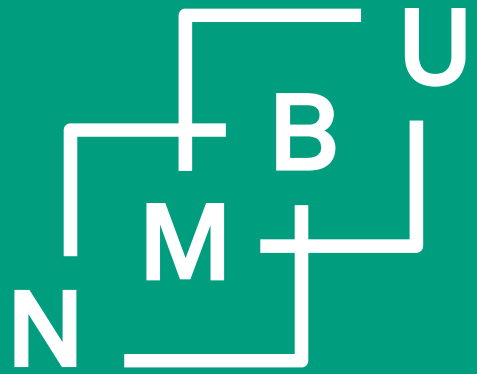

Norwegian University
of Life Sciences

Title: Elin Petterson Norwegian University of Life Sciences

Atlantic salmon was injected with two complementary non-viable SAV plasmids. Recombination of their non-viable genome transcripts resulted in a full-length viable genome and was confirmed by sequencing. A recombined virus was isolated from organ tissue by culture in cells and infectivity and pathogenicity confirmed by a second challenge of Atlantic salmon. Imprecise recombination resulting in deletion variants of the SAV genome was documented and found to be identical to previously identified deletions in the genome detected from natural infection of SAV.

Salmonid alphavirus 3 (SAV3) recombines in Atlantic salmon

Elin Petterson

Presented by Hetroney Mweemba Munangandu

Background

E. Petterson and others

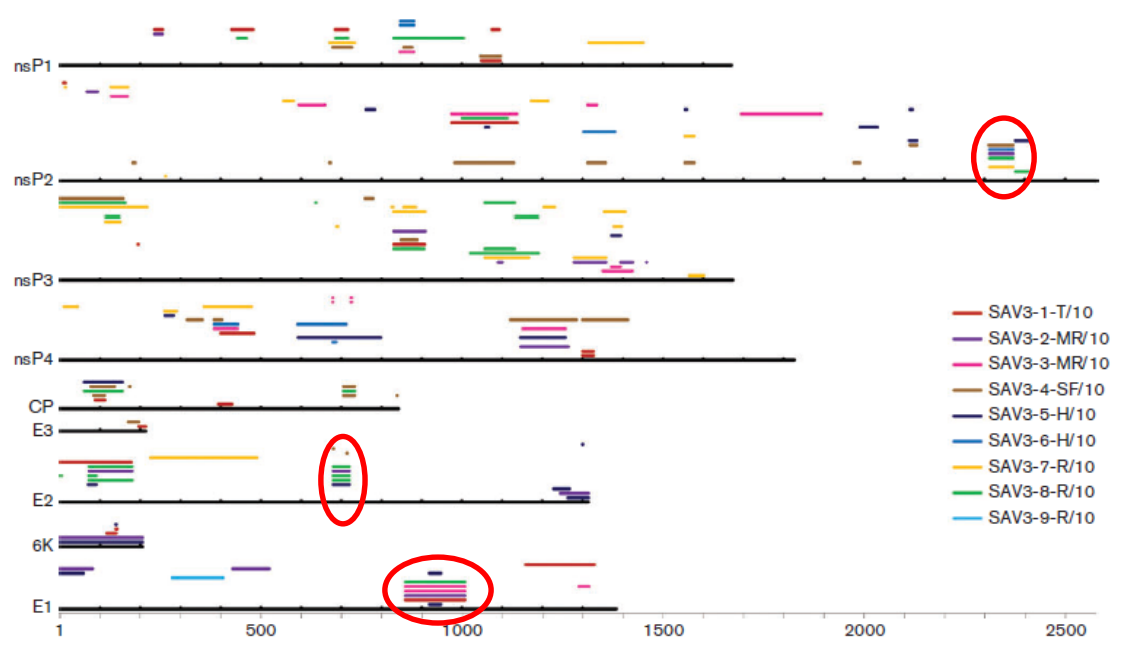

Fig. 2. Distribution of genome deletions (⩾2 nt) found in at least one clone out of 3–6 clones sequenced for each region of each virus strain. Deletion variants were found only in a subset of the clones for each population, and colour coding has been used for visualizing the distribution of deletion variants among different strains. The black lines indicate different coding regions of the contiguous genome, from 5′ to 3′, with deletions found in each region shown above each region. The consensus full-length sequences generated from a minimum of three clones per fragment did not contain deletions.

