The following supplement accompanies the article

Virulence marker candidates in N-protein of viral haemorrhagic septicaemia virus (VHSV): virulence variability within VHSV Ib clones

Takafumi Ito*, Jun Kurita, Koh-ichiro Mori, Helle Frank Skall, Niels Lorenzen, Niccolò Vendramin, Nikolaj Gedsted Andersen, Katja Einer-Jensen, Niels Jørgen Olesen

*Corresponding author: takafumi@fra.affrc.go.jp

Diseases of Aquatic Organisms 128: 51–62 (2018)

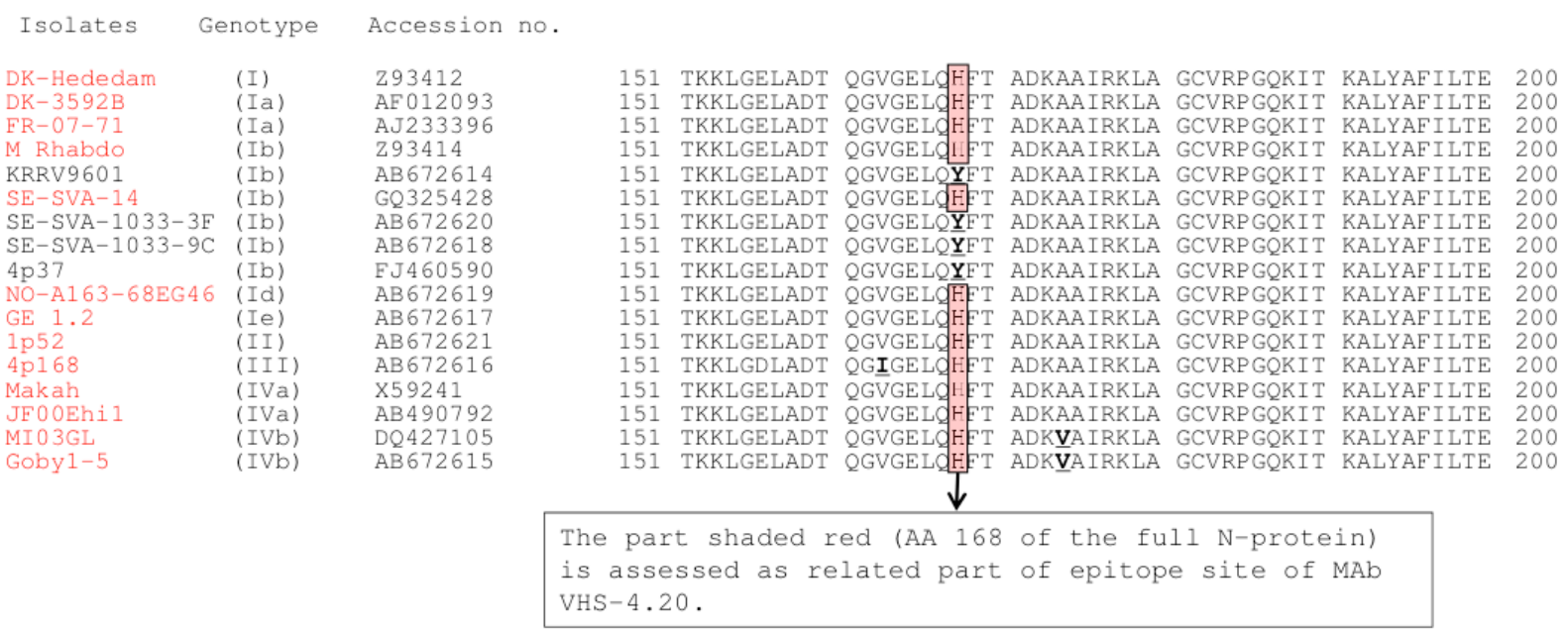

Fig. S1. Partial alignments of amino acid of N-protein for assessment of the epitope of MAb VHS-4.20. The isolates shown by red letters reacted with MAb VHS-4.20.

Table S1. VHSV isolates which was used for characterization of the MAbs from all over the world

