Thursday September 6th – Gray / Palmer / Pope Ballroom
Virology 4 & 5
Moderators – Suja Aarattuthodiyil (Mississippi State Univ.) Nick Phelps (Univ. of Minnesota)

9:30 AM	Virology 4	Gorgoglione - Differential Effects of VHSV and IHNV Genes on the Modulation of the Host Innate Immune Response
9:45 AM		Emmenegger - Virulence of Spring Viremia of Carp Virus (SVCV) Strains in Two Koi Stocks
10:00 AM		Kvamme - Susceptibility of Sea Trout (*Salmo trutta*) to Important Viral Pathogens (SAV3 and PRV1)
10:15 AM		**Wei - Persistence of Cyprinid Herpesvirus 2 in Asymptomatic Goldfish Surviving in Experimental Infection**
10:30 AM		**Refreshments**
10:45 AM	Virology 5	Al-Hussinee - Partial Validation of a Taqman Real-Time Quantitative PCR for the Detection of Tilapia Lake Virus
11:00 AM		Kim - Development of a New Real-Time RT-PCR Using Peptide Nucleic Acid (PNA) Probes for Detection and Genotyping of VHSV
11:15 AM		Raissy - Molecular Identification of Viral Hemorrhagic Septicemia Virus (VHSV) in Rainbow Trout, Iran
11:30 AM		Roberts - Hydrodynamic Dispersion Model of Ostreid Herpesvirus to Improve Surveillance and Emergency Responses
11:45 AM		**OPEN**

Differential Effects of VHSV and IHNV Genes on the Modulation of the Host Innate Immune Response

Bartolomeo Gorgoglione * and Douglas W. Leaman

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Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV) are highly contagious pathogenic Novirhabdoviruses (Fam. Rhabdoviridae) affecting a wide range of fish species, both causing OIE notifiable diseases. VHSV genotype -IVb is now endemic in the Laurentian Great Lakes region. IHNV is not yet reported in the Great Lakes area, but is of high concern due to the economic importance of salmonids in the American Northwest. This study analysed the relative capacities of the individual IHNV and VHSV genes to interfere with the general host cell transcription and explores the action of IHNV genes on regulation of the Type I IFN pathway.

An efficient cell transfection protocol was optimised, including the use of new transfection reagents, for EPC, BF-2, RTG-2 and RTgill-W1 cell lines, dramatically increasing their transient transfection efficiency. Single VHSV and IHNV genes, including N, P, M, G (structural) and Nv (non-structural), were cloned into expression plasmids. To assess their impact on the cell general transcription, single VHSV and IHNV genes were co-transfected with specific luciferase constructs with a modified Actin promoter (pCAG) and a luciferase reporter assay was performed at 24 (exclusively for EPC) or 48 hours post transfection (hpt). For both viruses, P and G showed mild suppression of transcriptional potential, whereas M was confirmed as a potent suppressor of the host transcriptional activity in all cell lines tested. In contrast, the Nv gene showed a consistent stimulation of host transcription (from 2 to 8 fold-increase). IHNV-N gene showed a potent anti-host effect in BF-2, RTG-2 and RTgill-W1, but not in EPC, nor for VHSV-N. To characterise the impact of IHNV genes on the innate antiviral response, single genes were co-transfected with specific luciferase constructs with promoters for rainbow trout (*Oncorhynchus mykiss*) Type I IFN, MX-1 and IFITM1. The basal IFN production was stimulated by co-transfecting MAVS, and the luciferase reporter assay was performed at 72 hpt. As for their effect on the general transcription, P and G showed minor effects, N and M showed consistently strong suppression of transcription. IHNV-Nv consistently showed a strong stimulatory effect in all cell lines tested. The precise action of Nv gene on the viral replication is still unclear, although previous studies available in literature identified its action in subverting the innate immune recognition. In our study, we have instead recorded a consistently marked positive effect of Nv, comparatively assessed for either VHSV and IHNV, in boosting the cell general transcription and the IFN pathway in four distinct cell lines. This study provides novel insights on the viral regulators of the innate signalling, helpful to identify potential key targets to design more efficient vaccination strategies.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Virulence of Spring Viremia of Carp Virus (SVCV) Strains in Two Koi Stocks

