
DTU

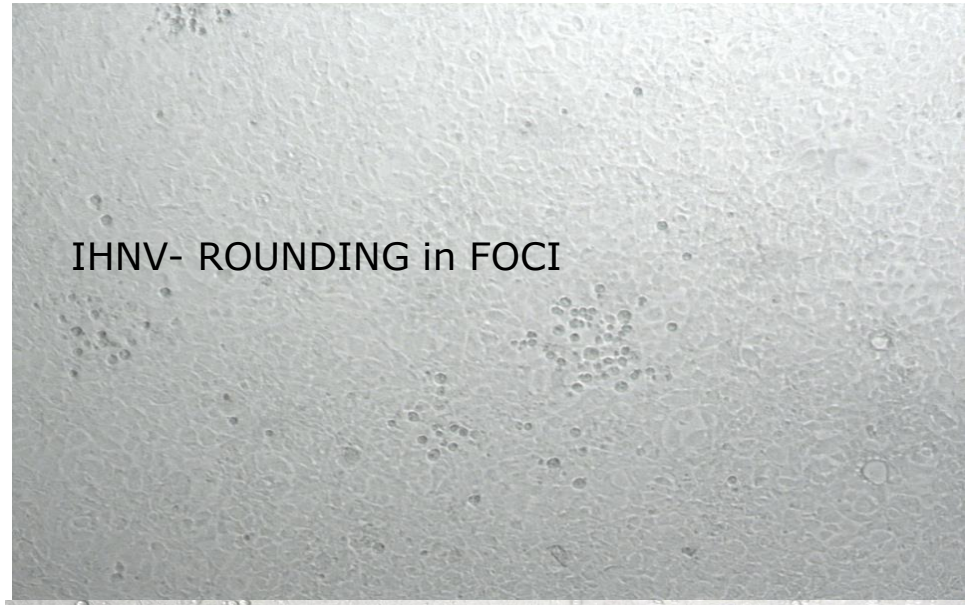
IHN in Denmark- the laboratory

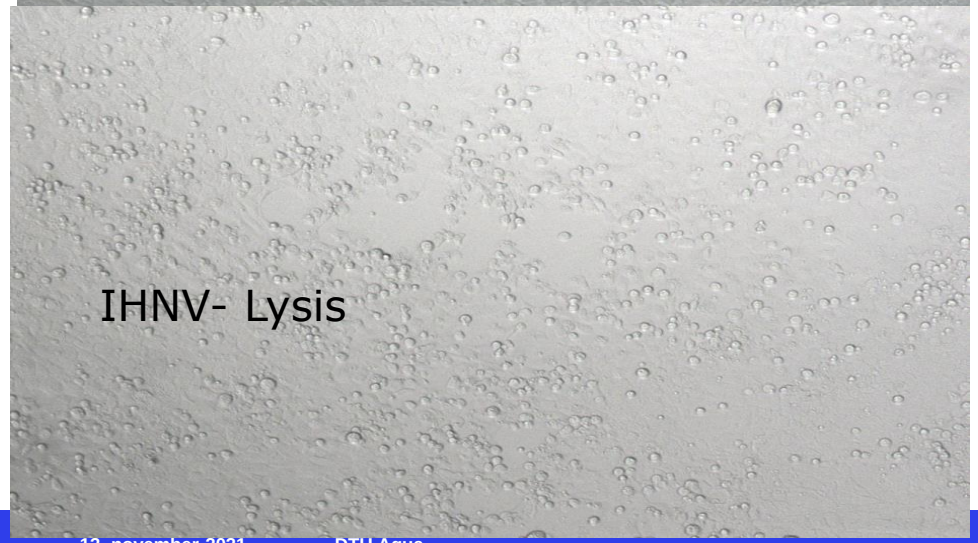
Niccolò Vendramin, Argelia Cuenca, Tine M. Iburg, Niels Jørgen Olesen
Unit for Fish and Shellfish Diseases, DTU Aqua

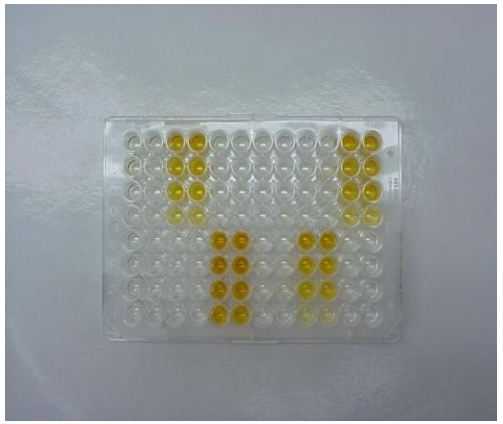
This presentation will cover today

- The new RT-qPCR method
- The choice of organs to be tested for detecting IHNV
- An experimental in vivo trial to assess virulence of IHNV in salmonids
- The persistence of the virus under farm condition

