GENETIC DIVERSITY OF PISCINE ORTHOREOVIRUS (PRV)

Frederick S. Kibenge[1], Marcos G. Godoy[2], Molly J. Kibenge[1], Yingwei Wang[3], Rudy Suarez[2], Camila Leiva[2], Francisco Vallejos[4]

[1]Department of Pathology & Microbiology, Atlantic Veterinary College, University of Prince Edward Island, PEI, CANADA
[2]Centro de Investigaciones Biológicas Aplicadas (CIBA), Puerto Montt, CHILE; [3]School of Mathematical & Computational Sciences, University of Prince Edward Island, PEI, CANADA; [4]Friosur, Aysen, CHILE

Introduction: Piscine orthoreovirus (PRV) is a newly emerging aquatic animal reovirus first described in 2010 in association with heart and skeletal muscle inflammation (HSMI) in marine-farmed Atlantic salmon in Norway (Palacio *et al.*, 2010). A new disease in rainbow trout similar to HSMI and associated with a PRV-related virus has also been reported in Norway (Olsen *et al.*, 2015). PRV belongs to the family *Reoviridae* (**Figure 1**), subfamily *Spinareovirinae*, and has been proposed as a member of the genus *Orthoreovirus*. The virus is ubiquitous in Atlantic salmon in Norwegian aquaculture, and has also been detected in wild Atlantic salmon, sea-trout and in certain marine fish species along the coast of Norway (reviewed in Kibenge and Godoy 2016). PRV was first reported outside of Europe in 2013 in farmed Atlantic salmon in Chile and in farmed and wild salmonids in British Columbia-Canada (Kibenge *et al.*, 2013), and recently in hatchery Chinook salmon and coho salmon in Washington state-USA (WFRC, 2014). Outside of Europe, HSMI in Atlantic salmon and HSMI-like disease in coho salmon associated with PRV have only been reported in Chile (Godoy *et al.*, 2016), but HSMI may also be present in farmed Atlantic salmon samples collected from an aquaculture facility in 2013-2014 in British Columbia-Canada (DFO, 2016). The genetic variation of PRV in relationship to the geographical distribution of pathogenic strains of PRV in salmonid aquaculture is poorly understood.

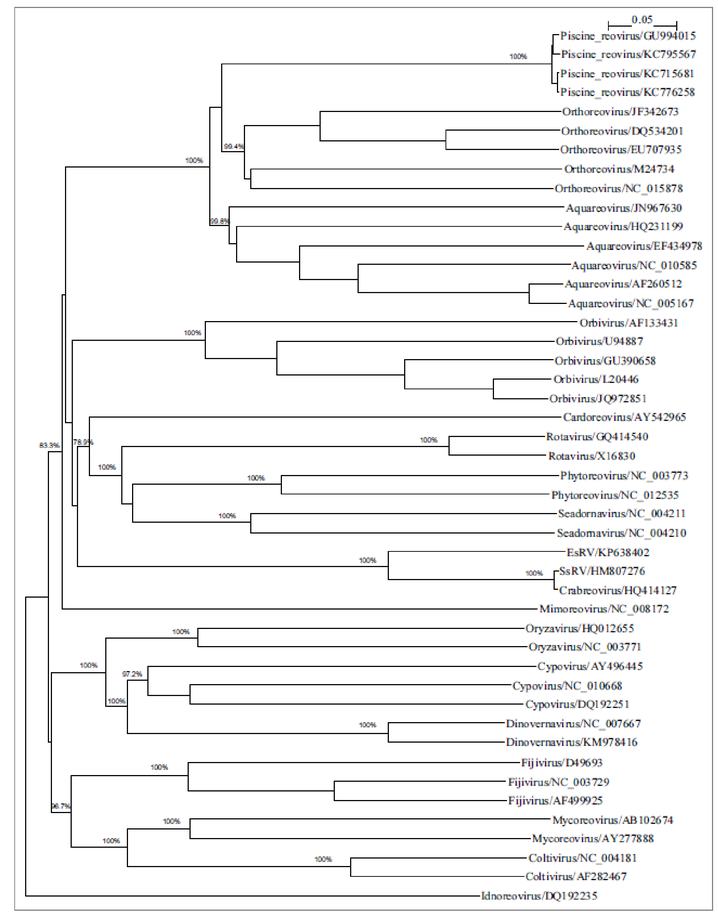

Figure 1: Phylogenetic tree showing the relationships within family *Reoviridae* using RdRp nucleotide sequences of selected members in the different genera. The sequences used for comparision are identified by the genus name/GenBank accession number (Original figure appears in Kibenge and Godoy 2016).

