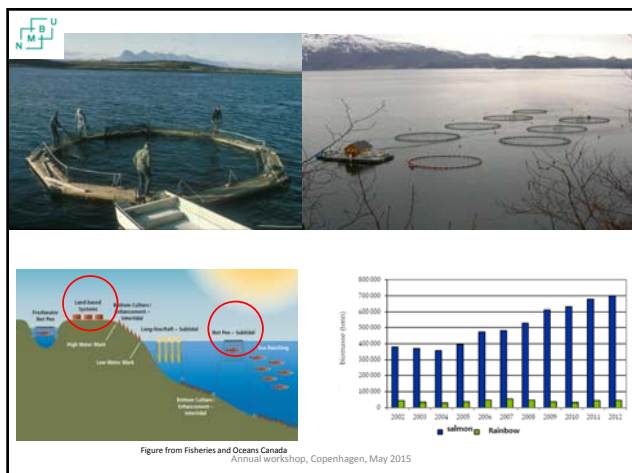


**Emerging virus diseases in fish globally**

Virus	Genome	Virus family	Geographic distribution
Infectious haematopoietic necrosis virus (IHNV)	-ssRNA	<i>Rhabdoviridae</i>	Europe, North America, Asia
Viral haemorrhagic septicaemia virus (VHSV)	-ssRNA	<i>Rhabdoviridae</i>	Europe, North America, Asia
Spring viraemia of carp virus (SVCV)	-ssRNA	<i>Rhabdoviridae</i>	Europe, Asia, North and South America
Infectious salmon anaemia virus (ISAV)	-ssRNA	<i>Orthomyxoviridae</i>	Europe, North America, Chile
Salmon pancreas disease virus (SAV)	+ssRNA	<i>Tagoviridae</i>	Europe
Viral nervous necrosis virus (VNNV)	+ssRNA	<i>Nodaviridae</i>	Australia, Asia, Europe, North America, Africa, South Pacific
Piscine orthoreovirus (PRV)	dsRNA	<i>Reoviridae</i>	Europe, North America, Chile
Piscine myocarditis virus (PMCV)	dsRNA	<i>Totiviridae</i>	Europe
Koi herpesvirus (KHV)	dsDNA	<i>Alloherpesviridae</i>	Asia, Europe, North America, Israel, Africa
Epizootic haematopoietic necrosis virus (EHNV)	dsDNA	<i>Iridoviridae</i>	Australia, Europe, Asia, North America, Africa
Red sea bream iridovirus (RSIV)	dsDNA	<i>Iridoviridae</i>	Asia

Modified from Walker and Winton, Vet Res 2010 Annual workshop, Copenhagen, May 2015





### Orthoreovirus structure

- The serotype-specific antigen of the orthoreoviruses is protein  $\sigma 1$ . Antigenic recognition of this protein is the basis for three major serotypes of MRV and 5–11 serotypes of ARV.

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### PRV- in RBC

**Isolated erythrocytes**

**PCR**

- High PRV load in RBC
- The main fraction of blood-PRV is associated with RBC

Wpc	RBC (Ct-value)	Plasma (Ct-value)
3	~35	~35
4	~35	~35
5	~25	~35
6	~20	~35
7	~18	~35
8	~18	~35
3-4	~35	~25
5-6	~25	~20
7-8	~20	~18

### PRV- in RBC

**Isolated erythrocytes**

- High PRV load in RBC
- The main fraction of PRV is in RBC

**Flow cytometry**

- PRV positive population
- Correlation with PCR
- > 50% PRV positive

Wpc	PRV $\sigma 1$ Positive (%)
5 wpc	2%, 28%, 43%
7 wpc	51%, 37%, 31%

### PRV- in RBC

**Virus inclusions in RBC**

- Variable size
- Cytoplasmatic
- PRV protein
- dsRNA

**Phase contrast**  
Co-localization w/PRV staining

**Viral factories**

### PRV- in RBC

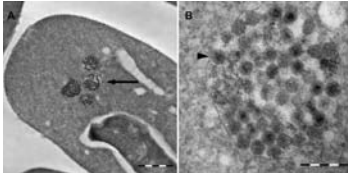
**Virus particles**

- Naked
- 73 nm

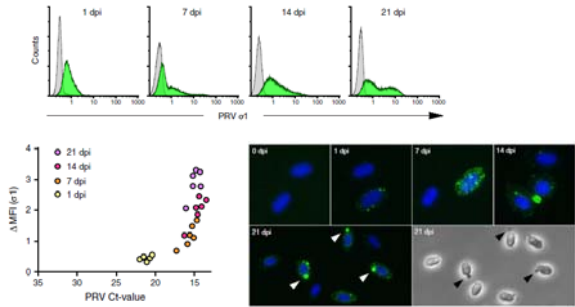
*Family Reoviridae*

**Inclusions**

- Lamellar structure
- Virus particles

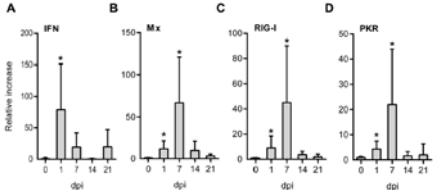


### PRV can be cultivated in RBC



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### RBC antiviral responses to PRV infection