Diagnosis by detection in cell culture



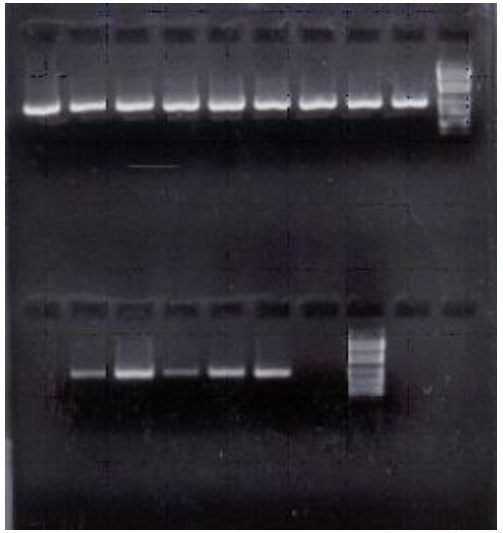


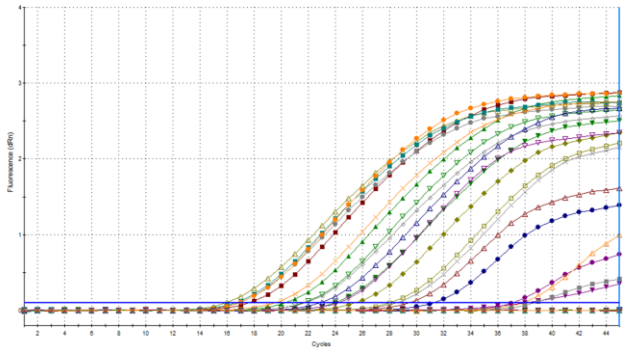


ELISA

RT-qPCR / RT-PCR

RT-PCR + sequencing



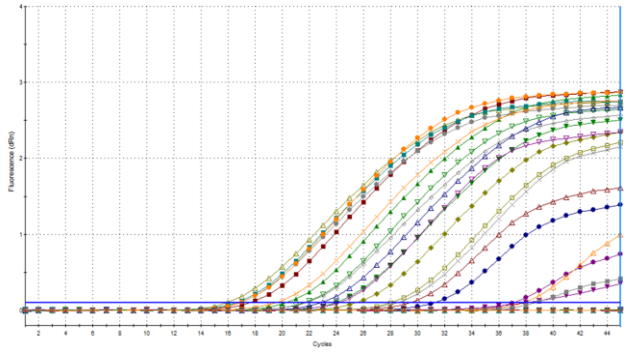


Diagnosis by real-time RT-PCR

260, Bull. Eur. Ass. Fish Pathol., 40(6) 2020

Analytical validation of one-step real-time RT-PCR for detection of infectious hematopoietic necrosis virus (IHNV)

A. Cuenca[1]*, N. Vendramin[1], N. Jørgen Olesen[1]



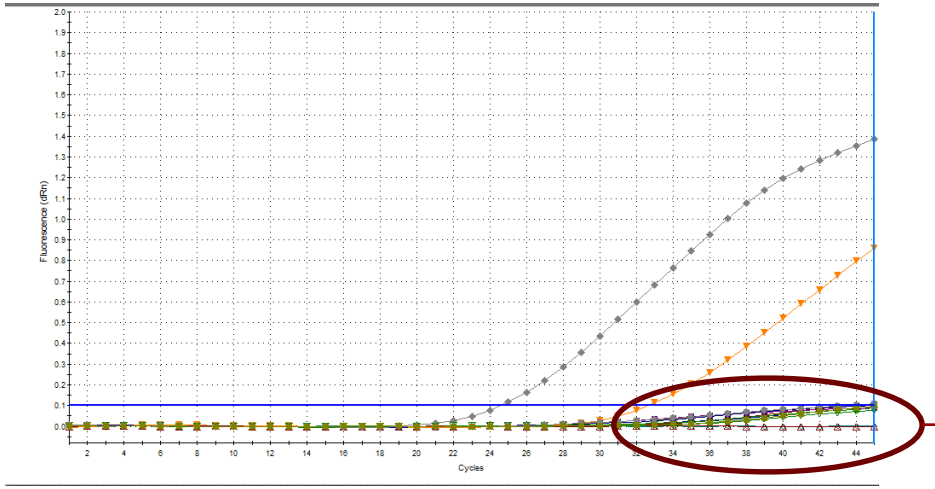
Target: conserved region of 97 NT in the N gene of IHNV

