PRVom
Emerging pathogen in Rainbow trout

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PLOS ONE

RESEARCH ARTICLE

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)

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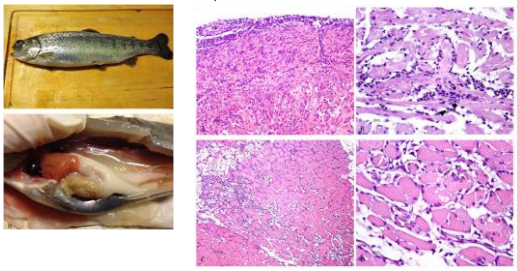
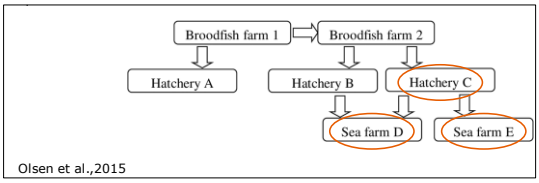
Background- 1 Disease outbreak

In fall 2013, mortality outbreak in FW Rainbow trout.
Diagnostic show no significant known pathogen
Inflammatory lesions in the heart similar to the ones observed with PRV/AS

Olsen et al.,2015



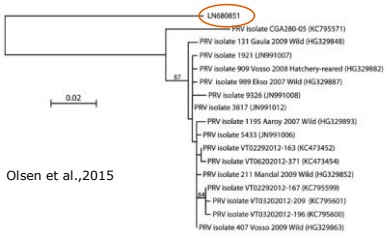
Background-2 Epidemiology



Olsen et al.,2015

Background-3 Aetiological agent?

Detection of new viral PRV-like viral RNA



Olsen et al.,2015

Fig 4. **Phylogenetic tree.** The phylogenetic tree is showing the genetic distance between the PRV-related virus (Acc. No. LN680851) and various piscine orthoreovirus (PRV) isolates. A neighbor-joining analysis was done using Kimura 2-parameter distances and GenBank accession numbers have been indicated. Bootstrap values above 80% are reported.

High load of PRV-like viral RNA in Blood cells

- Affected RT characterized by Anemia and lesion in heart

PRV-AS Wessel Finstad et al., 2014

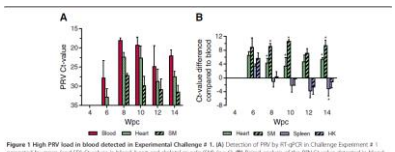


Figure 1 High PRV load in blood detected in Experimental Challenge # 1. (A) Detection of PRV by RT-qPCR in Challenge Experiment # 1 presented by mean (and SE) Ct-values in blood, heart and skeletal muscle (SM) (n = 6). (B) Paired analysis of the PRV Ct-value detected in blood compared with samples from heart, skeletal muscle (SM), spleen, head kidney (HK) of the same fish. The results are presented as the mean Ct-value difference at each time point. Data were analyzed using Wilcoxon matched pairs signed rank test, *p< 0.05.

Experimental work:

- Cooperative project between NVI and NMBU-Oslo and DTU-VET EURL Copenhagen to:
  - AT DTU:
    - confirmation of new viral aetiological agent causing disease in RT
    - pathogenicity assessment of new disease
    - pathogenesis investigation to provide guidelines for diagnostics procedure
  - AT NVI-NMBU:
    - Assess risk for Atlantic salmon production for new viral disease
  - JOINT EFFORT; investigate immune response in AS and RT to PRV-like infection

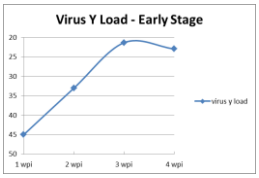
1st Trial: Production of reference material

1st trial – How do we produce infectious material of a virus that is not cultivable in vitro?

- Infected blood from preliminary trial produced at NVI
- 15 adult rainbow trout 250-300 gr
- IP injection
- Weekly monitoring of Ct values in blood samples

1st trial- Results

- 1 dead fish, qPCR for PRV-om positive in heart, spleen, kidney and blood
- 3-4 W.P.I peak of virus in blood



	1 wpi	2 wpi	3 wpi	4 wpi
sample 1	33,26	31,24	21,13	19,93
sample 2	25,48	33,04	45	23,26
sample 3	45	45	19,48	20,62
sample 4	45	45	45	22,94
sample 5	45	30,76	21,54	23,56
sample 6			18,37	
sample 7			23,12	
sample 8			18,98	
sample 9			26,23	
sample 10			45	
sample 11			20,68	
sample 12			21,8	
sample 13			20,84	
sample 14			20,4	

