

Påvisning af ILA på Færøerne

Debes Hammershaimb Christiansen

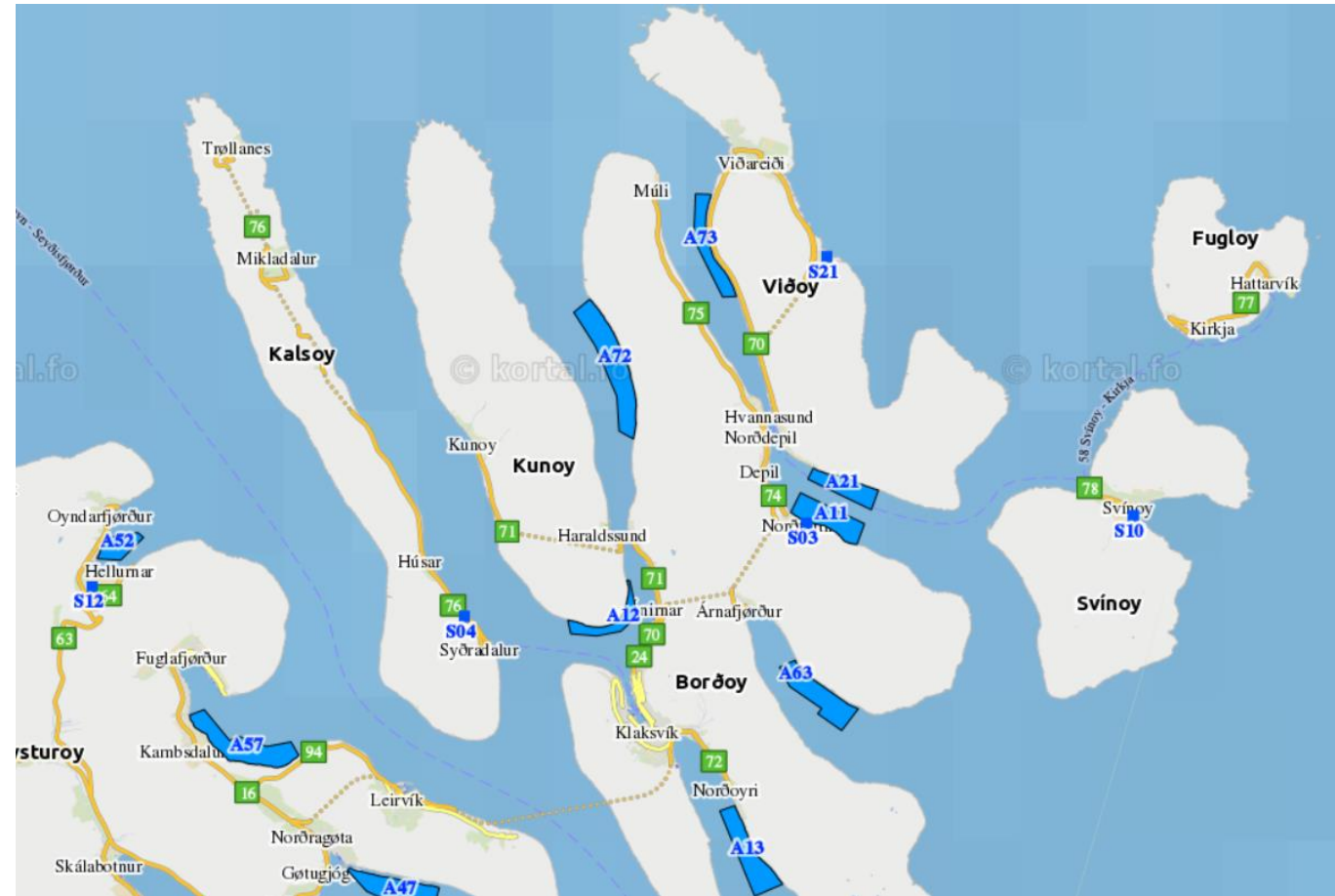
Seniorforsker & afdelingsleder

Færøernes Nationale Referencelaboratorium for Fiskesygdomme

ILA Workshop, Trondheim 3. – 4. April 2017

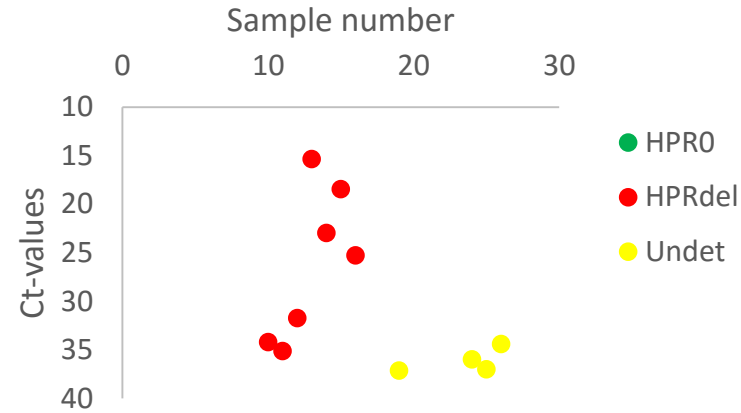
Case 2016

- 1,3 mill smolts transfered to 14 cages in feb-mar 2016
- Smolts delivered from 7 smolt farms