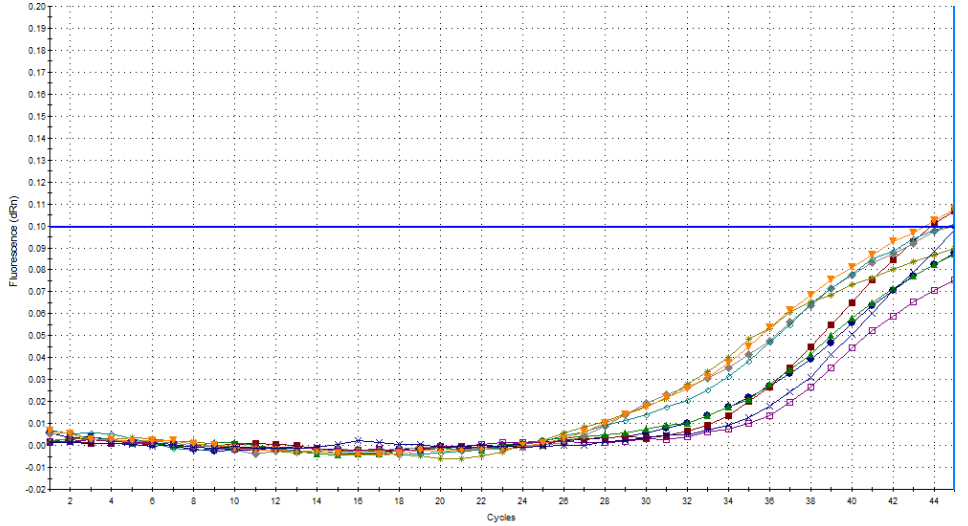
Takes over more and more due to demands for quick response

But cell culture always negative / positive

RT-qPCR: negative / suspect / positive

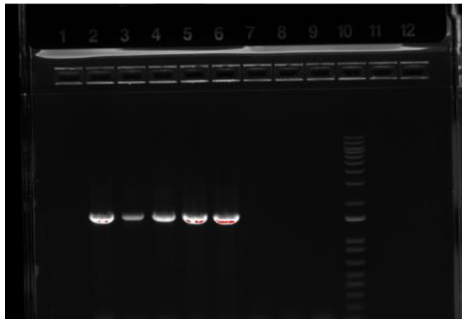
Why was IHNV only finally diagnosed 5 days after receipt in the laboratory?





12.05.2021

0-passage material



From Cuenca et al.,2020 – comparison RT-qPCR one step method VS EPC cell line for IHNV detection

Table 3. Analytical sensitivity for detection of five IHNV isolates. Samples were tested in duplicate by inoculation in EPC cells and by one-step real-time RT-PCR. Initial $TCID_{50}$/ml titers of each isolate are shown in parenthesis.

	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
BLK94 ($5.9*10^{8}$)									
EPC cells	++**	++	++	++	++	++	++	+	
One-step	++	++	++	++	++	++	++	(+)	
Average Ct	13.34	16.55	19.99	23.06	26.75	30.40	33.70	36.27*	
I-4008 ($1.9*10^{6}$)									
EPC cells	++	++	++	++	++	+			
One-step	++	++	++	++	++	+(+)			
Average Ct	18.23	21.68	25.08	28.34	31.78	34.96			
32/87 ($5.9*10^{7}$)									
EPC cells	++	++	++	++	++	++	++		
One-step	++	++	++	++	++	++	+(+)	(+)*	
Average Ct	17.14	22.07	25.53	29.08	32.02	34.51	36.23	38.7	
RBH (1.9×10^{5})									
EPC cells	++	++	++	++	++				
One-step	++	++	++	++	(+)(+)				
Average Ct	23.09	28.01	31.15	34.38	36.47				
Coleman ($1.3*10^{5}$)									
EPC cells	++	++	++	++					
One-step	++	++	++	+(+)	(+)*	(+)*			
Average Ct	25.16	30.18	33.82	36.22	39.83	38.44			

** ++ positive for IHNV in both replicates; (+) detection with a Ct value higher than Ct = 35

*Ct value for a single positive sample, as the other one is negative (no Ct)

What was happening to our RT-qPCR?

Improvement of a diagnostic procedure in surveillance of the listed fish diseases IHN and VHS

Marc Hoferer[1] | Valerij Akimkin[1] | Julia Skrypski[1] | Heike Schütze[2] | Reinhard Sting[1]