Dead fish 3w.p.i.	Heart	kidney	spleen
	26.28	28.96	29.18

2nd Trial:

Challenge model and long term pathogenesis study

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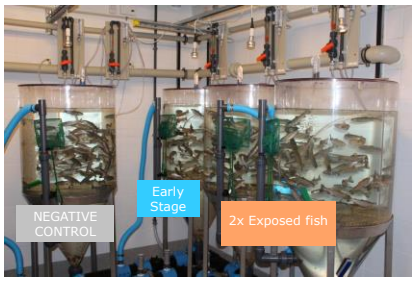
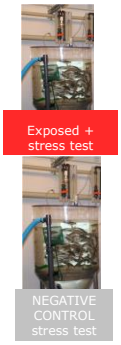
Can we reproduce the disease in experimental conditions?

Trial design





M&M

- Shedders injected IP with 0,1mL of inoculum and adipose fin clip for tagging (mock infection was performed with naive blood diluted in L15 media);
- Shedders and Cohabitants were kept in cohabitation for 14 weeks and sampling was performed in pre determined time points.

Groups
30 Virus Y injected shedders + 30 cohabitants
30 Virus Y injected shedders + 30 cohabitants
40 Mock infection shedders + 40 cohabitants
40 fish Virus Y injected for early stage infection studies
10 (5 shedders+ 5 cohab)+10 (5 shedders+ 5 cohab) from tank 1 and 2 12 WPI

Sampling-time points

Week		0	1	2	3	4	5	6	7	8	9	10	Stress-test	11	12	13	14
Treatment	Total #/gruppe																
# RT/ cohabitants	30					5				5							
# RTshedders injected i.p.	30					5				5							
# RT/ cohabitants	30							5				5					
# RT/shedders injected i.p.	30							5				5					
From each tank 5 shed + 5 cohab are moved													20		(5shed+ 5 cohab)		(5shed+ 5 cohab)
# RT/ cohabitants	40					10							20		10		10
# RT/shedders injected i.p.	40					10											
RT injected for early stage	40		5	5	5						Tank termi nated						
Total #	240					30		10		10		10			20		20

Sampling-Tissues analysis

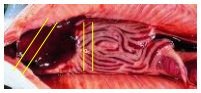
Number	Preservative	Organ	Code	User	Analysis
1	Heparin	Blood	BL	DTU	Virus y qPCR
2	RNALater	Gills	GI	NVI	Virus Y qPCR + immune gene expression
3	RNALater	Heart	HE	NVI	Virus Y qPCR + immune gene expression
4	RNALater	Spleen	SP	NVI	Virus Y qPCR + immune gene expression
5	RNALater	Liver	LI	NVI	Virus Y qPCR + immune gene expression
6	RNALater	Pancreas	PC	NVI	Virus Y qPCR + immune gene expression
7	RNALater	Mid-gut	MI	NVI	Virus Y qPCR + immune gene expression
8	RNALater	Head Kidney	HK	NVI	Virus Y qPCR + immune gene expression
9	RNALater	Muscle	MU	NVI	Virus Y qPCR + immune gene expression
10	Formalin	Organpacket *	F	NVI	Histopathology
11	L15	Blood	BL – B	DTU	BACKUP -80
12	L15	Gills	GI – B	DTU	BACKUP -80
13	L15	Heart	HE- B	DTU	BACKUP -80
14	L15	Spleen	SP – B	DTU	BACKUP -80
15	L15	Liver	LI – B	DTU	BACKUP -80
16	L15	Pancreas	PC- B	DTU	BACKUP -80
17	L15	Mid Gut	MI – B	DTU	BACKUP -80
18	L15	Head Kidney	HK – B	DTU	BACKUP -80
19	L15	Muscle	MU - B	DTU	BACKUP -80

INFECTION TRIAL – rt/virus Y - HISTOPATHOLOGY

GILL

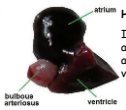
2nd arch-

whole or piece at least 1 cm



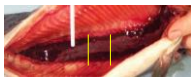
LIVER

PYLORIC CAECA



HEART

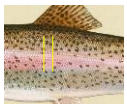
Include atrium, b.a. and ventricle



KIDNEY

mid-part

SPLEEN



MUSCLE

Include sideline, red and white muscle

A wish: Include brain at the two last samplings from some fish

In general: Piece thickness 3-4 mm, 10 x formalin to organs

Instruction from A.B.Olsen

VIB/abo

Sampling-Tissues analysis

- Blood qPCR to detect viral load according to protocol of Olsen et al.,2015

	Name	Sequence 5'- 3'
Forward primer	ORMV_1F	TCG TGG TTC CAA TGA CAG
Reverse primer	ORMV_1R	CCA ACC ACT AAA ACC GAG
Probe	ORMV probe	FAM-ACG CCT TAG AGA CAA CAT GCG AAG –BHQ-1