Findings: We show here that PRV occurs in 2 genotypes, I and II (**Figure 2**). Genotype I has 2 sub-genotypes, Ia which is very variable and occurs in Norway, Canada, USA and Chile, and Ib which is less variable and occurs in Norway and Chile. The geographical areas covered by PRV sub-genotypes Ia and Ib in Norway and Chile are overlapping (even in the same fish farms), but they appear to spread independently as if they were two different viruses. This is also evidenced by the relatively long branch separating the two sub-genotypes in the tree. Genotype II includes the Norwegian PRV-related virus associated with HSMI-like disease in rainbow trout and PRV isolates from coho salmon in Chile. To date Genotype II sequence has not been found in any fish species in Canada.

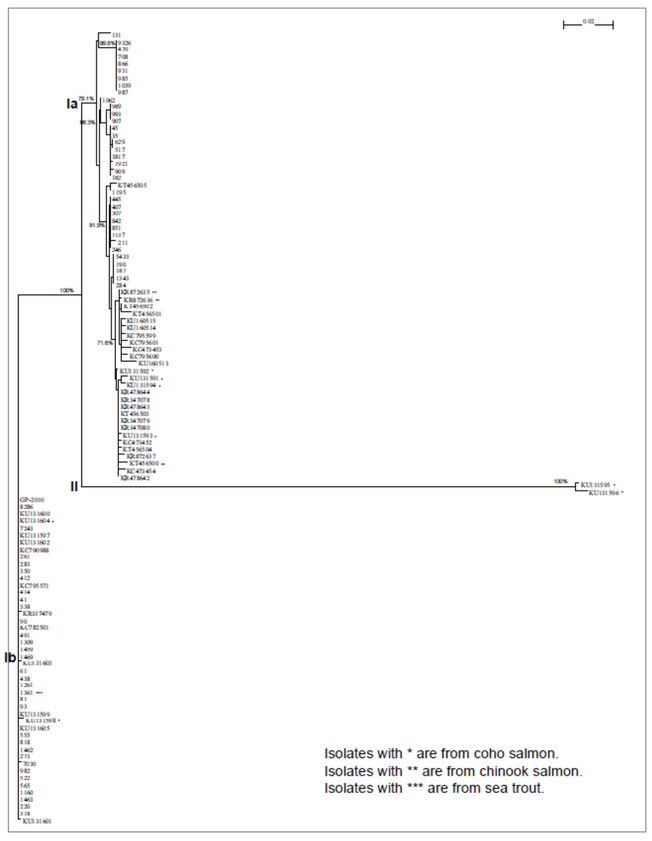

Figure 2: Phylogenetic tree of Segment S1 sequences from PRV isolates from Norway, Canada, USA and Chile. The phylogenetic tree was constructed using Maximum Likelihood method (software package PhyML 3.0). An outgroup (Genbank accession number: AF059720) was used to determine its root, but the outgroup itself was not included in the tree. Bootstrapping was performed 1000 times and branches with ≥60% bootstrapping support value are marked. The scale bar represents the number of substitutions per site.

In Chile, farmed Atlantic salmon with classical HSMI as described in Norway (**Figure3A-D**) had PRV sub-genotype Ib, whereas the PRVs in farmed coho salmon were more diversified, grouping as sub-genotype Ia or Ib or Genotype II, and the HSMI-like lesions were also unique (**Figure 3E-H**). The sub-genotype Ia from Chilean coho salmon clusters with Garseth *et al.* (2013) Group II Norwegian PRV isolates, and the Canadian PRV isolates (regardless of fish species source), whereas sub-genotype Ib with Garseth *et al.* (2013) Group I is distinctly a Norway-Chile subgroup (**Figure 2**).