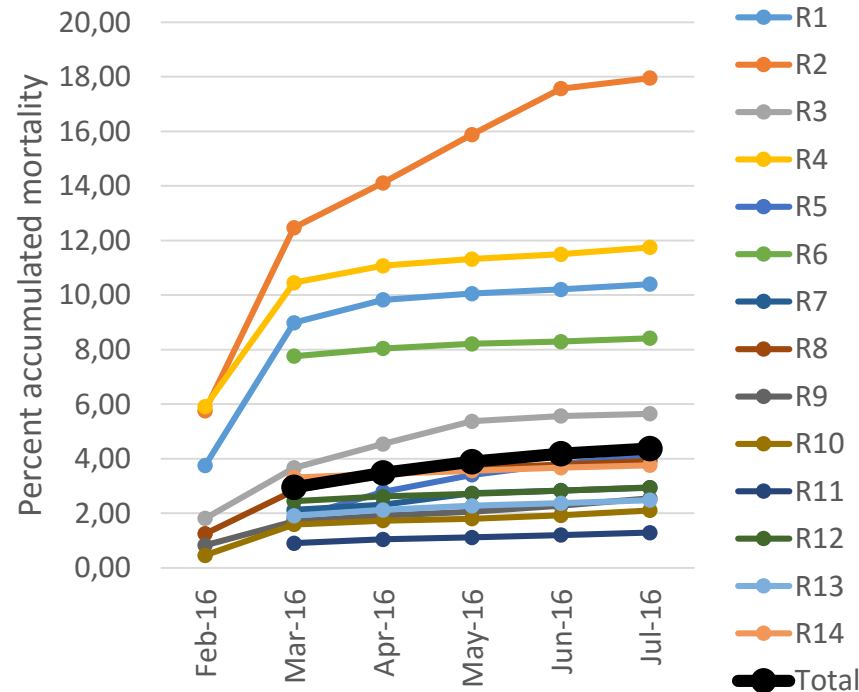


First detection of ISAV-HPRdel in July 2016

Date	Number of fish	ISAV RT-qPCR
08. July	30	11



Cage no.	Number of fish	ISAV+	HPR0	HPRdel	n/a
R1	2	2	0	2	0
R2	5	5	0	5	0
R4	4	0			
R5	5	0			
R6	1	0			
R7	1	1	0	0	1
R8	1	1	0	0	1
R9	4	0			
R10	3	1	0	0	1
R11	1	1	0	0	1
R12	3	0			
Total	30	11	0	7	4



No specific clinical signs of ISA

No confirmation of ISAV-HPRdel the next five months

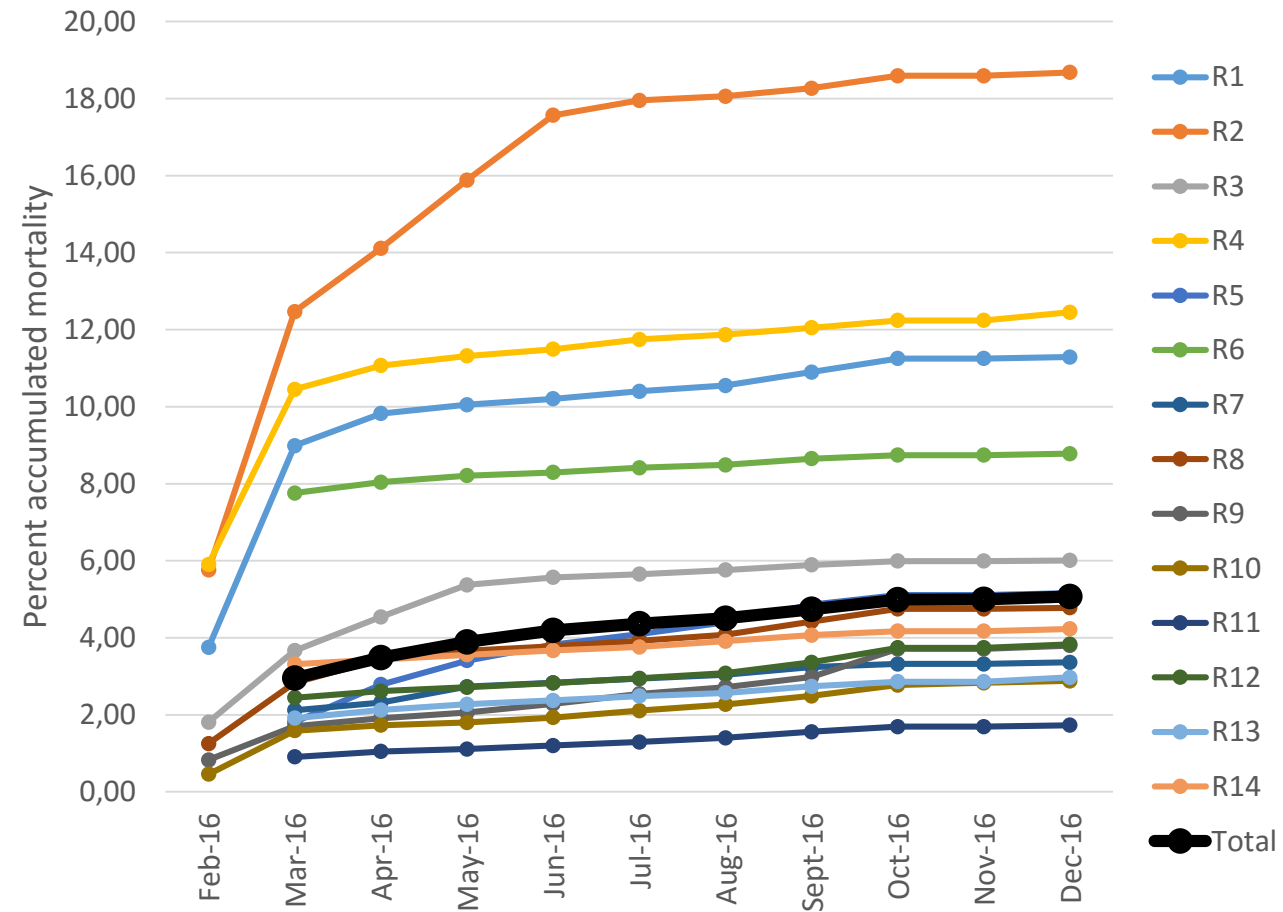
Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
Total	577	22	7	7	8