- Heart tissue qPCR for virus load and Histopathology, IHC (at NVI)
- Spleen tissue for immune gene response

2nd trial - Results

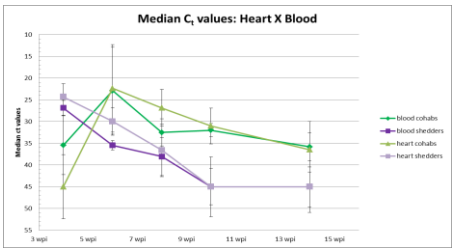
During 14 weeks trial only 1 shedder 25 days post infection (3-4wpi), which later had the infection confirmed by RT-qPCR on the heart, spleen and kidney tissues. Findings included pale gills, ascites and hemorrhages (similarity with gross lesions described in field outbreak).

Dead fish 3w.p.i.	Heart	kidney	spleen
	26.79	23.55	24.88

Experimentally infected fish

Diagnostic case

Virus Kinetics Heart and blood

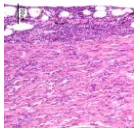


2 "waves" of infection 4wpi peak in shedders; 6wpi peak in the cohab.

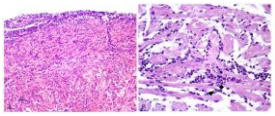
4wpi shedders peak of virus in blood and heart; in 6 weeks fish clear the infection.

6 wpi cohab peak of virus in blood and heart; still positive 14 wpi

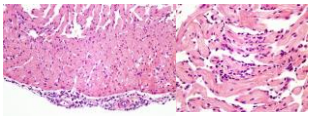
Histopathology



Field Outbreak
PRV-AS
AS



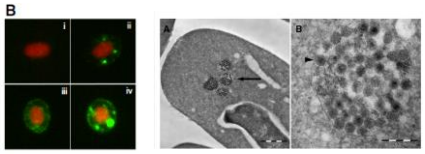
Field Outbreak
PRV-OM
RT



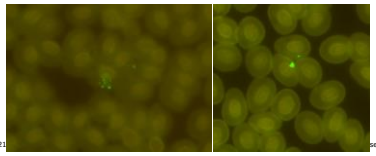
Experiment
PRV-OM
RT

Viral factories in RBC

PRV-AS in RBC (Finstad et al.2014)



PRV-OM in RT RBC



Conclusion

- PRV om can infect RT horizontally and induce lesions consistent with field cases report
- It is an acute infection characterized viral peak and clearence (VS PRV-AS)
- Under these experimental conditions, mortality is low
- Blood and heart are target organs for sampling for diagnostics and surveillance

Outlook

- Survey in Europe to describe prevalence?
- Vertical transmission?
- It is now possible to cultivate PRV-as in vitro, investigate PRV-om
- Use PRVom as vaccine for PRV as?

Thanks to all people that contributed into this project

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Norwegian Veterinary Institute

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NMBU

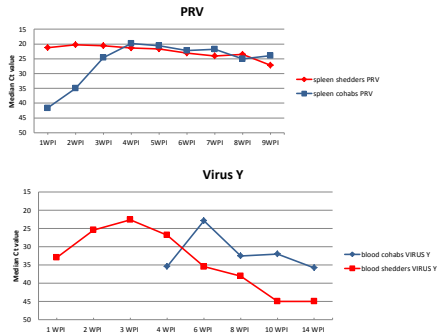
Thanks for your attention

2nd trial – Results
Stress test

- 10 fish from each replicate of the treated fish and 20 fish from the controls were net for 1 min. Fish were placed in 2 tanks respectively and sampled in the following week.
- Negative fish always tested negative, whereas only 1/5 fish blood tested for cohab and shedder tested positive 2 weeks post stress and; 2/5 fish blood tested positive 4 weeks after stressed.

This stress test does not seem to boost the infection; other options (salinity) could be considered in the future

PRV in AS and PRV-2/Virus Y in RT



2nd trial - weight