Isolates	Country or Location	Year	Genotype [a]	Serotype [b]	Host (Fresh;(F), Brackish;(B) or Sea;(S))	Reference or Source
DK-F1	Denmark	1962	I	1	Rainbow trout (F)	Jensen 1965
DK-Hededam	Denmark	1970	I	1	Rainbow trout (F)	Jørgensen 1974
DK-3592B	Denmark	1986	Ia	1	Rainbow trout (F)	Fish Pathogens Database 2009
DK-3971	Denmark	1987	Ia	NT [c]	Rainbow trout (S)	Einer-Jensen et al. 2005b
DK-3946	Denmark	1987	Ia	NT	Rainbow trout (F)	Einer-Jensen et al. 2005b
DK-5151	Denmark	1988	Ia	3	Rainbow trout (F)	Batts et al. 1993
DK-6137	Denmark	1991	Ia	3	Rainbow trout (S)	Jørgensen et al. 1995
DK-7974	Denmark	1995	Ia	NT	Rainbow trout (F)	Einer-Jensen et al. 2004
DK-9695377	Denmark	1996	Ia	3	Rainbow trout (F)	Einer-Jensen et al. 2004
DK-200051-1	Denmark	2000	Ia	NT	Rainbow trout (F)	Einer-Jensen et al. 2004
DK-200149	Denmark	2000	Ia	NT	Rainbow trout (F)	Einer-Jensen et al. 2004
FR-07-71	France	1971	Ia	1	Rainbow trout (F)	Thiery et al. 2002
FR-23-75	France	1975	Ia	2	Brown trout (F)	Thiery et al. 2002
FR-02-84	France	1984	Ia	1	Rainbow trout (F)	Benmansour et al. 1997
CZ-7738-R5	Czech Republic	1994	Ia	3	Rainbow trout (F)	DTU.Vet
CAPM V553	Czech Republic	1999	Ia	3	Rainbow trout (F)	Reschova et al. 2008
DE-10/90 (DK-5927)	Germany	1991	Ia	3	Tubot (S)	Schlotfeldt et al. 1991
AU-8/95	Austria	1995	Ia	3	Rainbow trout (F)	Einer-Jensen et al. 2004
CH-F1 262 BFH	Switzerland	1999	Ia	2	Rainbow trout (F)	Knuesel et al. 2003
PL-A	Poland	2002	Ia	3	Pike fry (F)	PIWet
M Rhabdo	Baltic Sea	1979	Ib	1	Cod (S)	Jensen et al 1979
1p8	Baltic Sea	1996	Ib	1	Herring (S)	Mortensen et al. 1999
1p40	Baltic Sea	1996	Ib	1	Rocking (S)	Mortensen et al. 1999
1p85	Baltic Sea	1996	Ib	NT	Herring (S)	Mortensen et al. 1999
1p86	Baltic Sea	1996	Ib	NT	Sprat (S)	Mortensen et al. 1999
1p93	Baltic Sea	1996	Ib	NT	Cod (S)	Mortensen et al. 1999
1p116	Baltic Sea	1996	Ib	NT	Cod (S)	Mortensen et al., 1999
1p120	Baltic Sea	1996	Ib	NT	Herring (S)	Mortensen et al. 1999

Table S1. (Continued)

Isolates	Country or Location	Year	Genotype [a]	Serotype [b]	Host (Fresh;(F), Brackish;(B) or Sea;(S))	Reference or Source
1p121	Baltic Sea	1996	Ib	NT	Sprat (S)	Mortensen et al. 1999
5p276	Kattegatt	1998	Ib	NT	Plaice (S)	Snow et al. 2004
SE-SVA-14	Kattegatt	1998	Ib	2	Rainbow trout (S)	Nordblom 1998
SE-SVA-1033	Kattegatt	2000	Ib	1	Rainbow trout (S)	Nordblom & Norell 2000
(3F and 9C)						Ito et al. 2012
UK-96-43	English Channel	1996	Ib	NT	Herring (S)	Dixson et al., 1997
4p37	North Sea	1997	Ib	1	Blue whiting (S)	Mortensen et al., 1999
KRRV9601	Japan	1996	Ib	NT	Japanese Flounder (S)	Isshiki et al. 2001
DK-2835	Denmark	1982	Ic	1	Rainbow trout (F)	Einer-Jensen et al. 2004
DK-5123	Denmark	1988	Ic	NT	Rainbow trout (F)	Einer-Jensen et al. 2004
DK-5131	Denmark	1988	Ic	2	Rainbow trout (F)	Batts et al. 1993
FiA01a.00	Finland	2000	Id	1	Rainbow trout (B)	Raja-Halli et al. 2006
FiP02b.00	Finland	2000	Id	1	Rainbow trout (B)	Raja-Halli et al. 2006
NO-A163-68 EG46	Norway	1968	Id	1	Rainbow trout (F)	Einer-Jensen et al. 2004
GE-1.2	Gerogia	1981	Ie	NT	Rainbow trout (F)	Einer-Jensen et al. 2004
TR206239-1	Turkey	2006	Ie	NT	Rainbow trout (F)	DTU.Vet
1p49	Baltic Sea	1996	II	1	Herring (S)	Mortensen et al. 1999
1p52	Baltic Sea	1996	II	1	Sprat (S)	Mortensen et al. 1999
1p53	Baltic Sea	1996	II	1	Herring (S)	Mortensen et al. 1999
1p54	Baltic Sea	1996	II	NT	Cod (S)	Mortensen et al. 1999
2p51	Skagerrack	1996	III	NT	Norway pout (S)	Mortensen et al. 1999
4p101	Skagerrack	1996	III	3	Whiting (S)	Mortensen et al. 1999
4p168	Skagerrack	1996	III	3	Atlantic herring (S)	Mortensen et al. 1999
4p51	North Sea	1996	III	NT	Lesser argentine (S)	Mortensen et al. 1999
UK-H17/5/93	UK	1993	III	NT	Cod (S)	Stone et al. 1997
UK-860/94	UK	1994	III	2	Turbot (S)	Ross et al. 1994
UK-H17/2/95	UK	1995	III	1	Haddock (S)	Snow et al. 1999