New organ samples for

1. Real-time RT-PCR
2. Histology
3. Cell culturing in the ASK cell line

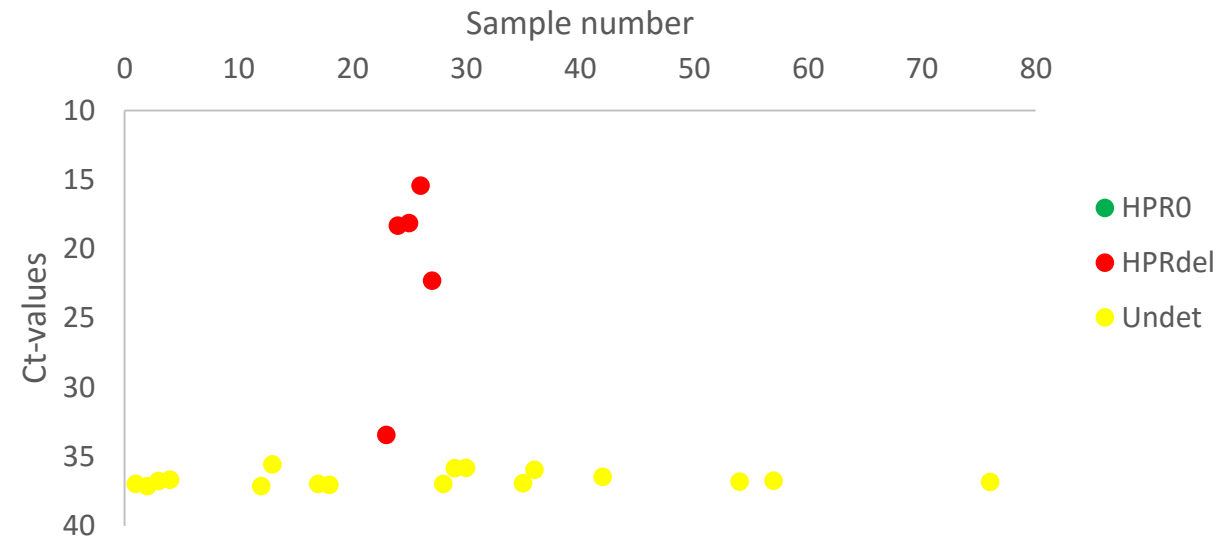
No increase in mortality the next five months

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
Total	577	22	7	7	8



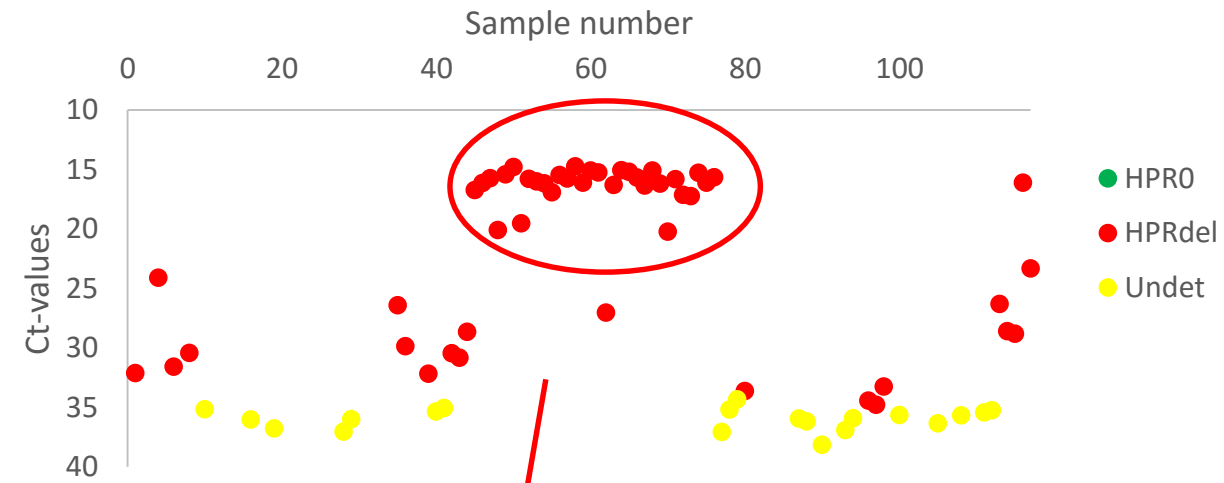
New detections of ISAV-HPRdel in Jan. 2017

