cleaner-fish i.e. ballan wrasse and lumpsuckers. New molecular typing methodology means that strain variation on farm can be readily understood relatively cheaply and bespoke vaccines can be formulated accordingly targeting the relevant variants on farm. Aspects relating to production and quality together with case studies will be presented. However autogenous vaccines are not an alternative to conventional vaccines if effective, good nutrition and good animal husbandry.

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

**Fish Vaccination – Factors to Consider**

Suja Aarattuthodiyil *, Todd Byars, Matt Griffin, Lester Khoo, Terrence Greenway, Ganesh Kumar, and David Wise

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Aquaculture industry is seriously impacted by several diseases. Although vaccination can minimize mortality due to some of these diseases, the effectiveness of a vaccine is dependent on the cross protection offered against multiple isolates. For example, in the case of Enteric Septicemia of Catfish (ESC), caused by the bacteria, *E. ictaluri*, traditionally, losses have been controlled by withholding feed from fish to reduce the oral route of infection combined with medicated feeds. Recently, a live attenuated ESC vaccine is delivered orally to catfish. Under laboratory conditions, the vaccine was shown effective against the parental wild-type strain and proved to be safe at 10 times the applied target dose. While live vaccines are very effective in providing long lasting immunity against disease, vaccine safety and efficacy could be compromised, if delivered to animals in suboptimal health or under stressful conditions. In a compromised animal, attenuated vaccine can cause infections leading to morbidity and mortality. Another key factor in field vaccinology is antigenic variation among pathogenic species, where immunization with a vaccine derived from one strain does not provide protection against genetic variants of the same species. Vaccinated and control fish were challenged with the wild type *E. ictaluri* isolate 30 days post-vaccination. Low oxygen stress did not induce any post-vaccination mortality in any of the vaccinated treatments. Similarly, all groups of vaccinated fish, regardless of stress treatment, were protected against virulent *E. ictaluri* infection. Data indicated that acute oxygen deprivation, before or after vaccination, does not alter vaccine safety and efficacy, however the effects of chronic long term stress have not been evaluated. Therefore, short acute stressors are unlikely to influence vaccine safety and efficacy and provides valuable insight in developing commercial vaccination protocols. Additional trials were conducted to determine if the attenuated isolate afforded protection against 23 archived field isolates collected over a time span of twenty years (1997-2016). Vaccination followed by bacterial challenge with archived isolates were conducted over a three year period. In all trials, vaccination was shown to protect catfish against all challenge isolates, regardless of host species, geographic region (state and farm location) or isolation year. While on farm vaccination greatly improved survival, yield and fish net-value, limited mortality was observed in vaccinated pond populations. Results indicated that mortality observed in farm vaccinated fish populations was not related to antigenic variations among isolates. The most likely cause of on-farm mortality was related to unequal distribution of vaccine laden feed to individual fish, an inherent problem with mass delivery of oral vaccines to large populations of animals. In order to differentiate between the 23 isolates, their clonal relation were determined. The PCR profile indicated relative homogeneity among the isolates dating back to 1997. This further confirmed the results which indicated no significant difference between the isolates. The clonal nature of *E. ictaluri* isolates demonstrated by our data negates the need to develop multivalent vaccines or construct new vaccines to account for antigenic variation occurring over time. Commercial vaccination trials are showing net economic benefit of $2000 to $3000/acre for channels and hybrid fingerling production phase.

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

**Economic Evaluation of Vaccination Against *Streptoccocus agalactiae* in Nile Tilapia Farms**

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*Streptococcus agalactiae* causes mortality and major economic losses in Nile tilapia (*Oreochromis niloticus*) farming worldwide. In Brazil, serotype strains Ia, Ib and III have been isolated in streptococcosis outbreaks, but serotype Ib is the most prevalent. Vaccination is considered an effective method to prevent economically-important diseases in aquaculture and has been associated with decreased usage of antibiotics and improvements in fish survival. We developed a simple and flexible partial-budget model to undertake an economic appraisal of vaccination against *Streptococcus agalactiae* in Nile tilapia farmed in net cages in large reservoirs. The model considers the benefits and costs that are likely to occur in one production cycle (time for fish to reach the marketable size), because of the proposed intervention. We analysed three epidemiological scenarios of cumulative mortality due to *S. agalactiae* (5%, 10% and 20%, per production cycle) in a non-vaccinated farm. For each scenario, we calculated the net return (benefits – costs) of vaccination, given a combination of values of "vaccine efficacy" and "gain in feed conversion ratio", in order to model uncertainty about the true value of such parameters. Results indicate that vaccination against *S. agalactiae* is likely be profitable in Nile tilapia farms, although in scenarios where cumulative mortality is lower than 10%, the profitability of vaccination would be more dependent on higher vaccine efficacy and/or better feed conversion ratio.

**Conference Session Designation:** ( Latin American Fish Health )
**Presentation Format:** ( Oral )
**Student Presentation:** ( Yes )

**Immersion Vaccination Research for Aquatic Animals Guided by Computer Assisted Laser Scanning Cytometry**

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Immersion vaccination can be a rapid and cost-effective method to stimulate the immune system of fish against pathogens. Enhancement of antigen uptake during immersion vaccination may lead to increased vaccine efficacy and improved survival against disease. A variety of methods and materials have been reported to facilitate the uptake of foreign particulate antigens in fish, and fluorescent microspheres have been used in studies of particulate antigen uptake and processing by the host. Recent advances in solid phase laser scanning cytometry enabled development of a method to more accurately quantify adhesion and uptake of fluorescent microspheres by mucosal fish tissues. One-micron fluorescent microspheres were applied in combination with immersion vaccine formulations to quantify their adhesion to gill and skin tissues of rainbow trout under different vaccination conditions. We were particularly interested in the effect of adipose fin clipping on particle uptake by skin tissue and whether the wound site would be more susceptible to particle binding. A substantial increase in particle uptake occurred at the site of the fin clip wound compared to unclipped control fins. The use and concentration of hyperosmotic saline immersion treatments, MS-222 anesthesia, and Seppic Montanide™ adjuvants were also investigated. The results are relevant to decisions regarding the timing of immersion vaccination and potential methods used to improve their efficacy.

