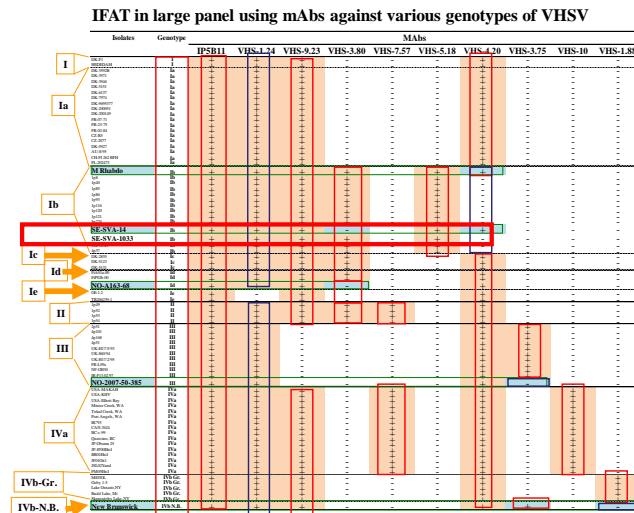


Molecular features of low and high pathogenic clones of VHSV Ib isolates- on the trace of pathogenicity markers

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Typing VHSV by monoclonal antibodies

Table. Summary of MABs reactions for all of the VHSV genotypes

MABs	Genotype of VHSV									
	I/1a	Ib	Ic/Id	Ie	II	III	III*	IVa	IVb-Gr.	IVb-N.B.
IP5B11	+	+	+	+	+	+	+	+	+	+
VHS-1.24	+	+	+	+	+	+	+	+	+	+
VHS-9.23	+	+	+	+	+	+	+	+	+	+
VHS-3.80	+	+	+	+	+	+	+	+	+	+
VHS-7.57	+	+	+	+	+	+	+	+	+	+
VHS-5.18	+	+	+	+	+	+	+	+	+	+
VHS-3.75	+	+	+	+	+	+	+	+	+	+
VHS-10	+	+	+	+	+	+	+	+	+	+
VHS-1.88	+	+	+	+	+	+	+	+	+	+

+:positive

-:negative

*; NO-2007-50-385; The rainbow trout pathogenic genotype III isolate

IVb-Gr.; Genotype IVb of the Great Lakes isolates

IVb-N.B.; Genotype IVb of New Brunswick isolate

MAB	VHSV genotype							
	I/1a	Ib	Ic/Id	Ie	II	III	IVa	IVb
IP5B11	+	+	+	+	+	+	+	+
VHS-3.80	-	+	+	-	+	-	-	-
VHS-4.20	+	-	+	+	+	+	+	+
VHS-5.18	-	+	-	-	-	-	-	-

Table. Results of IFAT for monoclonal antibodies against genotype Ib isolates of VHSV

G1b Isolates	MABs									
	IP5B11	VHS-1.24	VHS-9.23	VHS-3.80	VHS-7.57	VHS-5.18	VHS-4.20	VHS-3.75	VHS-10	VHS-1.88
M Rhabdo	+	+	+	+	-	+	+	-	-	-
1p8	+	+	+	+	-	+	+	-	-	-
1p40	+	+	+	+	-	+	+	-	-	-
1p85	+	+	+	+	-	+	+	-	-	-
1p86	+	+	+	+	-	+	+	-	-	-
1p93	+	+	+	+	-	+	+	-	-	-
1p116	+	+	+	+	-	+	+	-	-	-
1p120	+	+	+	+	-	+	+	-	-	-
1p121	+	+	+	+	-	+	+	-	-	-
5p276	+	+	+	+	-	+	+	-	-	-
SE-SVA-14	+	+	+	+	-	+	+	-	-	-
SE-SVA-1033	+	+	+	+	-	+	+	-	-	-
UK-96-43	+	+	+	+	-	+	+	-	-	-
4p37	+	+	+	+	-	+	+	-	-	-

Partial alignments of amino acids of N-proteins of the homologous Ib isolate and the 4 SVA clones.