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
05. Jan	80	22	0	5	16



Fast increase in prevalence and load of HPRdel within 14 days

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
05. Jan	90	22	0	6	16
17. Jan	117	73	0	54	19



Innoculated on ASK and sent to VI for Histology & IHC

The prevalence of HPRdel increased particularly in R2 after treatments in weel boat for sea lice

05.01.2017

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R2	68	14	0	6	8
R4	6	3	0	0	3
R5	4	0			
R6	3	0			
R8	3	3	0	0	3
R9					
R10	1	0			
R13	5	2	0	0	2
R14					
Total	90	22	0	6	16

17.01.2017

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R2	21	7	0	3	4
R2-b	59	46	0	41	5
R4	6	3	0	0	3
R5	5	0			
R6	4	3	0	0	3
R8	3	0			
R9	5	5	0	5	0
R10	5	2	0	0	2
R13	5	5	0	4	1
R14	4	2	0	1	1
Total	117	73	0	54	19

Stress-induced increase in HPRdel replication

The prevalence of HPRdel increased particularly in R2 after freash water treatments in weel boat for sea lice

05.01.2017

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R2	68	14	0	6	8
R4	6	3	0	0	3
R5	4	0			
R6	3	0			
R8	3	3	0	0	3
R9					
R10	1	0			
R13	5	2	0	0	2
R14					
Total	90	22	0	6	16

17.01.2017

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R2	21	7	0	3	4
R2-b	59	46	0	41	5
R4	6	3	0	0	3
R5	5	0			
R6	4	3	0	0	3
R8	3	0			
R9	5	5	0	5	0
R10	5	2	0	0	2
R13	5	5	0	4	1
R14	4	2	0	1	1
Total	117	73	0	54	19

The company desided to slaughther R2 and R9

No final confirmation of ISA in February ?

YES

- Repeated detection of ISAV-HPRdel by RT-qPCR & seq.
- Increased prevalence and replication of ISAV-HPRdel
- Replication of ISAV in kidney confirmed by IHC

NO

- No clear clinical signs of ISA
- No clear pathological signs of ISA
- No increased mortality associated with ISA
- No CPE in cell culture
 - *Have the ASK lost their sensitivity particularly when we compare with the 2014 case ?*
 - 2014: 50 passages
 - 2016: 90 passages

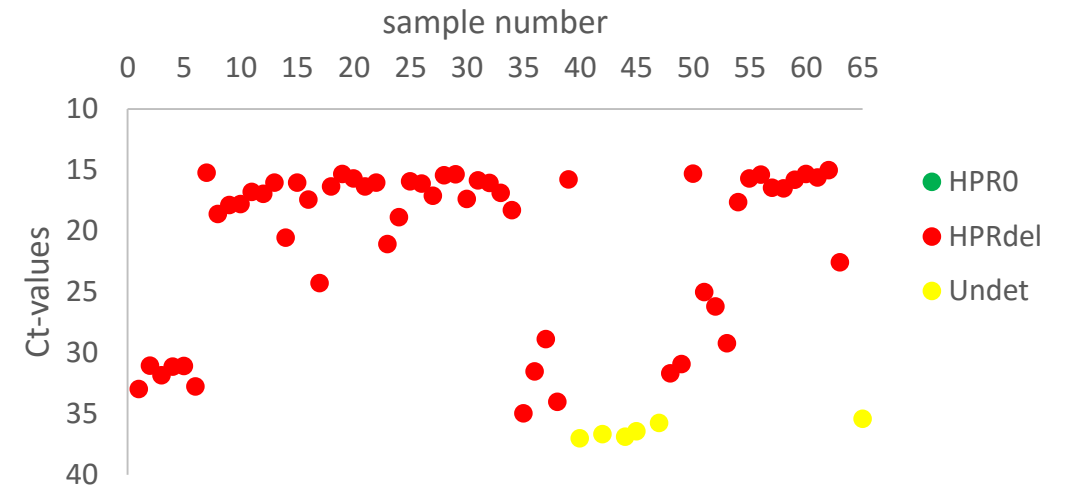
Only further suspicion of ISA

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
05. Jan	90	22	0	6	16
17. Jan	117	73	0	54	19