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

**Vaccine-Induced Protection Against Infection with *Aeromonas salmonicida* Subsp *salmonicida* in Arctic Charr *Salvelinus alpinus* Involves Pre-Emptive Priming of Humoral Immunity**

Laura M. Braden[1*], Shona K. Whyte[1], Alyson B. J. Brown[1], Carter VanIderstine[1], Corinne Letendre[2], David Groman[1], Jeff Lewis[1], Sara L. Purcell[1], Tiago S. Hori[3], Mark D. Fast[1]

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With respect to salmonid aquaculture, one of the most important bacterial pathogens in terms of economic loss due to mortality and antibiotic usage is the causative agent of typical furunculosis, *Aeromonas salmonicida* spp. *salmonicida* (*Asal*). In Atlantic salmon, *Salmo salar*, the host response during infections with *Asal* is well documented; however, less is known about the host-pathogen interactions in the emerging aquaculture species, Arctic charr, *Salvelinus alpinus*. Furthermore, there is no data on the efficacy or response of this species during vaccination with commonly administered vaccines against furunculosis. To this end, we were interested in examining the immunological response of *S. alpinus* during infection with *Asal*, with or without administration of vaccines (Forte Micro®, Forte Micro® + Renogen®, Elanco Animal Health). Artic charr (vaccinated or unvaccinated) were i.p.-injected with a virulent strain of *Asal* ($10^5$ CFUs/0.1mL) and tissues were collected pre-infection/post-vaccination, 8, and 29 days post-infection. By 8 dpi, *Asal* bacterial load in sham fish, as assessed by *aopO* qPCR, was 4-fold higher than both vaccinates. Unvaccinated charr were extremely susceptible to *Asal* with 72% mortalities observed after 29 days. However, there was 72-82% protection in fish vaccinated with either the single or dual-vaccine, respectively. Protection in vaccinated fish was concordant with significantly higher *Asal*-specific serum IgM concentrations, and following RNA sequencing and transcriptome assembly, differential expression analysis revealed several patterns and pathways associated with the improved survival of vaccinated fish. Most striking was the dramatically higher basal expression of complement/coagulation factors, acute phase-proteins (APRs), and metal homeostasis proteins in pre-challenged, vaccinated fish. Interestingly, following *Asal* infection, this response was abrogated and instead, the transcriptome was characterized by a much weaker immune response compared to that of non-vaccinated fish. Furthermore, where pathways of actin assembly and Fc□R-mediated phagocytosis were significantly dysregulated in non-vaccinates, vaccinated fish showed either the opposite regulation (ForteMicro®), or no impact at all (ForteMicro®+Renogen®). The present data indicates that vaccine-induced protection against *Asal* relies on the priming of complement and other APRs, which influences cell-cell interactions, possibly in favour of B-cell survival and enhanced serum IgM production following challenge.

**Conference session designation:** ( Immunology General )
**Presentation format:** ( Oral )

**Cross-protective Ability of a Live Attenuated Coldwater Disease Vaccine in Juvenile Rainbow Trout**

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Bacterial coldwater disease (BCWD) caused by *F. psychrophilum* remains one of the most significant bacterial diseases of salmonids worldwide and impacts many government and commercial operations across North America and globally. A live attenuated immersion vaccine (B.17-ILM) has been developed at the University of Idaho and has been shown to confer significant protection to salmonids. To further characterize this novel vaccine, a series of challenge trials were carried out to determine the cross-protective efficacy of this live attenuated vaccine against 9 virulent *F. psychrophilum* isolates in comparison with a domestic, wild-type virulent strain. The 9 *F. psychrophilum* isolates of various sequence types (STs) were assessed for virulence using an injection-based challenge model prior to the initiation of a vaccination trial. To assess protection in juvenile rainbow trout, two separate 28-day challenge trials were conducted following immersion vaccinations with the B.17-ILM vaccine. All vaccinated fish developed an adaptive immune response (as measured by *F. psychrophilum*-specific antibodies) that increased out to the time of challenge (8 weeks post-immunization). All isolates demonstrated virulence at the time of initial testing, with cumulative percent mortality (CPM) rates ranging from 25.3% to 88.0%. Following vaccination and subsequent challenge, the immersion vaccine was shown to provide significant protection against all *F. psychrophilum* strains tested, with relative percent survival (RPS) values ranging from 51-72%. Similar RPS values were observed in fish challenged with ST10 group isolates. The ability of vaccine-specific antibodies to bind to similar antigenic proteins or LPS components for all *F. psychrophilum* strains was determined by Western blot analyses. It was shown that serum antibodies recognize a common 65 kDa antigen across all isolates, suggesting that this protective antigen is shared widely. Results suggest that this live attenuated vaccine will elicit a protective immune response to fish and provide a valuable tool for BCWD control even in aquaculture operations affected by diverse strains of *F. psychrophilum*. Additional studies to assess the cross-protective ability of this vaccine against other emerging Flavobacterial and non-Flavobacterial pathogens are underway.

**Conference Session Designation:** ( Vaccines / Flavobacteria )
**Presentation Format:** ( Oral )