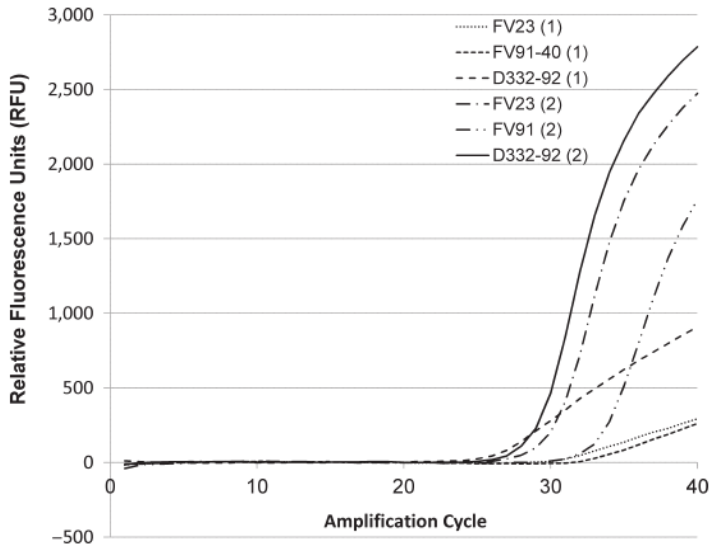
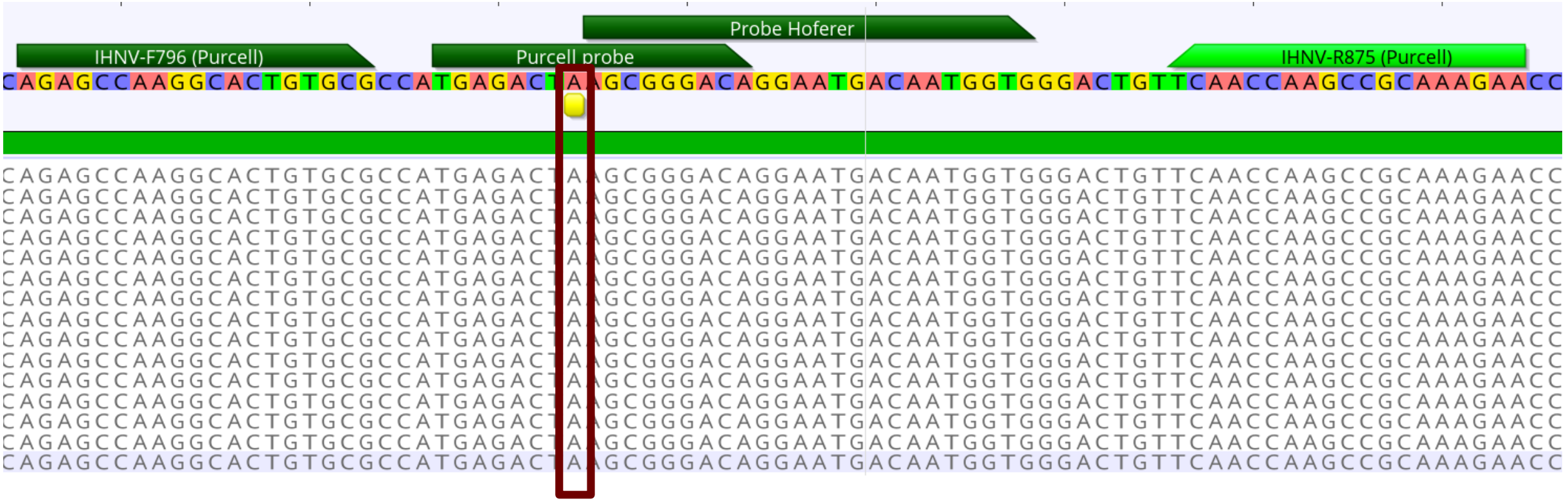


FIGURE 1 Amplification curves generated by the original IHNV RT-qPCR protocol provided by Purcell et al. (2013) using an MGB TaqMan® probe (RFU values <1,000) (1) in comparison with the improved protocol designed in this study using a TaqMan® probe run with a modified thermal profile in a one-step protocol (RFU values >1,000) (2)

Report few German isolates where the Purcell et al. 2013 method shows a lower sensitivity

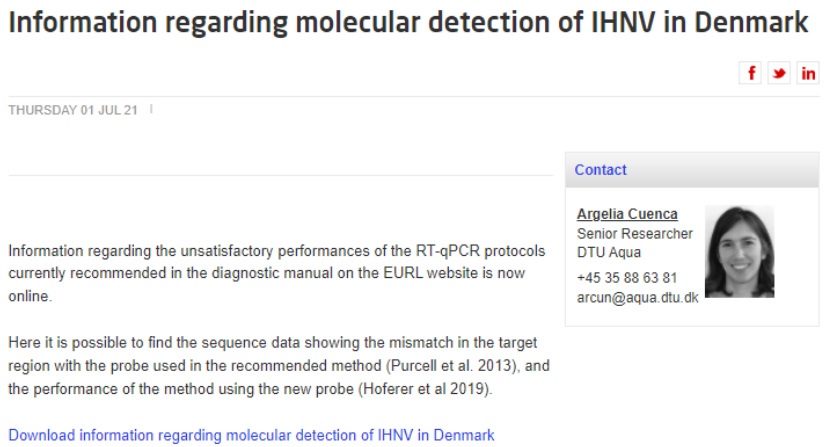
Modification of RT-qPCR method

Danish Isolates – N Gene sequences



All NRLs have been notified to update the qPCR probe to the one suggested by Hoferer et al.

This has been done by email,



Information regarding molecular detection of IHNV in Denmark

THURSDAY 01 JUL 21

Contact

Argelia Cuenca
Senior Researcher
DTU Aqua
+45 35 88 63 81
arcun@aqua.dtu.dk

Information regarding the unsatisfactory performances of the RT-qPCR protocols currently recommended in the diagnostic manual on the EURL website is now online.

Here it is possible to find the sequence data showing the mismatch in the target region with the probe used in the recommended method (Purcell et al. 2013), and the performance of the method using the new probe (Hoferer et al 2019).