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Spring viremia of carp virus (SVCV, species *Carp sprivivirus*), is considered one of the most lethal freshwater pathogens of cyprinid fish. Common carp *Cyprinus carpio carpio* and koi *C. carpio koi* are the most susceptible host fish species. The virus was formally described in the 1960's after outbreaks occurred in carp species on the European continent, but detections of SVCV have now also been reported in Asia, Middle East, South America, and North America. The virus genome contains five genes (N, M, P, G, and L) and phylogenetic analyses of the P and G genes separates SVCV isolates into four genogroups (Ia, Ib, Ic, and Id). In this study we compare the virulence of eight SVCV strains including representatives of each genogroup. These were tested in two koi breeds, long fin semi-scaled (Shusui) and short-fin fully scaled (Taisho Sanke). Koi fry challenged by immersion, with isolates from USA, China, and Europe, were observed for mortality and assessed for infection. Cumulative mortality ranged from 4 – 82% and 0 – 94% in the Shusui and Sanke koi breeds respectively. Each virus strain had similar levels of virulence (high, moderate, or low) in both of the koi breeds with the exception of the SVCV Fijan (genotype Id) isolate. Analysis of the virulence patterns and infection levels of the tested strains is ongoing. To our knowledge this is the first side-by-side virulence comparison of SVCV strains representing each genogroup in an infected primary host species.

Conference Designation: (Virology)
Presentation Format: (Oral)

Susceptibility of Sea Trout (*Salmo Trutta*) to Important Viral Pathogens (SAV3 and PRV1)

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Both PD and HSMI constitutes major disease problems in Norwegian Atlantic salmon (*Salmo salar*) farms. As open cages are the dominating production method there is a high probability of release of virus (e.g. SAV3, PRV1) to the environment. Wild fish may be exposed, contract infections and possibly develop disease. Diseased fish are particularly prone to predation, so there may be a potential for negative population levels effects. Due to the increased public concern about environmental impact of diseases in fish farming it is crucial to fill knowledge-gaps to support management decisions.

Due to its coastal residence, sea trout is at increased risk of exposure to, and infection by, disease agents released from farmed fish, when compared to wild Atlantic salmon. Despite this, surveys conducted annually by the Institute of Marine Research (IMR) has not detected elevated prevalence of these viruses in wild trout or salmon collected from areas of intense farming or many disease outbreaks.

Two important questions are to what extent sea trout is susceptible to the same pathogens as salmon, and if there are differences in susceptibility between different lifestages of these species to important pathogens found in aquaculture.

In order to address these questions, we have initiated a series of disease challenge experiments. Here we present the results of four challenge experiments, carried out in the disease challenge facilities of IMR, studying the susceptibility and time course of infection of SAV3 and PRV1 in sea trout and salmon post-smolts and fry. Viral load over time for each of the viruses was quantified using qPCR, and development of disease was evaluated by histology. The results suggest that both sea trout post smolts and fry are less susceptible to both PRV and SAV than salmon, and that the time course of the infections are different in these hosts. These results corroborate the findings of the wild fish surveys, and supports the conclusions in the IMR risk assessments for the environmental effects of Norwegian aquqaculture. The results are important for the evaluation of risk of population reducing effects on wild fish associated with the presence of SAV and PRV in farmed fish. It also demonstrates the complementary role of wild fish surveys and controlled disease challenge experiments.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Persistence of Cyprinid Herpesvirus 2 in Asymptomatic Goldfish Surviving in Experimental Infection

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Cyprinid herpesvirus 2 (CyHV-2) is the causative agent of herpesviral hematopoietic necrosis in goldfish *Carassius auratus* and Prussian carp *C. auratus gibelio*. Apparent healthy virus-carrier can be suspected as a source of the infection. In this study, we investigated virus persistence in the goldfish experimentally infected with CyHV-2. The virus DNA in the organs including spleen, kidney, heart, brain, gills and fin were monitored in fish groups reared constantly at a virus-permissive temperature (28°C). Cumulative mortality of the fish group was 89% in 2 weeks, and the spleen and kidney of the survivors showed high detection rate of virus DNA at 2 months after infection. We also monitored virus DNA in survivors, which had been treated with non-permissive water temperature (34°C) for 4 days initiated at 24, 48 and 72 h after virus infection and were subsequently reared at 25°C to make the fish with different virus loads. The results showed that DNA-positive rates in the organs were high in severely infected fish (72 h-group) even at 30 days after infection and the spleen and kidney showed commonly positive by PCR in all the groups. In addition, some organs dissected from the four of five asymptomatic survivors at 5 months after virus infection, where the virus DNA was negative by PCR initially, turned positive after being incubated *in vitro* in a medium for 5 days. The spleen, kidney and heart showed highest virus detection rates. By inoculation of the homogenate of the PCR-positive kidney tissue after being incubated *in vitro*, one of three fish died due to the virus infection. These results suggest that the hematopoietic tissues, spleen and kidney, can be the most potential persistent site of CyHV-2 and asymptomatic healthy surviving fish can be the potential source of the infection.

