keywords: Flagellin, cytokines proinflammatory, immune response, adjuvant

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P-065.

Development of a modular oral vaccine based in outer membrane vesicles for rainbow trout and characterization of the systemic and mucosal B and T cell response assembled

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Abstract
Infectious diseases cause serious economic losses due to the high-density fish stocks. Diversevaccines were developed to prevent this; however, they are not effective enough and theinjection route is linked to side effects and stress. It is known Gram-negative bacteria produce Outer Membrane Vesicles (OMVs) and are used for human research purposes; therefore, fish bacteria OMVs could be also used as vaccines platforms. The present work is focused on the development of an oral vaccine based on recombinant *Aeromonas salmonicida* OMVs, expressing the G-protein of VHSV, and the evaluation of the B and T cell response at mucosal and systemic level in Rainbow trout. In order to validate the oral stimulation formula, first fish were stimulated with inactivated bacteria intra-peritoneally or orally using vaccine pellets. Distribution and proliferation of B and T-cell populations were analyzed in gut, peritoneum, spleen and head kidney using monoclonal antibodies by flow cytometry. Additionally, cell populations were sorted for characterization of membrane and secreted markers, expressed cytokines and transcription factors. The immune response is characterized by an early proliferation of intraperitoneal B and T-cells (24-48h post stimulation). Comparing the kinetics of the cell populations observed in the peritoneum and in the gut as well as the recruitment of cells from spleen or head kidney will be further analyze. The next upcoming trial will be done with the OMVs from *A. salmonicida* to further studies.
keywords: Rainbow trout, oral vaccine, OMVs, *Aeromonas salmonicida*, adaptive immunity.

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P-066.

Immune and pathogen interactions during experimental co-infection with *Piscirickettsia salmonis* and Piscine Orthoreovirus in *Salmo salar*

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Abstract
Piscine Orthoreovirus (PRV) infections are widespreadly distributed in Chilean salmon cultivation. And it is estimated that over 80% of freshwater Atlantic salmon is infected predominantly with PRV-1. In this scenario, mixed infections with other viruses or bacteria are likely to occur, and typical clinical signs could be misdiagnosed due to different responses triggered during a simultaneous infection with two or more pathogens. On the other side, *Piscirickettsia salmonis (P. salmonis)* is the most important bacterial pathogen for Chilean salmon cultivation. The objective of our pilot study was investigating viral and bacterial presence, some aspects of innate immune responses and histopathological features during an experimental challenge with P. salmonis in a population of Atlantic salmon smolt infected with PRV-1.
From a population of 240, PRV-1 positive smolt (100g), 84 shedder fish were intraperitoneally infected with *P. salmonis* and then allocated with 156 co-habitant smolts. Sampling was carried out at 14, 21, and 30 days post-challenge (dpc). Co-habitant fish were euthanized and denervated. Blood, head kidney and spleen samples were directed to molecular analysis and head kidney, spleen, liver, heart, and gills were obtained for histological examination.
Our results showed that viral loads diminished significantly from 14dpc to 21dpc and to 30dpc, but they did not disappear. Meanwhile, the percentage of *P.salmonis* positive fish increased from 21 to 30dpc. In accordance with the decreasing viral load, a significant drop of IFN-I transcripts was detected from 21-fold change at 14dpc to 11,7 at 21 and to 1,4-fold change at 30dpc. On the other hand, Mx transcripts did not show any considerable change during the experiment. Cytokine transcripts related to inflammatory bacterial infections such as IL-8 transcripts were upregulated 12,4-fold change at 14 and 7,9-fold change at 21dpc and decreased 2,7-fold change at 30dpc. However, IL-12 and IL-1β _transcripts showed no variation at any time point evaluated. Most of the fish showed no lesion, and just a few evidenced only mild to moderate lesions concordant with HSMI or SRS at different time points. These results suggest that PRV-1 infection could exert an apparent protective effect on the host against the bacterial infection diminishing the severity of SRS clinical and histopathological signs. Grants: FIE-Sernapesca 2015-V014, VIDCA UACH.
keywords: Co-infection, Piscine Orthoreovirus, Piscirickettsia salmonis, antiviral immune response, antibacterial immune response

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P-067.

Stress regulation and tolerance in shrimp: The transcriptomic and physiological response to chronic ammonia exposure in the black tiger shrimp, *Penaeus monodon*

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Abstract
Elevated ammonia (NH3) is a significant challenge in penaeid shrimp aquaculture worldwide, and can compromise shrimp osmoregulation, growth, immune-competency, leading to substantial crop loss. Despite the impact of elevated NH3 levels to the well-being of farmed penaeids, little is known about physiological and transcriptomic responses to chronic NH3 exposure. NH3 is a toxic byproduct of the break-down of uneaten feed, faeces and metabolic processes, and high NH3 levels are often difficult to mitigate quickly in ponds. This study investigated the physiological and transcriptomic response of sub-adult black tiger shrimp, *Penaeus monodon*,