Virus clone isolates		Region of epitope of Mab VHS-3.80					
KRRV9601 (Genotype Ib)	1	MEGGIRAAFS	GLNDVRIDPT	GSEGRVLVPG	EVELIVVVG	FG EDGK IV	50
SE-SVA-14-3D	1	MEGGIRAAFS	GLNDVRIDPT	GSEGRVLVPG	EVELIVVVG	FG EDGK IV	50
SE-SVA-14-5G	1	MEGGIRAAFS	GLNDVRIDPT	GSEGRVLVPG	EVELIVVVG	FG EDGK IV	50
SE-SVA-1033-3F	1	MEGGIRAAFS	GLNDVRIDPT	GSEGRVLVPG	EVELIVVVG	FG EDGK IV	50
SE-SVA-1033-9C	1	MEGGIRAAFS	GLNDVRIDPT	GSEGRVLVPG	EVELIVVVG	FG EDGK IV	50
KRRV9601 (Genotype Ib)	51	DALSALGGPQ	TVQALS V LLS	VVLQNTQED	LGMRCKVLTD	MGFKVTQAA	100
SE-SVA-14-3D	51	DALSALGGPQ	TVQALS V LLS	YVLQNTQED	LGMRCKVLTD	MGFKVTQAA	100
SE-SVA-14-5G	51	DALSALGGPQ	TVQALS V LLS	YVLQNTQED	LGMRCKVLTD	MGFKVTQAA	100
SE-SVA-1033-3F	51	DALSALGGPQ	TVQALS V LLS	YVLQNTQED	LGMRCKVLTD	MGFKVTQAA	100
SE-SVA-1033-9C	51	DALSALGGPQ	TVQALS V LLS	YVLQNTQED	LGMRCKVLTD	MGFKVTQAA	100
KRRV9601 (Genotype Ib)	101	ATSEAGIMM	PMRELALTVN	DDNLMEIVKG	TLMTCSELLTK	YSVDRMIKYI	150
SE-SVA-14-3D	101	ATSEAGIMM	PMRELALTVN	DDNLMEIVKG	TLMTCSELLTK	YSVDRMIKYI	150
SE-SVA-14-5G	101	ATSEAGIMM	PM G ELALTVN	DDNLMEIVKG	TLMTCSELLTK	YSVDRMIKYI	150
SE-SVA-1033-3F	101	ATSEAGIMM	PMRELALTVN	DDNLMEIVKG	TLMTCSELLTK	YSVDRMIKYI	150
SE-SVA-1033-9C	101	ATSEAGIMM	PMRELALTVN	DDNLMEIVKG	TLMTCSELLTK	YSVDRMIKYI	150
KRRV9601 (Genotype Ib)	151	TKKLGELADT	QGVGELQYFT	ADKAAIRKLA	GCVRPQGRIT	KALYAFILTE	200
SE-SVA-14-3D	151	TKKLGELADT	QGVGELQYFT	ADKAAIRKLA	GCVRPQGRIT	KALYAFILTE	200
SE-SVA-14-5G	151	TKKLGELADT	QGVGELQYFT	ADKAAIRKLA	GCVRPQGRIT	KALYAFILTE	200
SE-SVA-1033-3F	151	TKKLGELADT	QGVGELQYFT	ADKAAIRKLA	GCVRPQGRIT	KALYAFILTE	200
SE-SVA-1033-9C	151	TKKLGELADT	QGVGELQYFT	ADKAAIRKLA	GCVRPQGRIT	KALYAFILTE	200

Region of epitope of Mab VHS-4.20

Fig. Mortality of rainbow trout after infection with Swedish Ib variants by intraperitoneal (i.p.) injection

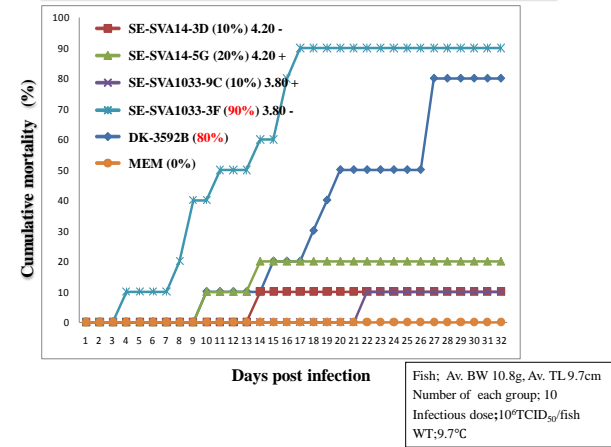


Fig. Mortality of amago trout (*Oncorhynchus masou ishikawae*) after infection with Swedish Ib variants by i.p. injection