- New organ and blood samples for
 1. Real-time RT-PCR
 2. Histopathology
 3. Cell culturing in the ASK cell line
 4. Haematokrit
- No sampling in February because of new treatments for sea lice

Further increase in HPRdel prevalence

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
05. Jan	90	22	0	6	16
17. Jan	117	73	0	54	19
09. Mar	65	61	0	55	6



Most cages are now HPRdel positive

09.03.2017

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
05. Jan	90	22	0	6	16
17. Jan	117	73	0	54	19
09. Mar	65	61	0	55	6

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R1	5	5	0	5	0
R3	4	4	0	4	0
R4	5	5	0	5	0
R5	10	10	0	10	0
R6	17	17	0	17	0
R8	5	3	0	3	0
R10	4	2	0	0	2
R11	2	2	0	2	0
R12	4	3	0	0	3
R13	7	7	0	6	1
Total	65	61	0	55	6

Significantly increased prevalence of HPRdel in R4, R5 & R6

17.01.2017

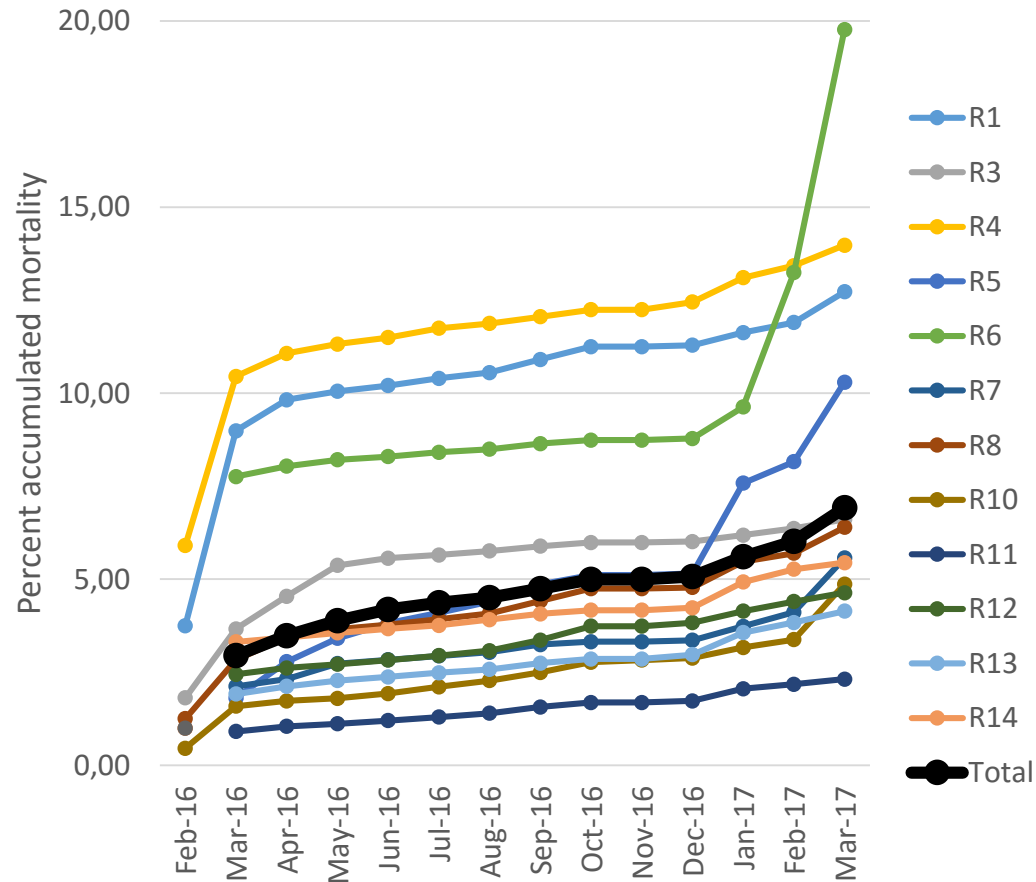
Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R1					
R3					
R4	6	3	0	0	3
R5	5	0			
R6	4	3	0	0	3
R8	3	0			
R10	5	2	0	0	2
R11					
R12					
R13	5	5	0	4	1
Total	28	13	0	4	9

09.03.2017

Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R1	5	5	0	5	0
R3	4	4	0	4	0
R4	5	5	0	5	0
R5	10	10	0	10	0
R6	17	17	0	17	0
R8	5	3	0	3	0
R10	4	2	0	0	2
R11	2	2	0	2	0
R12	4	3	0	0	3
R13	7	7	0	6	1
Total	65	61	0	55	6