Background

- We suggested these deleted RNA copies to be a result of imprecise recombination

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PLOS ONE

A 6K-Deletion Variant of Salmonid Alphavirus Is Non-Viable but Can Be Rescued through RNA Recombination

Tz-Chun Guo[1], Daniel X. Johansson[2], Øyvind Haugland[1], Peter Liljeström[2], Øystein Evensen[1]*

1 Norwegian University of Life Sciences, Department of Basic Sciences and Aquatic Medicine, Oslo, Norway, 2 Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

Background

In summary:

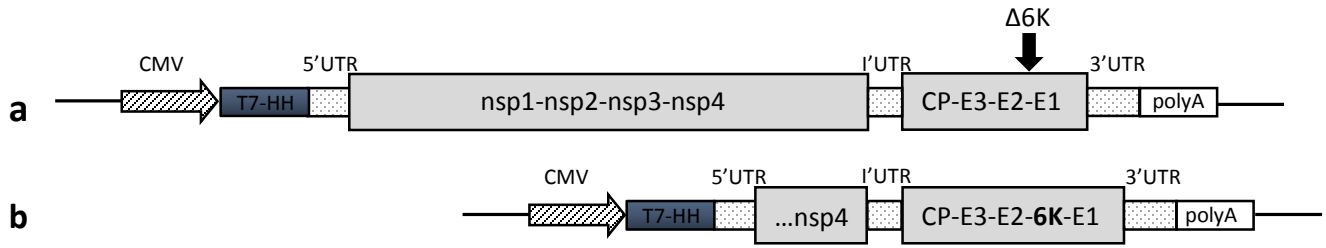
- Field samples show SAV3 deletion mutants

- RNA recombination is confirmed *in vitro*

Aim of the study

Document SAV3 RNA recombination *in vivo*

Material and methods



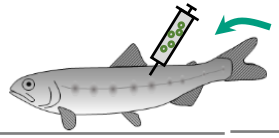
50 fish injected i.m. with a+b

50 fish injected i.m. with a

10 fish injected i.m. with dH_2O

Sampling of internal organs 0, 1, 2, 3, 4 and 5 weeks post infection

Results



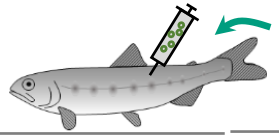
In vivo injection with both cDNA plasmids

Sampling	Fish #	Organ	SAV3 E2 specific real-time PCR (Cp)	Presence 6K-gene
3wpi	3w-1	Heart	25.3	+
		Kidney	25.0	+
	3w-2	**Heart**	**31.6**	**+**
		Kidney	30.0	+
	3w-7	Heart	27.9	+
		Kidney	31.3	+
4wpi	4w-5	Heart	21.8	+
		Kidney	27.9	
5wpi	5w-4	Heart	23.4	+
		Kidney	27.7	
	5w-7	Heart	25.3	+
		Kidney	30.3	
	5w-10	Heart	24.6	+
		Kidney	29.9	

Results

A product of 2.9kb of this combination can only be a result of recombination

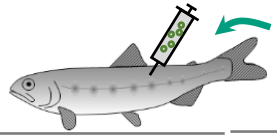
Results



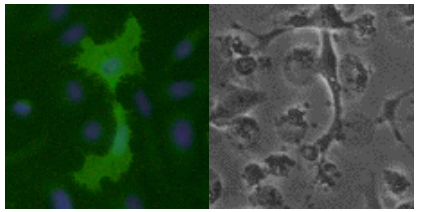
In vivo injection with both cDNA plasmids