Download information regarding molecular detection of IHNV in Denmark

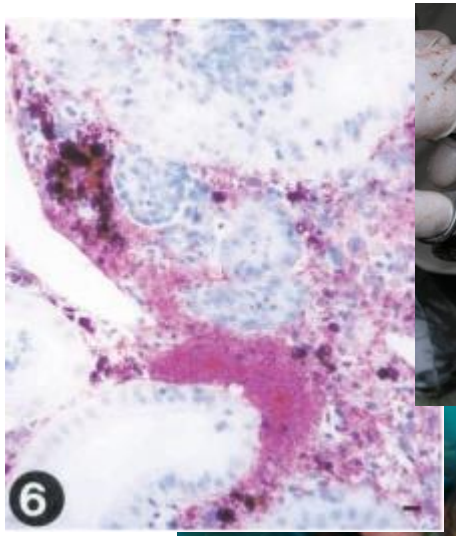
by advertising changes on our website https://www.eurl-fish-crustacean.eu/news/nyhed?id=3ad8a31a-1412-45a7-b96b-53a930f99bcc

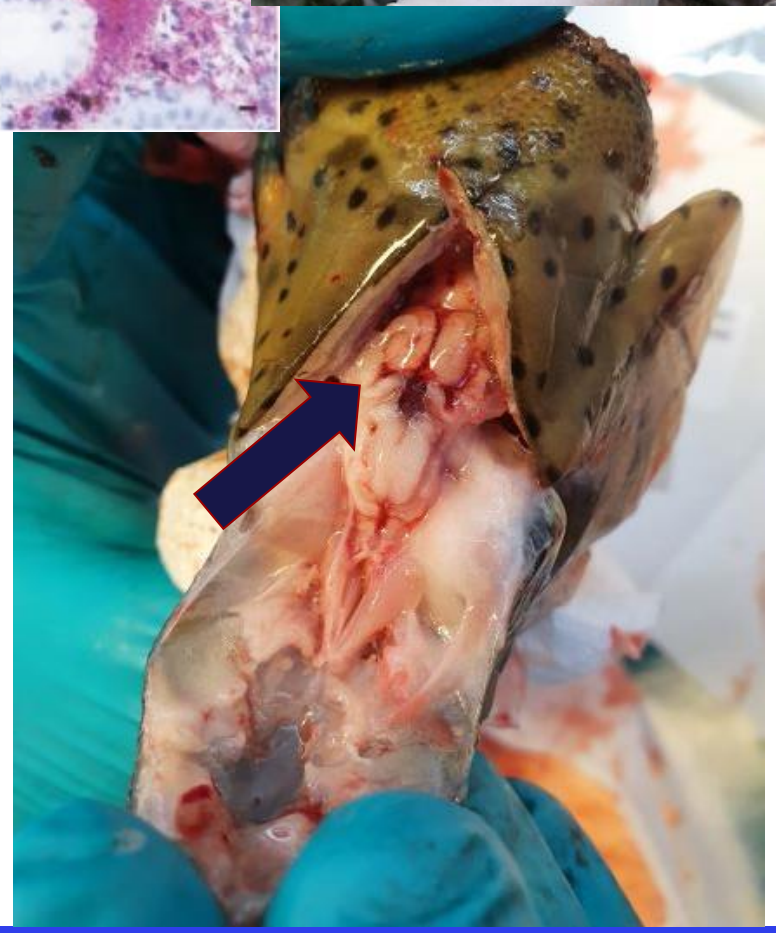
MAJOR CHANGES FROM THE PREVIOUS VERSION (V.1 21-04-2021)

Amendment of the probe sequence and reference for the detection of IHNV RNA by RT-qPCR in Paragraph 1.6.3.3; furthermore the two steps RT-qPCR for detection of IHNV RNA (Purcell et al., 2013) has been removed.

By amending the DIAGNOSTIC MANUAL

Tissues for examination





Highest viral titres found in **anterior kidney, heart, spleen and brain.**

Low mortality observed at several outbreaks with no significant excess mortality ➡ Risk of IHNV infection hiding under other diseases ?

Comparison of organ pool and pool of brain

- Received entire fish or severed heads of fish together with tubes of organ pools in the beginning of the outbreak

- This allowed us to separate the brains in their own tubes. Typically for one organ pool with 10 fish, two pools of brain with five fish in each was prepared. This happened for one or more pools pr. case.

- Cases with this possibility was 15 (10 negative and 5 positive cases)
- Plus repeated sampling at two positive farms additional ”4 cases”

- Method: Real-time RT PCR (Hoferer et al. 2019) Evaluation: Ct <30 = Pos Ct 30-35: weak pos Ct > 35: Suspect Ct > 40 Most likely negative. Sigmoid curves as well as the Ct values

- In 10 negative cases - a complete match between organ and brain.

- In 4 positive cases - a good match, meaning when organ pool is positive, the brain pools are positive or weakly positive.

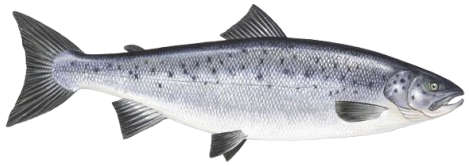
- In one case plus the repeated sampling cases minor discrepancy were seen. See the following table.