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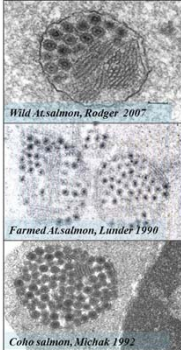
### PRV- in RBC


**Erythrocytic inclusion body syndrome (EIBS)**

- Virus inclusions in RBC
- Salmonids
- Healthy and diseased fish

*Virus unknown*

**PRV associated with EIBS**






### Summary- HSMI - an emerging disease?

- PRV is ubiquitous in Atlantic salmon SW farms
- Transmission routes are not well described
  - Horizontally
  - Vertically?
- Virulent and less virulent strains.
- Vaccination
  - Difficult to cultivate
- Selective breeding?


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### Amount of PRV in Atlantic salmon population

- The weight of an average farmed salmon infected with PRV is set to  $\approx 1$  kg,
  - 6 % is blood (60ml); Number of RBC is  $1 \times 10^6$  /ml i.e.
  - Number of RBC per kg fish is:  $60 \times 10^6$ .
- Number of salmon per year-class is  $300 \times 10^6$ .
  - Number of RBC per year-class is:  $60 \times 10^6 \times 300 \times 10^6 = 1.8 \times 10^{14}$
- 50% of RBC is infected.
  - $0.9 \times 10^{14}$  per year-class
- Number of PRV produced per cell (guessing)  $10^5$ / infected cell
  - Number of PRV in farmed salmon in Norway per year is  $0.9 \times 10^{14} \times 10^5 = 0.9 \times 10^{19}$

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### Amount of PRV in Atlantic salmon population

- The diameter of PRV is 72 nm ( $7.2 \times 10^{-8}$  m)
- The length of total PRV per year in farmed Atlantic salmon in Norway is in the range  $0.9 \times 10^{19} \times 7.2 \times 10^{-8}$  m =  $6.5 \times 10^{11}$  m
- Distance to the Sun is  $1.5 \times 10^{11}$  m.
- It can't be a very virulent virus

➔ A tiny change in virulence may impact the performance of the farmed salmon population

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### PRV - an emerging virus

- Other species?
  - A new disease was described (NVI, 2013), in Rainbow trout, freshwater (weight 25-100 g). Inflammation in heart, skeletal muscle and liver
  - A virus (Virus Y) was detected using  $\sigma 3$  PRV primers. 80-85% homology to PRV (pers comm, several at NVI).
  - Can be detected in erythrocytes in Rainbow, serological cross reaction to PRV (own observation)

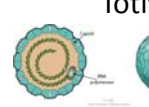
➔ If the genetic and serological homology are confirmed, this is a new strain of PRV. (PRV2).

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### Cardiomyopathy syndrome- detection of another "new" virus

Can't be cultivated in cell cultures  
Isolate RNA from fish with CMS

454-pyrosequencing etc.



**Totivirus**

dsRNA linear genome  
Non enveloped, icosah

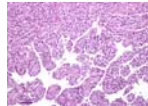

**Table 2. Piscine totivirus PCR results**

Sample no.	Type of sample	Tissue	Positivity
440	CMS outbreak	Heart/ kidney	4/4
479	CMS outbreak	Kidney	5/5
48	CMS outbreak	Heart/ kidney	5/5
89	CMS outbreak	Heart/ kidney	4/4
110	CMS outbreak	Heart/ kidney	10/10
12	CMS outbreak	Heart/ kidney	4/4
19	CMS outbreak	Heart	10/10
223	HSMI outbreak	Heart/ kidney	9/9
684	HSMI outbreak	Heart/ kidney	9/9
7607	HSMI outbreak	Heart/ kidney	9/9
PE501	Healthy, farmed Atlantic salmon	Heart/ kidney	9/9
PE1311	Healthy, farmed Atlantic salmon	Heart/ kidney	5/5
PE1	Healthy, farmed Atlantic salmon	Heart/ kidney	9/10
88	Healthy, farmed Atlantic salmon	Heart	9/9
98	Healthy, wild Atlantic salmon*	Heart	9/9

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### PMCV

- Mainly the spongy part of myocard that is affected.
- No particular reservoir has been found
- Genetically homogenous virus
- The probability of getting CMS increases with (From Jensen et al 2013)
  - increasing time in the sea,
  - Infection pressure
  - cohort size
  - Previous HSMI diagnosis
  - CMS in previous cohorts

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### Do PRV and PMCV cause notifiable diseases?

- Short answer: No
- International spread
  - Yes/no
- Zoonotic potential
  - No
- Significant spread in naive population
  - Yes/no
- Emerging disease- rapid spread
  - Yes/no

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## Thank you for your attention

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