Sampling	Fish #	Organ	SAV3 E2 specific real-time PCR (Cp)	Presence 6K-gene	RNA recombination confirmed
3wpi	3w-1	Heart	25.3	+	-
		Kidney	25.0	+	+
	3w-2	**Heart**	**31.6**	**+**	**-**
		Kidney	30.0	+	+
	3w-7	Heart	27.9	+	-
		Kidney	31.3	+	(+)
4wpi	4w-5	Heart	21.8	+	+
		Kidney	27.9		
5wpi	5w-4	Heart	23.4	+	+
		Kidney	27.7		
	5w-7	Heart	25.3	+	+
		Kidney	30.3		
	5w-10	Heart	24.6	+	+
		Kidney	29.9		

Results

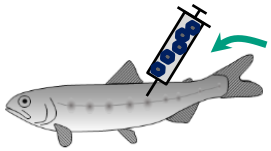


In vivo injection with both cDNA plasmids

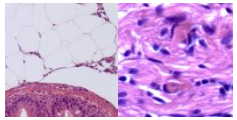


Sampling	Fish #	Organ	SAV3 E2 specific real-time PCR (Cp)	Presence 6K-gene	RNA recombination confirmed	Isolation of recombined SAV3 in cell cultur	
						3rd passage	5th passage
3wpi	3w-1	Heart	25.3	+	-	+	+
		Kidney	25.0	+	+	-	-
	3w-2	**Heart**	**31.6**	**+**	**-**	**+**	**+**
		Kidney	30.0	+	+	+	+
	3w-7	Heart	27.9	+	-	-	
		Kidney	31.3	+	(+)	-	
4wpi	4w-5	Heart	21.8	+	+	-	
		Kidney	27.9			-	
5wpi	5w-4	Heart	23.4	+	+	-	
		Kidney	27.7			-	
	5w-7	Heart	25.3	+	+	-	
		Kidney	30.3			-	
	5w-10	Heart	24.6	+	+	-	
		Kidney	29.9			-	

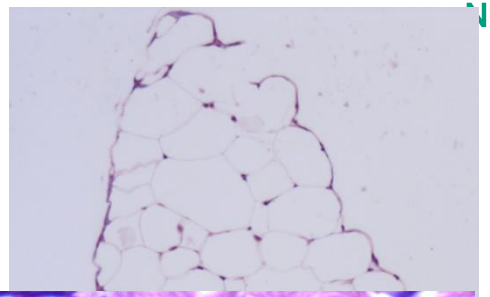
Results

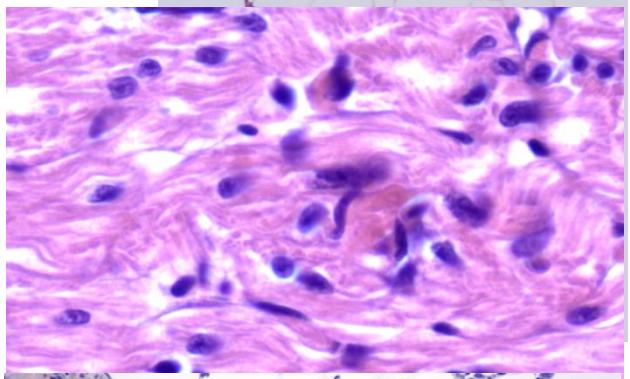


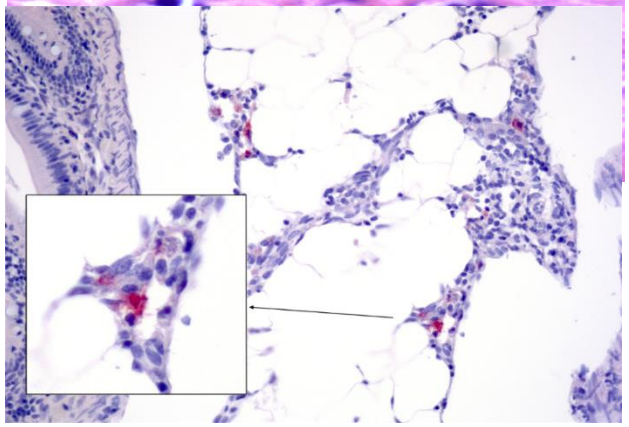
In vivo study with recombined SAV3, 3w-2H



