Popular science summary of the PhD thesis

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Title of the PhD thesis	Study of virulence markers in Viral Haemorrhagic Septicaemia Virus (VHSV)
PhD school/Department	DTU Aqua

Science summary

Viral haemorrhagic septicaemia virus (VHSV) is the causative agent of a very serious, listed and threatening disease affecting rainbow trout (*Oncorhynchus mykiss*) in aquaculture. It causes a systemic disease with various mortality rates in rainbow trout. VHSV belongs to the Rhabdoviridae family and possess a RNA genome of approximately 11000kb consisting of 6 genes encoding the viral proteins, nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G), non-virion (NV) and the polymerase (L). VHSV has been isolated from several species of fish, including marine species which can be infected and carry the virus without clinical symptoms. It has been previously found that VHSV isolated from marine species causes low to no mortality in rainbow trout, while VHSV isolated from rainbow trout has similar results in challenges with marine species. Many earlier studies have tried to identify genetic markers of VHSV to rainbow trout with limited success. The development of new technologies for DNA sequencing such as Next Generation Sequencing (NGS) has recently supported the full genome sequencing of a large number of VHSV field isolates while the development of reverse genetics techniques allowed the manipulation of the entire genome of VHSV.

In this project, we have assessed *in vivo* virulence by immersion challenges in rainbow trout of several VHSV isolates obtained from the DTU Aqua VHSV repository. These isolates were also subjected to full genome sequencing by NGS and analyzed together with other mixed virulence isolates generating the largest dataset of VHSV full genome sequences up to date. Based on early studies and these analyses results we hypothesized that virulence markers of VHSV to rainbow trout are located in the nucleoprotein gene. By the establishment of a new reverse genetics system based on the low virulent VHSV isolate SE SVA 1033-9C as a backbone, we were able to rescue three mutant recombinant VHSVs (rVHSVs) with specific amino acid residue changes in the nucleoprotein and two chimeric rVHSVs with parts containing the nucleoprotein gene exchanged from the high virulent VHSV DK-3592B to the low virulent SE SVA 1033-9C. The virulence of these rVHSVs was evaluated by *in vivo* challenges in rainbow trout by intraperitoneal injection (IP) and by immersion. Results indicated that both strategies caused gain-of-function in IP injected rainbow trout but not in immersion infected. We show evidence that the nucleoprotein is implicated in VHSV virulence to rainbow trout. However, the results also show that these alterations are probably not acting alone and therefore further investigation is necessary to look into the role of other genes and intergenic regions that could be associated with virulence to rainbow trout.