Comparison of organ pool and pool of brain (2)

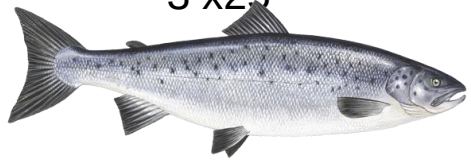
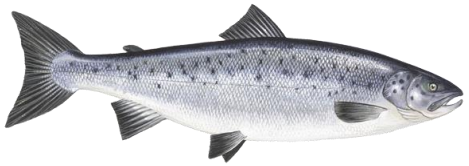
	Organ pool	Brain pool	Remarks
Case 1a (G) (6 pools of 5 fish)	6 pos	4 pos and 2 weak pos	
Case 1b (6 pools of 5 fish)	3 pos 1 susp 2 neg	2 weak pos+ 1 susp 1 susp 1 neg + 1 susp	Duplicate: Ct 38.77 and no ct
Case 2 (H) Three 10 fish organ, six 5 fish brain	3 pos	3 positive and 2 weak pos and 1 susp	
Case 2a (6 pools of 5 fish)	1 weak pos 2 susp 3 neg	1 susp 1 weak pos and 1 susp 3 neg	Ct organ 37.9/ Brain 31.69
Case 2b (6 pools of 5 fish)	2 susp 4 neg	2 neg 4 neg	

Of 19 cases, only 2 cases had single pools, where suspective or weakly positive values of IHNV was seen in the brain, but with higher or no ct organs and in both cases, it meant nothing for the status of the farm (both farms positive)

How contagious is IHNV from Denmark for rainbow trout and salmon?

Bath challenge	IP Injection
3 x25 	2x20 
3 x25 	2x20 

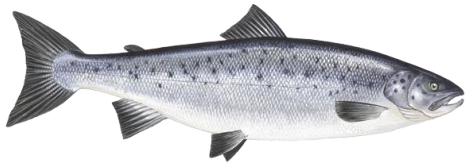
DF 04/99 Germany

Bath challenge	IP Injection
3 x25 	2x20 
3 x25 	2x20 

DK 2021 isolate

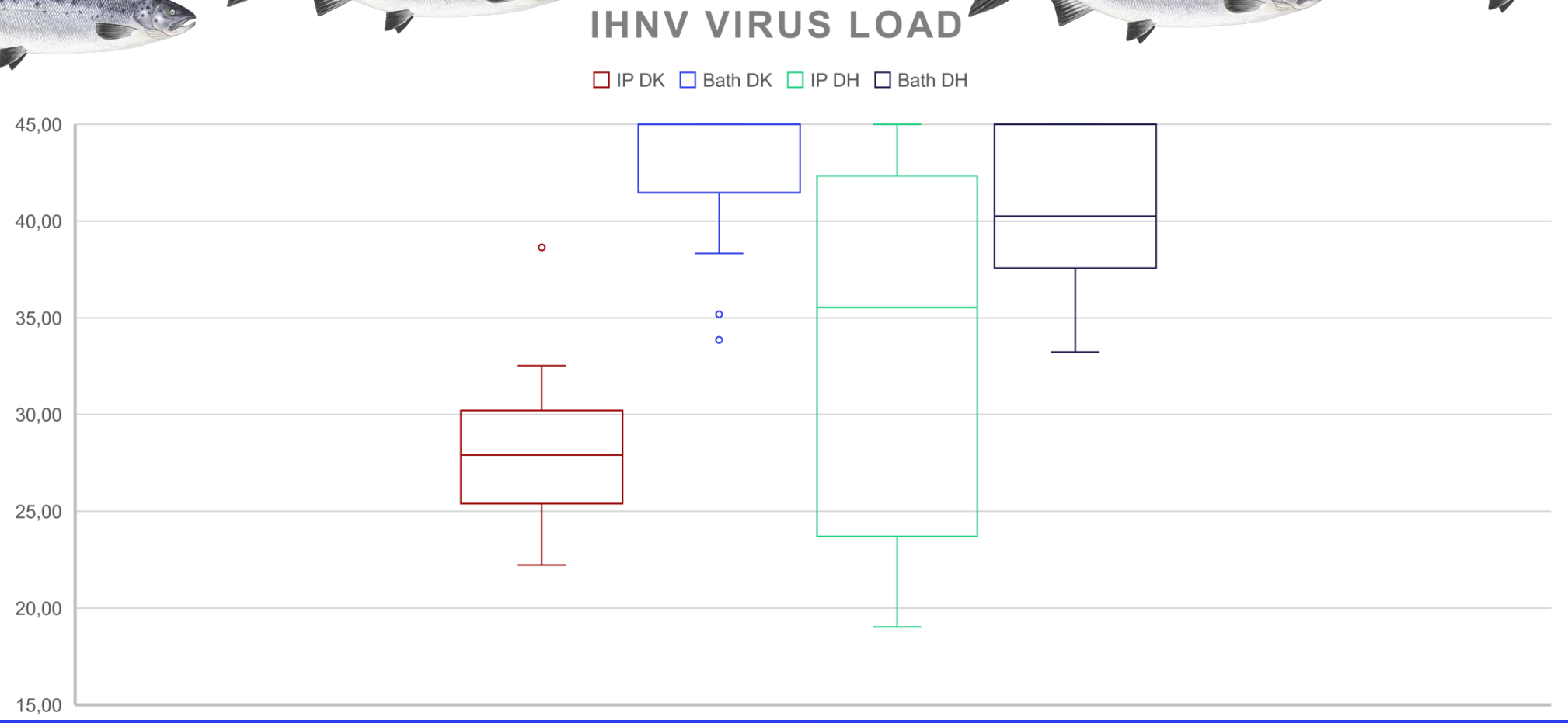
Experimental trial to assess virulence (1-%mort)