Conference Session Designation: (Virology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Partial Validation of a Taqman Real-Time Quantitative PCR for the Detection of Tilapia Lake Virus

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Since 2014, tilapia lake virus (TiLV) has emerged as a significant threat to tilapia production in Asia, Africa, and South America. Clinical signs associated with TiLV infections include lethargy, anorexia, and swimming at the surface away from schooling tankmates. Infected fish display gross lesions including gill pallor, exophthalmia, body discoloration (darkening), scale protrusion and loss, and ascites. The most common microscopic lesions associated with TiLV infections include a syncytial hepatitis and an encephalitis. To date, TiLV has not yet been detected in North America and there are no surveillance programs in the United States or Canada due to the unavailability of validated diagnostic assays. However, producers in the United States are increasingly being asked to ensure tilapia exports are TiLV-free. To fill this industry need, we developed a TaqMan real-time PCR assay targeting a conserved region within segment 9 of the TiLV genome. A series of experiments using a serially diluted standard revealed high assay analytical sensitivity and efficiency. The assay did not amplify other fish orthomyxoviruses including infectious salmon anemia virus and an uncharacterized orthomyxovirus isolated from koi. The reported TiLV qPCR assay is not only critical to ensure US producers continue to be able to export tilapia, but could also serve as an integral part of a coordinated US surveillance program aimed at protecting the naïve US tilapia industry from this globally emerging virus.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Development of a New Real-Time RT-PCR Using Peptide Nucleic Acid (PNA) Probes For Detection and Genotyping of VHSV

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Viral haemorrhagic septicaemia (VHS) is one of the most serious viral diseases in salmonid and olive flounder farms. The causative agent VHS virus (VHSV) is classified into four genotypes, I-IV, based on the sequence analysis of the N, G and NV-genes, respectively. Among the various diagnostic methods, gene detection by real-time reverse transcription PCR method (RT-qPCR) based on TaqMan-probe is a stable, rapid, specific and highly sensitive method for viral gene detection. However, the method can only check the amplification curves and Ct values. Peptide nucleic acid (PNA) is artificially synthesized DNA analogs with an uncharged peptide backbone. PNA probes can effectively detect a target gene by amplification and a melting temperature signal. It was reported that PNA probes can effectively distinguish between mis-matched sequences by their different melting temperatures in amplified PCR products. The present study report a new RT-qPCR method using the 3F2R primer set towards VHSV N-gene [a validated primer set of the conventional RT-PCR (320 bp) for detection of all genotypes of VHSV, Aquaculture 492 (2018) 170-183] for simultaneous detection and genotyping of VHSV using PNA probes which was designed based on genotype specific sequences of VHSV.

In the sensitivity test, the new RT-qPCR was compared with virus titration on fish cell cultures, and included 6 VHSV isolates representing the VHSV genotypes Ia, Ib, II, III, IVa and IVb, respectively. For 5 isolates the cell culture and the RT-qPCR showed almost same detection levels except the IVa isolate. In case of this isolate, the virus titration revealed 10 fold higher sensitivity compared to the new RT-qPCR method. Thus, it was confirmed that the sensitivity of the new RT-qPCR for detection of VHSV showed almost same level as the cell culture method.

For the melting point analysis, the new RT-qPCR reactions were performed using extracted viral RNA from 81 VHSV isolates including all known genotypes. The melting temperatures of 40 genotype I VHSV isolates using FAM and ROX was 70.0° and 72.0°C, respectively. In the case of 4 genotype II isolates, FAM and HEX showed temperatures at 70.5° and 65.5°C while the melting temperatures of 11 genotype III isolates with FAM and HEX was 70.0° and 58.0°C . In the case of genotype IV, ROX showed a melting point of 72.0 °C in all 26 isolates, while FAM showed 58.0 °C in 20 subtype IVa, isolates) and 44.5 to 49.5 °C in 6 subtype IVb, isolates.