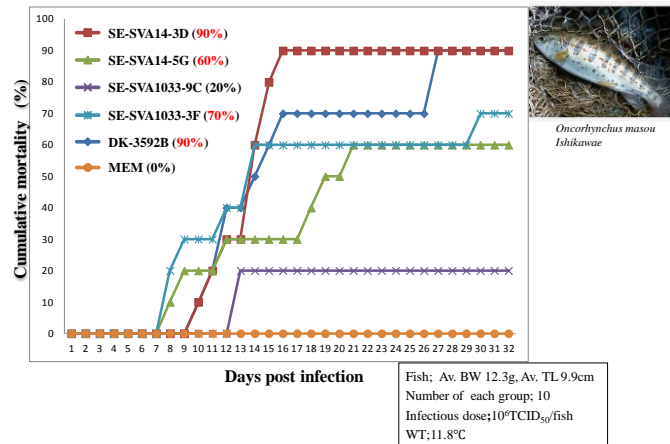


Fig. Mortality of rainbow trout after infection with Swedish Ib variants in DTU

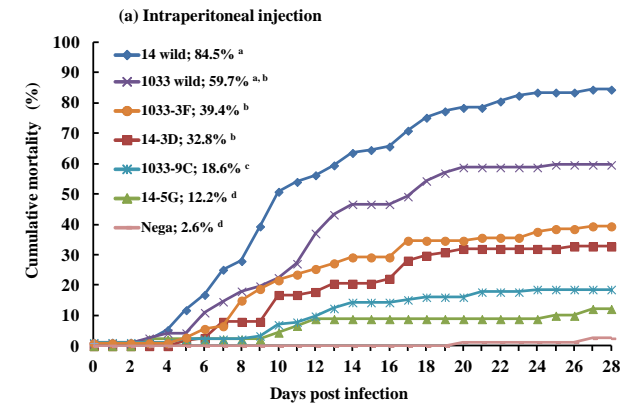


Fig. Mortality of rainbow trout after infection with Swedish Ib variants in DTU

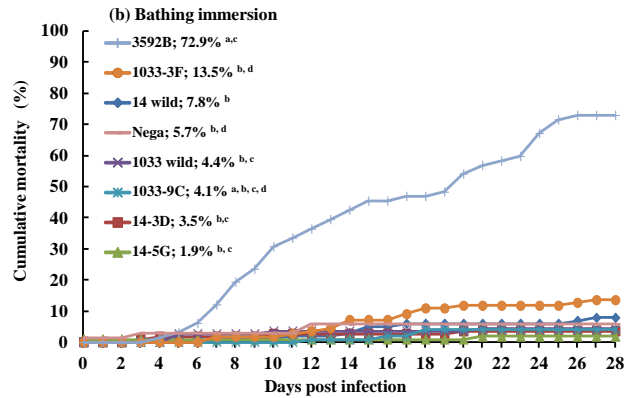


Table 1. Results of titration of isolates or variant clones to cell lines.

Isolates/Variant clones (Genotype)	Titer (TCID ₅₀ /ml)		
	BF-2	EPC	RTG-2
DK-3592B (Gla)	10 ^{7.2}	10 ^{8.9}	10 ^{7.4}
4p37 (GIb)	10 ^{7.0}	10 ^{4.7*}	10 ^{4.5}
SE-SVA-14-3D (GIb)	10 ^{7.5}	10 ^{7.2}	10 ^{5.0}
SE-SVA-14-5G (GIb)	10 ^{8.2}	10 ^{7.8}	10 ^{4.8}
SE-SVA-1033-3F (GIb)	10 ^{7.3}	10 ^{6.1}	10 ^{5.6}
SE-SVA-1033-9C (GIb)	10 ^{7.1}	10 ^{4.1*}	10 ^{4.8}

*: CPE is appeared, but not progressed to complete cell destruction.