- 100% survival in AS Negative control groups
- 100% survival in AS challenged by bath
- Low reduction of survival in AS challenged IP
  - 75-85% survival in AS challenged with 2021 Danish Isolate
  - 90-95% survival in AS challenged with DF 04/99



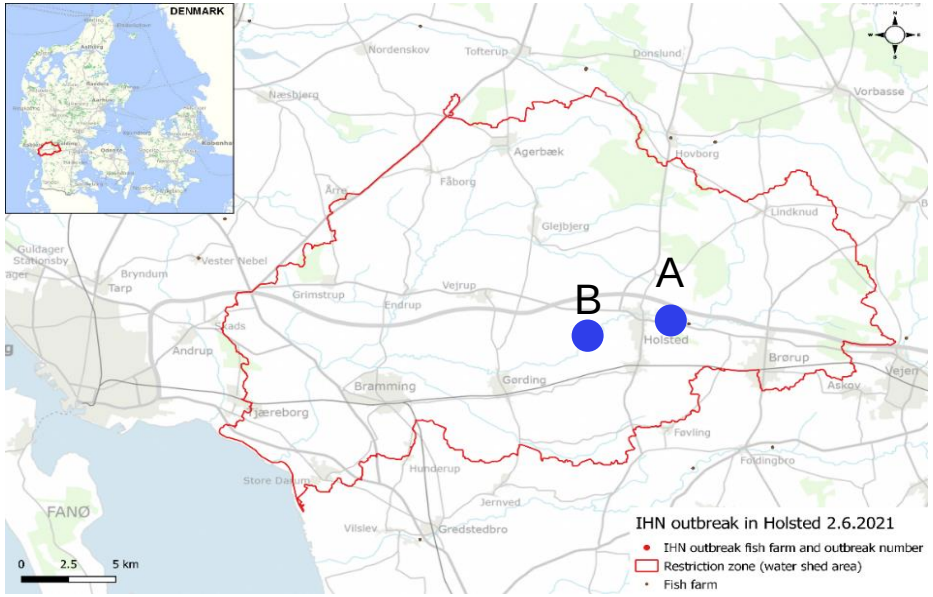
- 100% survival in RT negative control groups
- Difference in RT survival challenged by bath
  - 0 % in RT challenged with 2021 Danish Isolate
  - 72-83 % survival in RT challenged with DF 04/99
- No survival in RT challenged IP
  - 0 % in RT challenged with 2021 Danish Isolate
  - 0 % in RT challenged with DF 04/99