It was thereby showed this PNA based RT-qPCR can be a useful tool to distinguish between all VHSV genotypes by checking their melting temperatures.

Conference Sesson Designation: (Virology)
Presentation format: (Oral)

Molecular Identification of Viral Hemorrhagic Septicemia Virus (VHSV) in Rainbow Trout, Iran

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Viral Hemorrhagic Septicemia (VHS) is one of the most important viral diseases of fish, especially rainbow trout. This research aimed to evaluate the rate of VHSV infection in rainbow trout in Chaharmahal va Bakhtiari, Lorestan, Esfahan and Kohkilooye va Boyerahmad Provinces which are the most important trout producing Provinces of Iran. For this purpose, between 2014 and 2016, suspected fish samples were collected from fish farms and studied by RT-PCR, after transportation to the laboratory in appropriate conditions. According to the results, it was found that 103 samples (10.6%) of a total of 964 studied samples were infected with VHSV. The rate of infection in 2014, 2015 and 2016 was 16.98, 8.92 and 7.82%, respectively. In three (3) years of study, the highest and lowest infection rates were observed in Chaharmahal va Bakhtiari and Kohkilooyeh va Boyerahmad Provinces, respectively, showing a significant difference ($p<0.05$). On the other hand, fishes with 1-50 g weight had the highest infection rate and the lowest infection rate was observed in fishes with more than 200 g weight ($p<0.05$). Considering the results, a decrease in disease incidence was recorded from 2014 to 2016, although full-scale actions are necessary for the eradication of VHS.

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Hydrodynamic Dispersion Model of Ostreid Herpesvirus to Improve Surveillance and Emergency Responses

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In 2014 a toxic algae bloom of *Karenia mikimotoi* caused a significant fish kill in South Australia, with concerned nearby fisheries and aquaculture sectors wanting to know its predicted spread over those coming days and weeks. Subsequently, the eSA-Marine system was developed to provide a hydrodynamic model of ocean conditions for future emergency responses (see www.pir.sa.gov.au/research/esa_marine).

The system maps past 'hind-cast', present 'now-cast' and future 'forecast' ocean conditions in South Australia. Conditions include sea surface height 'sea level', sea temperatures, sea salinity, ocean currents and wind speeds and directions. The system is directly applicable to aquatic animal health in South Australia providing predictions on the trajectories of harmful algal blooms, toxins, oil spill, and viral particles to allow for rapid emergency response actions.

Most recently the system was used to model the dispersal of ostreid herpesvirus (OsHV-1) particles from an outbreak in the Port Adelaide River estuary. This was the first incursion of OsHV-1 into South Australia, which put at risk the States $32 million / year oyster farming industry. The closest farming region is ~60km from Port Adelaide, while the closest oyster hatchery is ~25km away. Known biological parameters of the virus were used to run a series of hydrodynamic models to predict particle dispersal based on real time data. At the start of each run the model was seeded with 10,000 particles randomly arranged in a Gaussian distribution with a radius of approximately 1km of the mouth of the Port Adelaide River. The model demonstrated that live viral particles do not spread any greater than ~5km, while viral DNA may travel further at up to ~45km before deteriorating. Viral dispersal was dominated by tidal movements. The model informed the emergency response planning team of the likelihood of viral DNA reaching nearby regions and potentially interfering with PCR results of state-wide proof of freedom surveillance.

A current project funded by the Fisheries Research and Development Corporation is now utilising the system further by running a series of models in each of the oyster farming regions, and at other known feral oyster populations at ports and harbors. The hydrodynamic particle tracking outputs will assist with designing future surveillance activities by identifying epidemiologically relevant geographical areas (biosecurity zones) and thus defining sampling populations. Historically biosecurity zones have been defined by a conservative distance of 5 nautical miles (<10km) based on previous literature. Model outputs will also enhance future emergency responses to OsHV-1.

Conference Session Designation: **(** Epidemiology / Aquatic Animal Health Management)
Presentation Format: **(** Oral)