TABLE 1. Presence of virus in BF-2 cells*

Hours p.i.	No. of wells positive for:	
	fw-DK-3592B	ma-1p8
2	1	0
4	2	0
8	2	0
24	2	0
48	3	2

* GEC filter insert cultures were infected with fw-DK-3592B and ma-1p8 on the apical side, and samples were collected on the basal side (see Fig. 1 for details) and examined for the presence of virus in BF-2 cells. The numbers of positive wells (of three per time point) over an incubation period of 48 h after virus was added to the apical side are indicated.

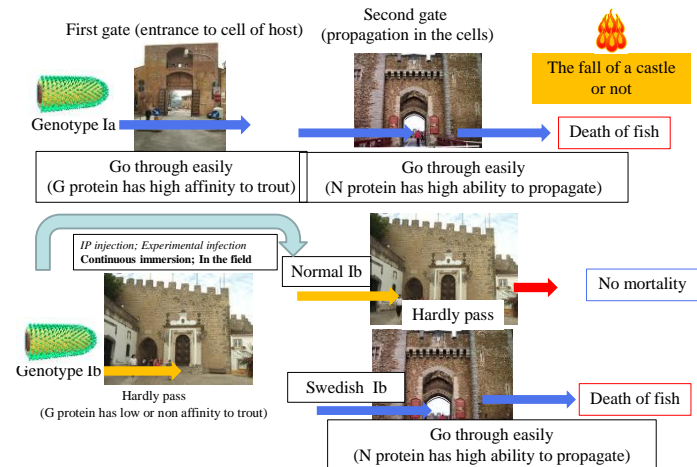
(Brudeseth *et al.* 2008)

Table Summary of the aa alignment of the epitopic part of each mAbs and pathogenicity to trout of each variant and isolate.

Isolates	Variants	AA43-48 in N-protein	AA168 in N-protein	Pathogenicity to trout
SE-SVA-14 (Ib)	3D	K E D G K V	H	++
	5G	K E D G K V	Y	++
SE-SVA-1033 (Ib)	3F	E E D R K V	Y	++
	9C	E E D G K V	Y	-
DK-3592B (Ia)		E E D R K V	H	+++

9C+/ 3F -

Image of virulence to trout of Swedish genotype Ib



Before final conclusions on pathogenicity mechanism can be made, the entire genome of these viruses must be analyzed in order to be sure that only substitutions in the N-protein is sufficient to influence the virulence of variants of G1b. This information will be provided soon.

Table 3. Comparative analysis of amino acid (AA) substitution rates among Swedish VHSV Ib variants (SE-SVA-14-3D vs. SE-SVA-14-5G) and (SE-SVA-1033-3F vs. SE-SVA-1033-9C) with different virulence properties which is pathogenicity towards rainbow trout.

Amino acid substitution

SE-SVA-14-3D vs. SE-SVA-14-5G

Gene 3' - 5'	Product size (AA)	Substitution (n)	Identity (%)	Substituted (AA)	Substitution position (AA)
N	404	2	99.5	Arg-Gly, His - Tyr	103, 168
P	222	x	xxx	-	-
M	201	x	xxx	-	-
G	507	x	xxx	-	-
NV	122	x	xxx	-	-
L	1985	x	xxx	-	-
Combined	3446	x	xxx	-	-

SE-SVA-1033-3F vs. SE-SVA-1033-9C

Gene 3' - 5'	Product size (AA)	Substitution (n)	Identity (%)	Substituted (AA)	Substitution position (AA)
N	404	2	99.5	Arg-Gly, Ile-Thr	46, 49
P	222	x	xxx	-	-
M	201	x	xxx	-	-
G	507	x	xxx	-	-
NV	122	x	xxx	-	-
L	1985	x	xxx	-	-
Combined	3446	x	xxx	-	-

Conclusions

- Pathogenicity is a question of host-pathogen interaction, even closely related *Oncorhynchus* species (*mykiss* and *masou*) respond differently
- Reverse genetics might help us to assess if these substitutions in the N-protein are sufficient to increase mortality in Rainbow trout significantly
- Virulence = Entrance (host defence)+ propagation (cell level defence)