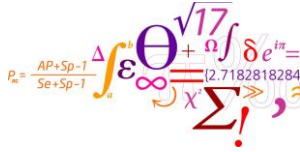# Risk assessment of new VHSV from Lumpfish for Rainbow Trout, Atlantic Salmon and Lumpfish

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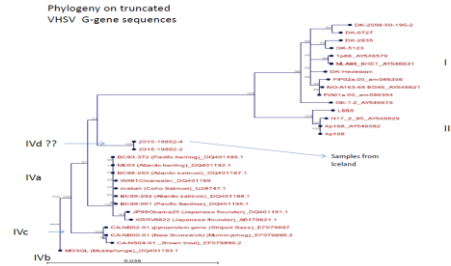
DTU Vet
National Veterinary Institute

Background

- Outbreak in Iceland rearing facility for lumpfish
- EURL confirmed diagnosis on cell supernatant provided by NRL Iceland by ELISA and qPCR (Jonstrup et al 2012)
- Seq analysis of G gene reveal new Genotype IV

Phylogeny on truncated VHSV G-gene sequences

AIM- risk assessment of this new VHSV genotype for salmonid aquaculutre in Europe

- Cooperative project between EURL at DTU-VET and instute for experimental pathology as Keldur (Iceland)

Pathogenicity trial
- Infection trial in RT, AS juveniles by bath and IP
- Infection trial in Lumpfish juveniles by bath and IP

Pathogenesis
- Transmission study Lumpfish – Atlantic salmon

Pathogenicity trial RT-AS M&M

Virus isolates:

- Icelandic strain 15-19852 was passaged once in BF-2
- Positive controls: The VHSV strain DK-3592B
- VHSV strain isolated from A.S. genotype IVa isolated from Port Angles WA
- As a negative control, EMEM with tris-buffer and 10% newborn calf serum (dilution medium) was likewise used by immersion only.
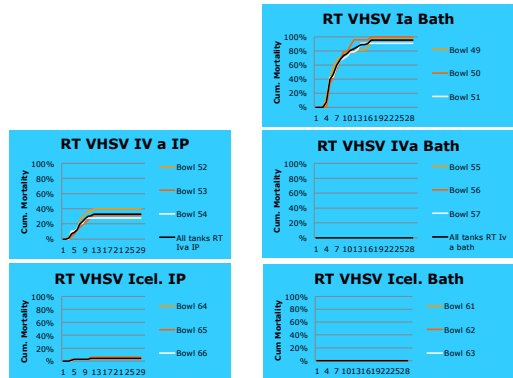
IP injection

Virus	AS	RT
VHSV Ia	x	
VHSV Iva	X	X
VHSV Iceland	X	X
Sterile MEM	X	X

10^5 TCID$_{50 / Fish}$
50 µl/Fish
Each treatment
triplicate of 31 fish

Bath infection (5h)

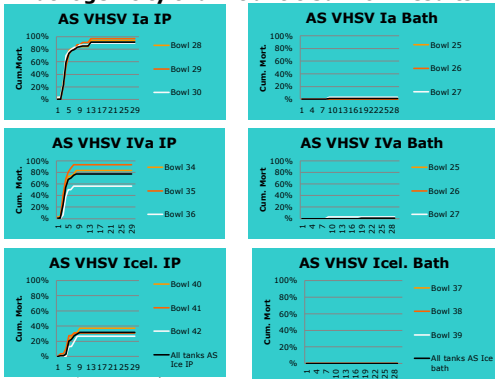| Virus	AS	RT
VHSV Ia	x	X
VHSV Iva	X	X
VHSV Iceland	X	X
Sterile MEM	X	X

10^5 TCID$_{50}$ /ml
5h
Each treatment
triplicate of 31 fish

Pathogenicity trial RT-AS

Pathogenicity trial Rainbow Trout Results

Pathogenicity trial Atlantic Salmon Results

AS VHSV Ia IP
Cum.Mort. — Bowl 28, Bowl 29, Bowl 30

AS VHSV Ia Bath
Cum. Mort. — Bowl 25, Bowl 26, Bowl 27

AS VHSV IVa IP
Cum. Mort. — Bowl 34, Bowl 35, Bowl 36

AS VHSV IVa Bath
Cum. Mort. — Bowl 25, Bowl 26, Bowl 27

AS VHSV Icel. IP
Cum. Mort. — Bowl 40, Bowl 41, Bowl 42, All tanks AS Ice IP

AS VHSV Icel. Bath
Cum. Mort — Bowl 37, Bowl 38, Bowl 39, All tanks AS Ice bath

Conclusion for Rainbow Trout- Atlantic Salmon

- Bath infection. No mortality registered for the Icelandic strain in RT and AS

- No virus isolated on BF-2 in 10 single fish per bowl of (i.e. 30 AS and 30 RT) collected at the end of the trial