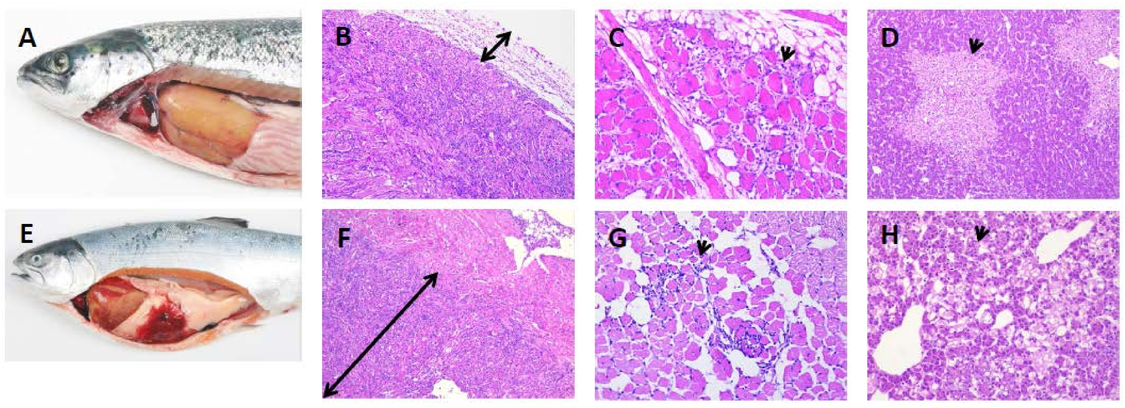

Figure 3: Images of HSMI lesions in farmed Atlantic salmon *Salmo salar* (panels A-D) and HSMI-like lesions in farmed coho salmon *Oncorhynchus kisutch* (panels E-H). **(A)** Atlantic salmon with haemopericardium, pale liver, liver pseudomembrane and petechial haemorrhage in liver. **(B)** Heart section stained with haematoxylin and eosin (H&E) showing diffuse infiltration of mononuclear cells in epicardium (arrow) and infiltration of mononuclear cells in both the compact and spongious layers of myocardium. **(C)** Red muscle section stained with H&E showing infiltration of mononuclear cells, degeneration and necrosis of muscle fibers (arrow head). **(D)** Liver section stained with H&E showing focal necrosis of hepatocytes (arrow head). **(E)** Coho salmon with haemopericardium and blood clot in abdominal cavity. **(F)** Heart section stained with H&E showing diffuse infiltration of mononuclear cells in the spongious layer of myocardium. **(G)** Red muscle section stained with H&E showing infiltration of mononuclear cells, degeneration and necrosis of muscle fibers (arrow head). **(H)** Liver section stained with H&E showing focal necrosis of hepatocytes (arrow head).

Pairwise amino acid sequence analysis of p13 protein showed no amino acid identity cut-off value between sub-genotypes Ia and Ib and Genotype II (**Table 1**). Since p13 is predicted to be cytolytic, both PRV genotypes are potentially pathogenic.

Table 1: Pairwise comparision of range of amino acid sequence percent identity of p13 protein of PRV genotypes I and II strains

PRV Genotype	I		II
	Ia	Ib	
Ia	96.0-100		
Ib	90.3-93.5	99.2-100	
II	78.2-80.6	77.4-78.2	97.6-100

Conclusions: Because both PRV sub-genotypes Ia and Ib contain isolates sampled in the period 2007 to 2015, we conclude that Ia and Ib diverged before 2007. PRV sub-genotype Ib is suggested to be the original virus that gave rise to sub-genotype Ia and Genotype II later since Ib strains have the least genetic variation of the three groups.

References:
1. DFO, 2016. Potential diagnosis of heart and skeletal muscle inflammation in Atlantic salmon at a B.C. fish farm. http://news.gc.ca/web/article-en.do?nid=1069579 (Accessed May 22, 2016)
2. Garseth *et al.*, 2013. Phylogenetic evidence of long distance dispersal and transmission of piscine reovirus (PRV) between farmed and wild Atlantic salmon. PLoS ONE, 2013 8:e82202.
3. Godoy *et al.*, 2016. First description of clinical presentation of piscine orthoreovirus (PRV) infections in salmonid aquaculture in Chile and identification of a second genotype (Genotype II) of PRV. Virol. J. *in press*.
4. Kibenge *et al.*, 2013. Whole-genome analysis of piscine reovirus (PRV) shows PRV represents a new genus in family *Reoviridae* and its genome segment S1 sequences group it into two separate sub-genotypes. Virol J 2013, 10:230.
5. Kibenge FSB, and Godoy MG. 2016. Reoviruses of aquatic organisms. In: Kibenge, F.S.B., and Godoy, M.M. (Eds.), Aquaculture Virology. Elsevier, Amsterdam.
6. Olsen *et al.*, 2015. First Description of a new disease in rainbow trout (*Oncorhynchus mykiss* (Walbaum)) similar to heart and skeletal muscle inflammation (HSMI) and detection of a gene sequence related to piscine orthoreovirus (PRV). PLoS ONE 2015, 10:e0131638.
7. Palacios *et al.*, 2010. Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLoS One 2010, 5:e11487.
8. Wiik-Nielsen *et al.*, 2012. First detection of piscine reovirus (PRV) in marine fish species. Dis Aquat Org 2012, 97:255–258.
9. WFRC (Western Fisheries Research Center) Information Sheet, May 2014. Piscine reovirus. Accessible at: http://wfrc.usgs.gov/fieldstations/hq/viruspdf/piscinereovirus05272014.pdf

Funding: FSK's lab is funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant.

American Society for Virology 35th Annual Meeting, June 18-22, 2016. Virginia Tech, Blacksburg, Virginia. Program/Board Number P10-5.