Molecular characterization of infectious pancreatic necrosis virus and its contribution to coinfection

1. Research project objectives/Research hypothesis:

The purpose of the study is the molecular characterization of the infectious pancreatic necrosis virus (IPN) , the identification of serotypes of the virus present in Poland using molecular techniques and the sequencing of selected isolates to create a phylogenetic tree and characterize the origin of the Polish strains of the IPN virus. Next Generation Sequencing of the VP1, VP2, VP3, VP5 genes of the virus will be carried out and determine the role of these genes in virulence, as well as definition by flow cytometry of the role of the IPN virus in dual infections for example by defining apoptosis in cell lines and blood cells of salmonids, as well as the influence of factors such as interferon on viral infections and expression of TLR3 receptor gene in rainbow trout internal organs as a response to single and double viral infection.

2. Research project methodology:

BF-2 and EPC cell lines will be required to amplify archived IPN virus isolates. When the cytopathic effect occurs, isolation of the genetic material of the IPN virus will be carried out using the commercially available RNA kit "Total RNA" A&A Biotechnology. Next, a confirmatory identification of the genetic material by reverse transcription and Rt-q-PCR will be performed. The resulting reaction products will be used for NGS sequencing, and the results will be used for phylogenetic studies to identify the origins of the Polish strains of the IPN virus. With appropriate bioinformatic software such as Geneious, a phylogenetic tree with Polish sequences and sequences from the Genebank date will be created. On this basis, the Polish isolates will be checked for the relevant serotypes, as well as for potential mutations in the A segment, VP2, which contains virulence markers and is particularly taxonomic for genotyping and VP5, VP4, VP3. The BF-2, EPC, CHSE-214 and RTG cell lines (rainbow trout gonads) will be infected with selected strains of the IPN virus (various serotypes) and then infected with IHN, VHS, SAV viruses to determine cell line cel apoptosis during coinfection and check Defense mechanisms that occur in the cell - induction of interferon by viral infection (molecular method - real time PCR). In in vivo studies will be analyze blood cells - apoptosis by flow cytometry and expression interferon and receptor TLR3 genes by real time RT-PCR.

3. Expected impact of the research project on the development of science:

The reason for taking up a given research topic was the lack of information on the constantly occurring strains of infectious pancreatic necrosis virus in Poland. It is only possible to draw conclusions about whether or not the strains present in Poland are pathogenic, but it is not known if they belong to European isolates (A2-A5) or whether isolates from the United States (A1) and Canada (A6-A9). Between different serogroups / serotypes there is a high level of antigenic diversity and differences in virulence and pathogenicity. The VP2 gene plays a major role in virulence, but is also attributed to other genes such as VP1, VP3 and VP5. Knowing this information would allow to characterize the Polish strains of the IPN virus and assign them to the relevant serogroup / serotype. In addition, the mechanisms that are induced during infection with infectious pancreatic necrosis virus (IPNV), infectious haematopoietic necrosis virus (IHNV), viral haemorrhagic septicemia virus (VHSV), sleeping alphavirus (SAV). It would be important to the health of fish because IPNV infection probably protects the vectors against the propagation of another virus. This is a topic that is extremely important for salmonid fish farmers (mainly rainbow trout farms, which in Poland is a significant amount) who are suffering from the above listed pathogenic organisms.