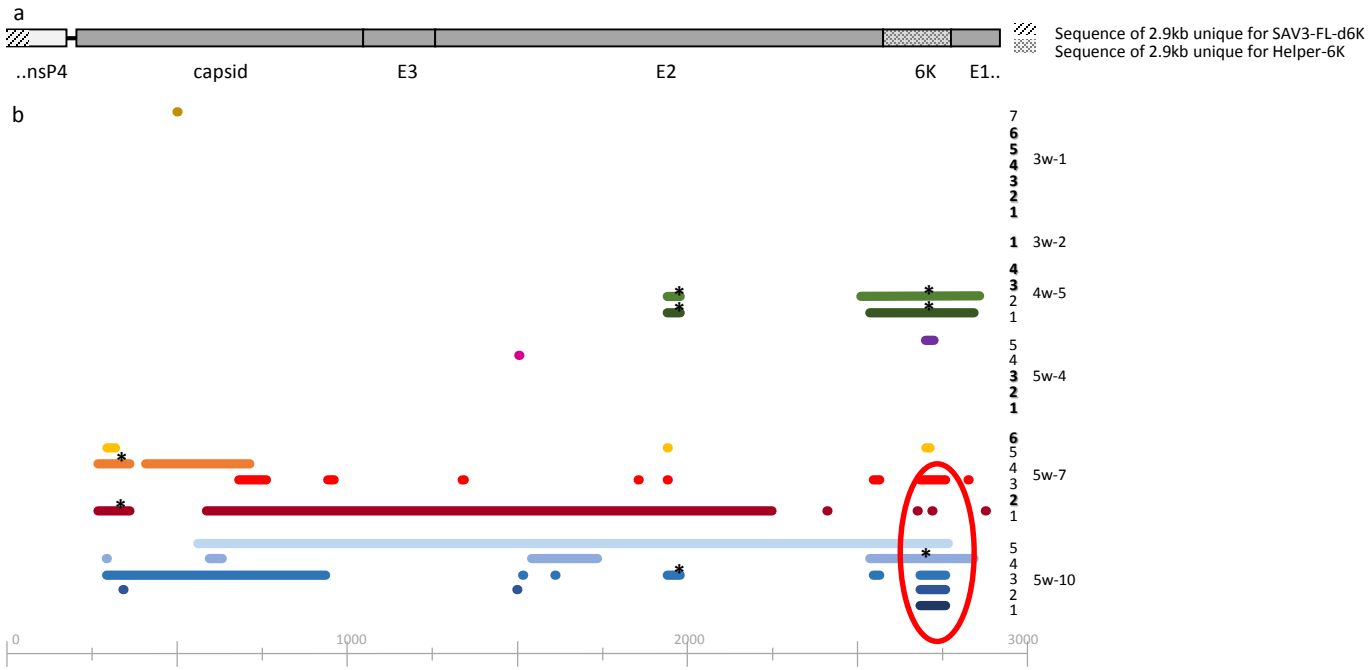
Sampling	Fish #	SAV3 specific PCR heart	SAV3 pathology Pancreas/Heart
6wpc	1	+	+/+
	2	+	+/+
	3	+	+/+
	4	+	+/+
	5	+	+/+
	6	+	+/+