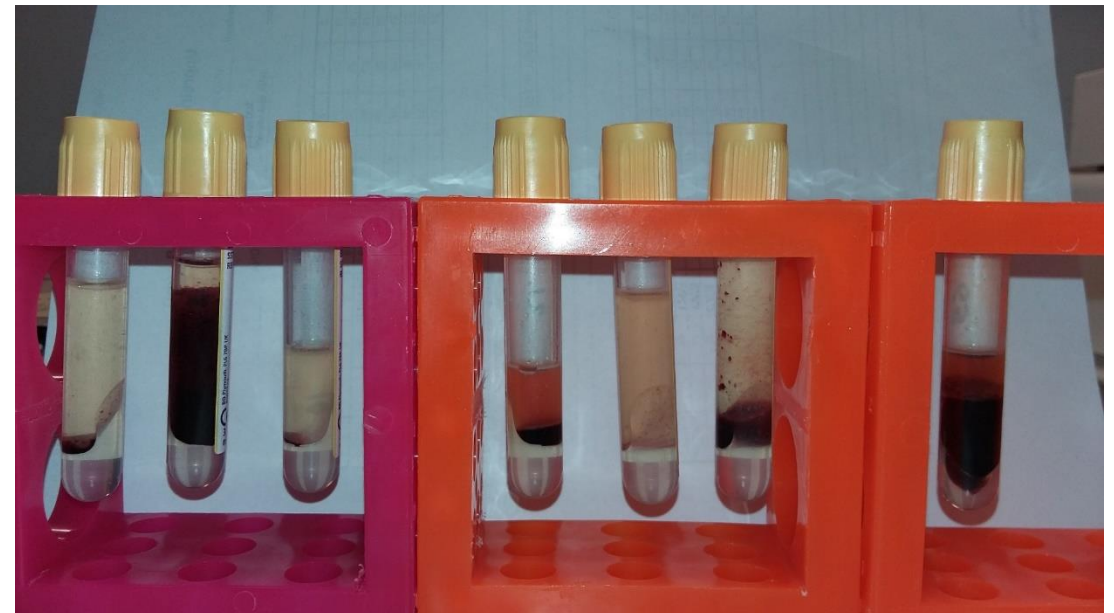
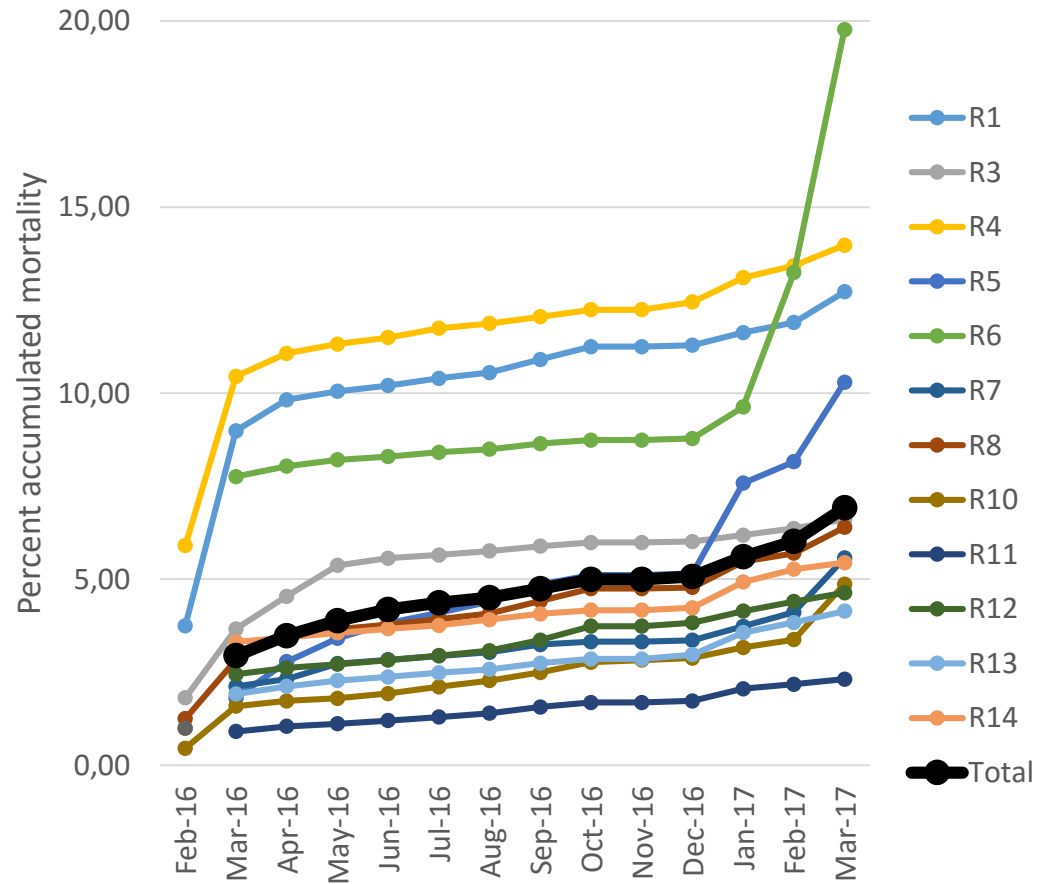
Significantly increased mortality in R5 & R6 after treatment for sea lice