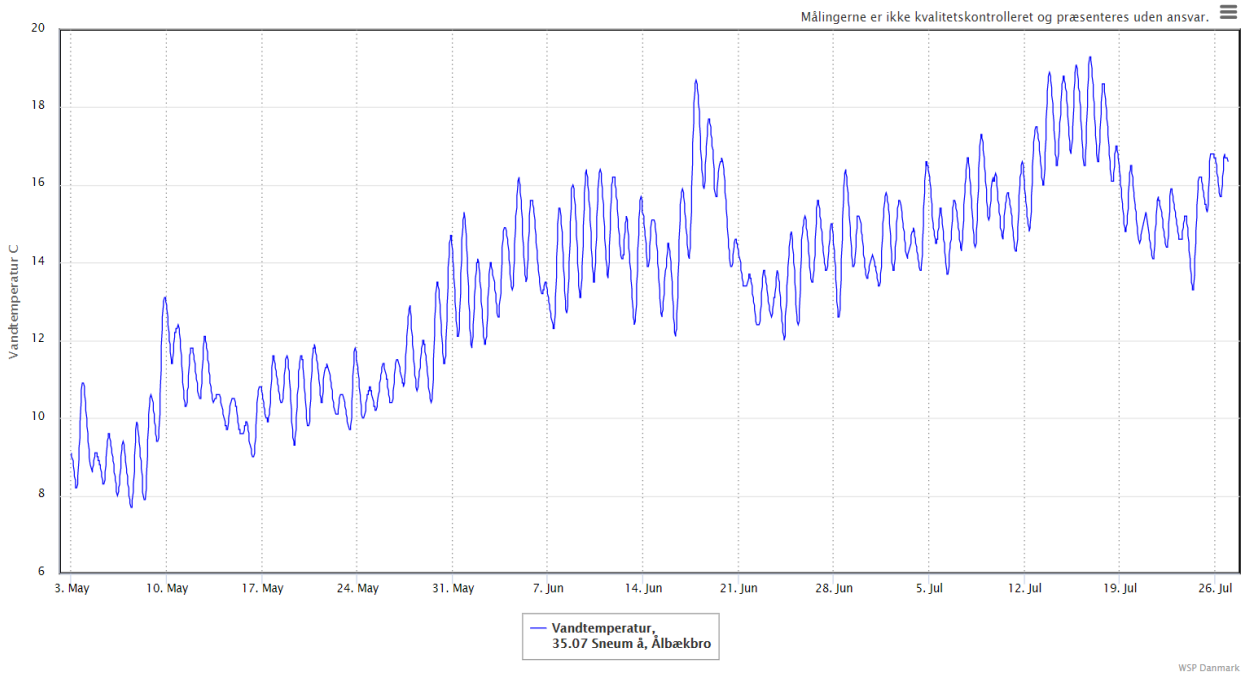
Virus load


IHNV VIRUS LOAD
IP DK
Bath DK
IP DH
Bath DH
45,00
40,00
35,00
30,00
25,00
20,00
15,00

Will it be possible to detect IHN at infected farms over time / Temperature ?

Two sites selected at Holsted Å





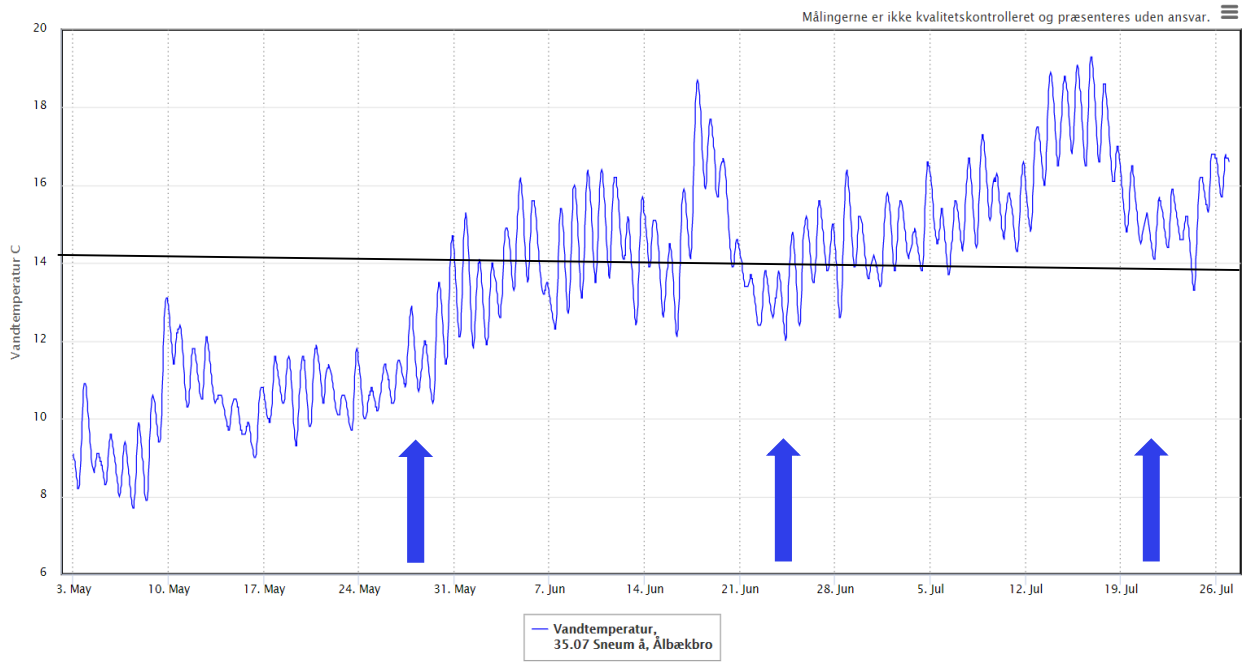
2 sites – A and B

Site A only RT, flow through farm production of large fish for export

Site B 2 types of rainbow trout (traditional rainbow and golden trout), flow through farm. Higher density of fish

samplings

- End of May DFVA samples 30 fish – 3 pools all positives
- 24th June. Sampling of 30 fish. 6 pools (five fish each) of organs, and 6 corresponding pools of brains and bacteriology from 10 fish
- 22nd July. Sampling of 30 fish. 6 pools (five fish each) of organs, and 6 corresponding pools of brains. Organs and brain (separately) are also collected from individual fish and bacteriology from 10 fish



Results

SITE / Sampling	IHNV RT-qPCR	IHNV cell culture	Bacteriology
Site A 1st follow up	3/12 positive	2/12 positive	1/10 fish with F.psychr.
Site A 2nd follow up	0/12 positive	0/12 positive	1/10 fish with F.psychr. 1/10 fish with A.salm.
Site B 1st follow up	12/12 positive	12/12 positive	3/10 fish with Y.ruck. 2/10 fish with F.psychr.
Site B 2nd follow up	4/12 positive	4/12 positive	3/10 fish with Y.ruck. 3/10 fish with F.psychr.

Conclusion:

Without introduction of new naive fish IHNV persisted on the farm during summer

It is possible to detect IHNV at temperatures >14C (up to 19C in put&take lakes)

Thank all of you for your attention
Questions?