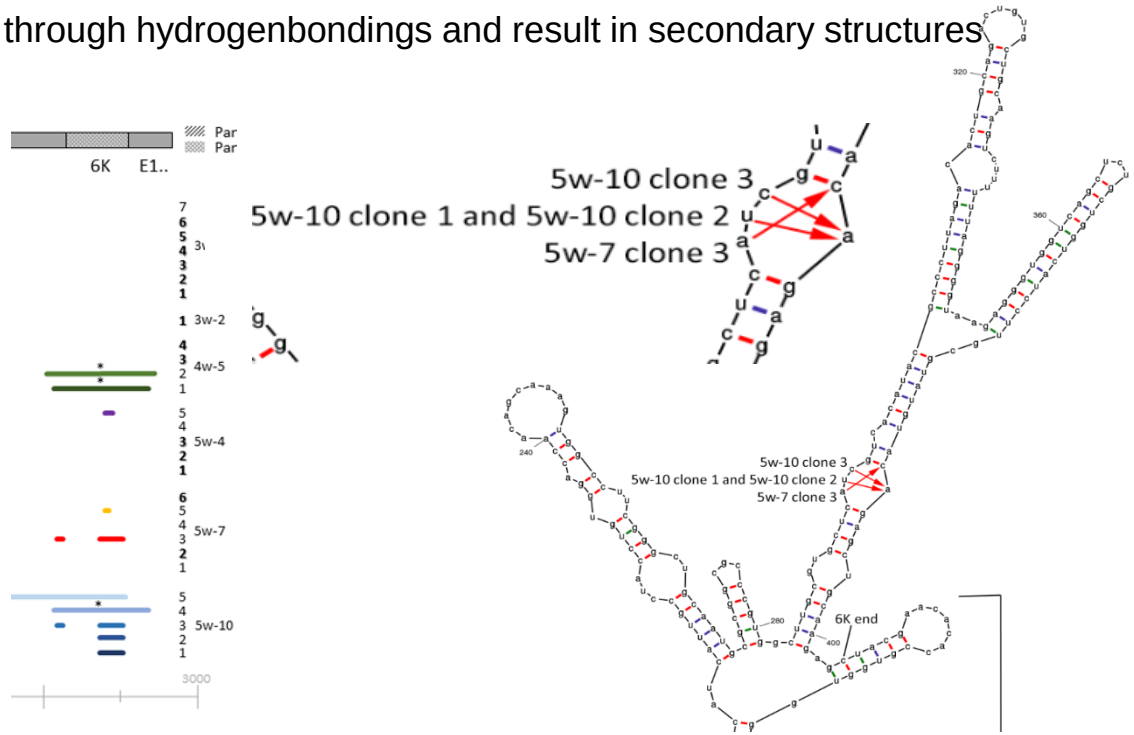


Results


a
..nsP4
capsid
E3
E2
6K
E1..
Sequence of 2.9kb unique for SAV3-FL-d6K
Sequence of 2.9kb unique for Helper-6K
b
3w-1
3w-2
4w-5
5w-4
5w-7
5w-10
0
1000
2000
3000

Results

- Secondary structures can be one of several possible underlying mechanisms involved in production of deletions
- RNA can basepare through hydrogenbondings and result in secondary structures



Conclusive remarks

- Recombination occurred *in vivo* in Atlantic salmon and the resulting virus was shown to be infectious in cell culture
- The isolated virus was infectious and resulted in specific pathology in the target organs
- The recombination is shown to also be imprecise as several viral RNA deletion mutants were found after plasmid injection

Conclusive remarks

- The deletions were of varying size and genome positions but the location in viral genome was identical or similar for several sequenced RNA copies
- Some positions were identical to deletions previously seen in SAV3 RNA in field infections (Paper II)
- A high mutation/recombination rate might provide a genetic plasticity that will benefit the virus in terms of cross-species transmission

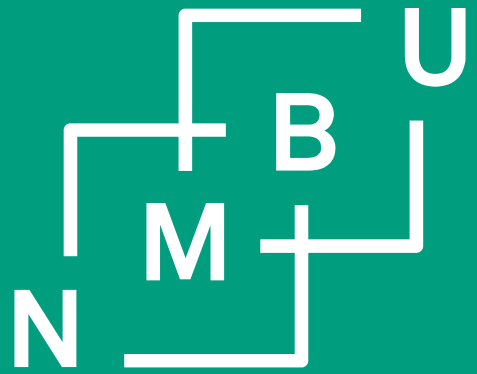
Conclusive remarks

- There is a potential of recombination occurring between SAV subtypes infecting the same invidual
- The recombination frequence needs further exploring
- What are the potential consequence of recombination
  - More virulent
  - Less virulent
  - No change
- The safety regarding attenuated vaccines and vaccines containing full-length or partially deleted genomes should be evaluated against the frequency and potential consequences of recombination

This work is accepted for publishing in Scientific Reports with the title:

Experimental piscine alphavirus RNA recombination in vivo yields both viable virus and defective viral RNA

Elin Petterson, Tz-Chun Guo, Øystein Evensen* and Aase B. Mikalsen*



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