09.03.2017

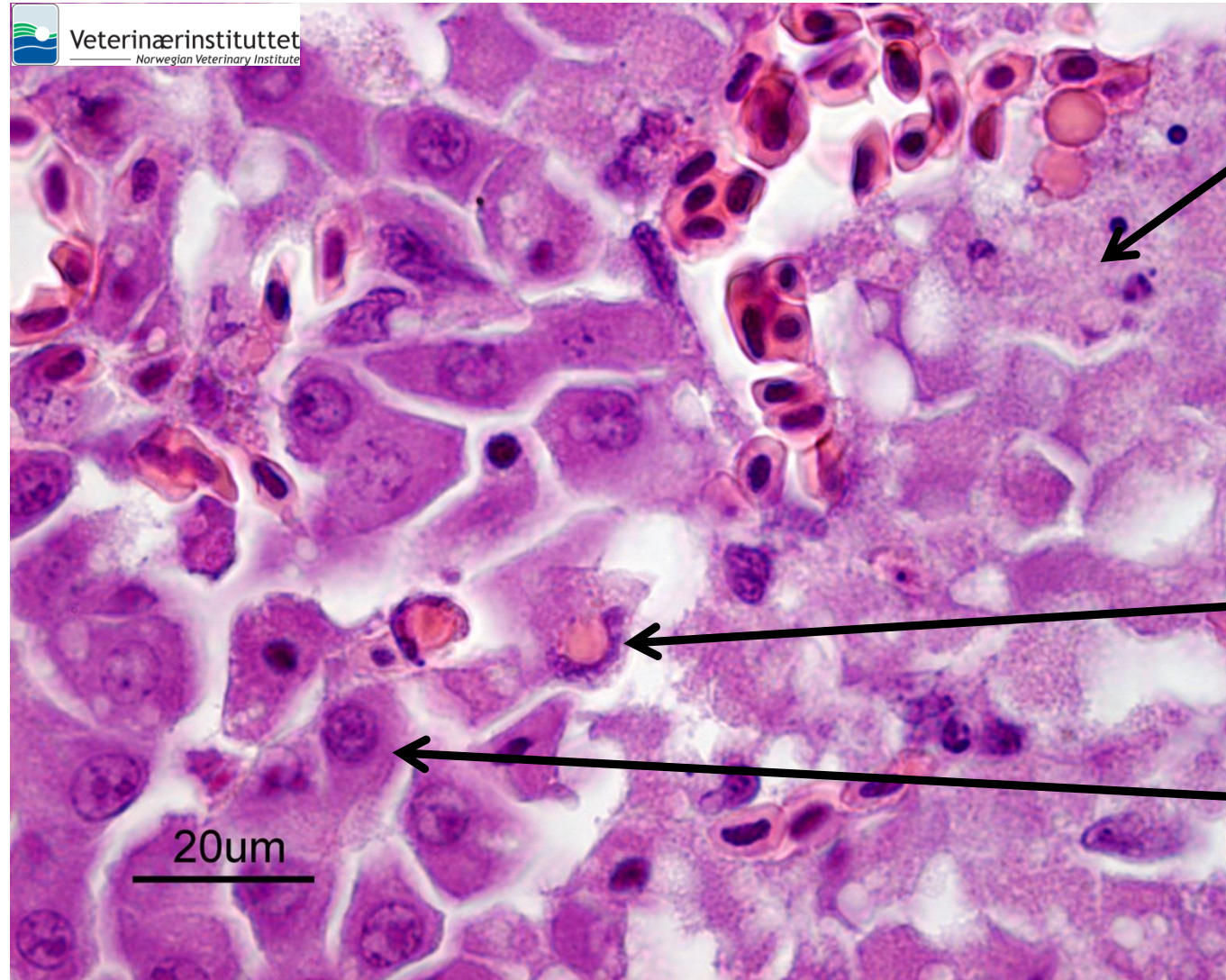


Cage	Number of samples	ISAV+	HPR0	HPRdel	n/a
R1	5	5	0	5	0
R3	4	4	0	4	0
R4	5	5	0	5	0
R5	10	10	0	10	0
R6	17	17	0	17	0
R8	5	3	0	3	0
R10	4	2	0	0	2
R11	2	2	0	2	0
R12	4	3	0	0	3
R13	7	7	0	6	1
Total	65	61	0	55	6