- IP infection. Significant mortality (40 %) in AS Ip injected

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Pathogenicity trial Cleaner fish (Cyclopterus lumpus) M&M

Virus isolates:

- Icelandic strain 15-19852 was passaged once in BF-2

- As a negative control, EMEM with tris-buffer and 10% newborn calf serum (dilution medium) was likewise used by immersion only

- To compare results a triplicate of AS IP injected with the same isolate is included in the trial

IP Injection	Bath
10^5 TCID$_{50 / Fish}$	10^5 TCID$_{50}$ /ml
50 µl/Fish	7h
Each treatment	Each treatment
triplicate of 31 fish	triplicate of 31 fish

Pathogenicity trial CF

Pathogenicity trial RT Results

CF VHSV Icel. IP
Cum. Mort. — Bowl 65, Bowl 66, Bowl 67, All tanks

Virus re-isolated from all dead fish

CF VHSV Icel. Bath
Cum. Mort. — Bowl 62, Bowl 63, Bowl 64, All tanks
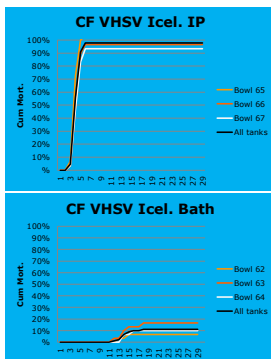
No Virus re-isolated from all dead fish

CF trial – Clinics?

Cohab. Trial CF- AS M&M

2 EXP Tanks + 1 control tank same setting

30 x Shed

IP Injection
10^5 TCID$_{50}$ / Fish
50 µl/Fish
Dorsal fin clip

50 AS cohab

30 x Cohab.
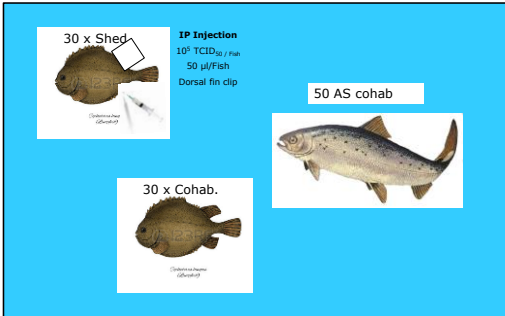
Cohab. Trial CF- AS M&M

At days 3-5-7-10-14-21-28 one tank is sampled alternatively

3 CF shedders
3 CF cohab
3 AS cohab

Are euthanized by hiperdose of anesthesia. From these fish spleen samples are tested by qPCR for vhsv to assess viral load; while the whole fish is sampled for histpathology.

At the end of the trial, 2 x 10 single AS are sampled . A pool of Heart, kidney, spleen, brain and gills stored in MEM and freeze -80.Samples are inoculated on BF-2 cells and tested on qPCR for VHSV

Results. qPCR

Cohab trial CF-AS VHSV Icel. Inf. Continous sampling
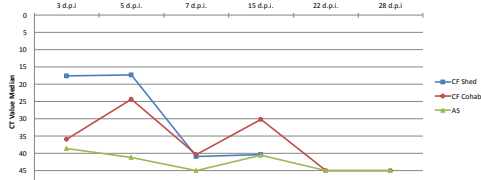
VHSV Inf	3 d.p.i	5 d.p.i.	7 d.p.i.	15 d.p.i.	22 d.p.i.	28 d.p.i
CF Shed	17,59	17,25	40,84	40,37		
ST Dev.	0,504997	0,530471	2,635602	-		
CF Cohab	35,89	24,36	40,34	30,15	45	45
ST Dev.	8,761689	5,826322	2,931045	5,130672	-	-
AS	38,61	41,21	45	40,51	45	45
ST Dev.	0,392032	2,202458	1,697056	1,294321	-	-

Results. Mortality and clinics

Cohab. CF-AS Tank 73

Cohab. CF-AS Tank 76
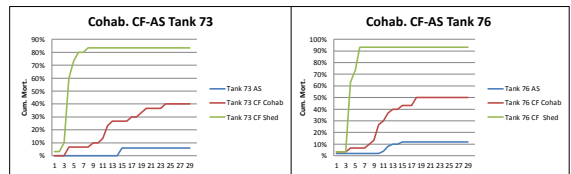
Conclusions

- Cohabitation model gave satisfactory results to reproduce disease in cleanerfish, showing horizontal transmission
- It was possible to detect VHSV in spleen of 3 AS cohabiting with CF 3 d.p.i. at low level
- Low mortality was observed in salmon the experimental tanks (NOT in the control tank) without being able to re-isolate the virus from the affected fish
- It was **NOT** possible to re-isolate and or detect VHS virus from 20 surviving salmon

Thanks for your attention