Dark liver and low hematocrit



Lever – hemoragisk, sonal nekrose med erythrofagocytose: detaljbilde, HE

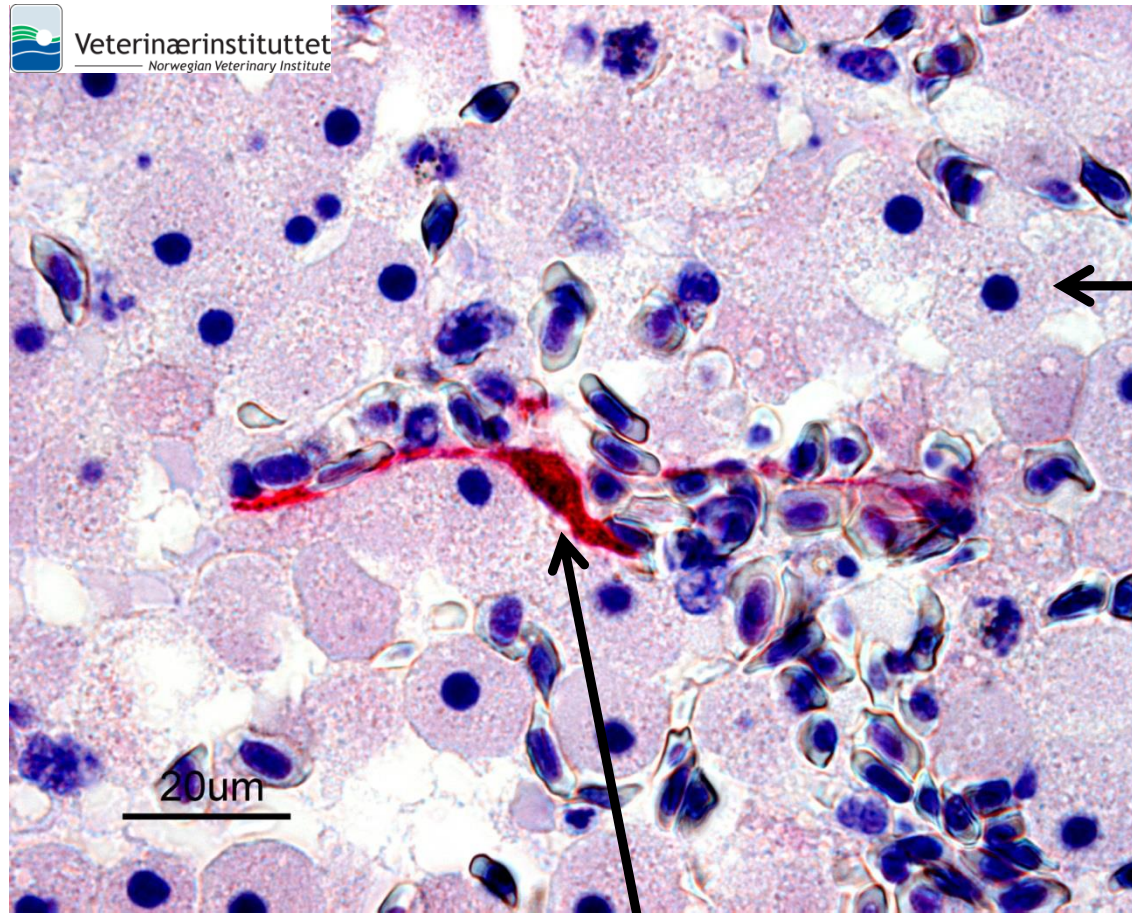


Nekrotiske leverceller

Erythrofagocytose

Intakte leverceller

Lever – hemoragisk nekrose: detaljbilde immunhistokjemi ILA-virus



← Nekrotiske leverceller: er ikke infiserte og nekrosene skyldes trolig lav oksygen i levervevet pga ekstrem anemi og ødelagt blodsirkulasjon: derfor et sonalt mønster

↑ ILA-virus infiserte karvegg-endotelcelle, rødfarget

Final confirmation on March 24th, despite no CPE in ASK

Fish no.	RT-qPCR			Sekven-tering	Hemato-krit %	Histologi			IHK	CPE i ASK
	Nyre + Hjerte	Serum	Blod			Hemoragisk levernekrose	Nyre-blødning	Erythro-fagocytose		
16	17			HPRdel		+	-	+	+	-
19	15	15	17	HPRdel	< 10	+	-	+	+	-
21	16	19	18	HPRdel	< 20	+	-	+	+	-
24	19	19	29	HPRdel	< 10	-	+	+	+	-
34	18	19	23	HPRdel	< 20	+	(+)	+	+	-
64	35	26	32	-	normal	-	-	(+)	-	-

Final confirmation of ISA on Mach 24th

YES

- Clinical signs of ISA ?
- Pathological signs of ISA ?
- Increased mortality associated with ISA and/or treatment ?

NO

- No CPE in ASK
 - *Have ASK lost their sensitivity ?*

Final confirmation of CPE by RT-qPCR & IFAT on March 27th

Fish no.	RT-qPCR			Sekven-tering	Hemato-krit %	Histologi			IHK	CPE i ASK
	Nyre + Hjerte	Serum	Blod			Hemoragisk levernekrose	Nyre-blødning	Erythro-fagocytose		
16	17			HPRdel		+	-	+	+	-
19	15	15	17	HPRdel	< 10	+	-	+	+	+
21	16	19	18	HPRdel	< 20	+	-	+	+	-
24	19	19	29	HPRdel	< 10	-	+	+	+	+
34	18	19	23	HPRdel	< 20	+	(+)	+	+	-
64	35	26	32	-	normal	-	-	(+)	-	-

The site will be empty in mid-April

Final confirmation of ISA on March 24th

- *Men hvorfor tog det så lang tid at confirmere ISA ved case 2016 fx. i forhold til case 2014 ?*
 - *Lav-virulent HPRdel*
- *Var vi for langsomme/forsigtige ?*
- *Ventede vi for længe på dyrkning ?*
- *Hvilken rolle spiller vaksinerings mod ILA ?*
 - *Har den besværliggjort ILA diagnostisering ?*
 - *Har den ændret det kliniske ILA billede ?*

Tak for deres opmærksomhed