Table S1. (Continued)

Isolates	Country or Location	Year	Genotype [a]	Serotype [b]	Host (Fresh;(F), Brackish;(B) or Sea;(S))	Reference or Source
F-L59x	France	1987	III	2	Eel (F)	Castric et al. 1992
GH30	Flemish Cap	1994	III	2	Greenland halibut (S)	Dopazo et al. 2002
IR-F13.02.97	Ireland	1997	III	NT	Turbot (S)	Snow et al. 1999
NO-2007-50-385	Norway	2007	III	NT	Rainbow trout (S)	Dale et al. 2009
Makah	USA	1988	IVa	1	Coho salmon (F)	Batts et al. 1993
KHV	USA	1988	IVa	1	Chinook salmon (F)	Einer-Jensen et al. 2005b
Elliot Bay herring	USA	1993	IVa	1	Pacific herring (S)	Einer-Jensen et al. 2005b
Minter Creek, WA	USA	2002	IVa	NT	Coho salmon (F)	USGS
Tokul Creek, WA	USA	2006	IVa	NT	Steelhead (F)	USGS
Port Angels, WA	USA	2007	IVa	NT	Atlantic salmon (F)	USGS
BC'93	Canada	1993	IVa	1	Pacific herring (S)	Meyers & Winton 1995
CAN-3624	Canada	1995	IVa	2	Atlantic salmon (S)	Traxler et al. 1995
BC-s-99	Canada	1998/99	IVa	1	Pilchard (S)	Hedrick et al. 2003
Quatsino, BC	Canada	2002	IVa	NT	Sardines (S)	USGS
JP-Obama 25	Japan	1999	IVa	1	Japanese Flounder (S)	Takano et al. 2000
JF00Ehi1	Japan	2000	IVa	1	Japanese Flounder (S)	Nishizawa et al. 2002
BR01Ehi1	Japan	2001	IVa	NT	Black rockfish (S)	Ito et al. 2010
JF01Oit1	Japan	2001	IVa	NT	Japanese Flounder (S)	Ito et al. 2010
JSL02Yam1	Japan	2002	IVa	NT	Pacific sandeel (S)	Ito et al. 2010
PM05Ehi1	Japan	2005	IVa	NT	Red sea bream (S)	NRIA FRA
MI03GL	USA	2003	IVb	NT	Muskellunge (F)	Elsayed et al. 2006
Goby 1-5	USA	2006	IVb	NT	Round goby (F)	Groocock et al. 2007
Lake Ontario, NY	USA	2007	IVb	NT	Gizzard Shad (F)	USGS
Budd Lake, MI	USA	2007	IVb	NT	Bluegill (F)	USGS
Skaneateles Lake, NY	USA	2007	IVb	NT	Smallmouth bass (F)	USGS
CA-NB00-01	Canada	2000	IVb	NT	Mummichog (F)	Gagné et al. 2007

[a] Classification of genotype of virus is based on the follow reference of Einer-Jensen K.et al. (2004 and 2005), Snow et al.(2004), López-Vázquez et al.(2006), Elsayed et al. (2006), Gagné et al. (2007), Groocok et al. (2007).

[b] Classification of serotype of virus is based on the described by Olesen et al. (1993)

[c] NT, Not tested

DTU Vet; National Veterinary Institute, Technical University of Denmark

PIWet; National Veterinary Institute in Poland

USGS; United States Geological Survey

NRIA FRA; National Research Institute of Aquaculture, Fisheries Research Agency

References

Batts WN, Arakawa CK, Bernard J, Winton JR (1993) Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. Dis Aquat Org 17:67–71

Benmansour A, Basurco B, Monnier AF, Vende P, Winton JR, De Kinkelin P (1997) Sequence variation of the glycoprotein gene identifies three distinct lineages within field isolates of viral haemorrhagic septicaemia virus, a fish rhabdovirus. J Gen Virol 78:2837–2846

Castric J, Jeffroy J, Bearzotti M, De Kinkelin P (1992) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild elvers *Anguilla anguilla*. Bull Eur Assoc Fish Pathol 12:21–23

Dale OB, Ørpetveit I, Lyngstad TM, Kahns S, Skall HF, Olesen NJ, Dannevig BH (2009) Outbreak of viral haemorrhagic septicaemia (VHS) in seawater-farmed rainbow trout in Norway caused by VHS virus Genotype III. Dis Aquat Org 85:93-103

Dixon PF, Feist S, Kehoe E, Parry L, Stone DM, Way K (1997) Isolation of viral haemorrhagic septicaemia virus from Atlantic herring *Clupea harengus* from the English Channel. Dis Aquat Organ 30:81–89

Dopazo CP, Bandín I, López-Vazquez C, Lamas J, Noya M, Barja JL (2002) Isolation of viral hemorrhagic septicemia virus from Greenland halibut *Reinhardtius hippoglossoides* caught at the Flemish Cap. Dis Aquat Org 50:171-179

Einer-Jensen K, Ahrens P, Forsberg R, Lorenzen N (2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. J Gen Virol 85:1167-1179

Einer-Jensen K, Winton J, Lorenzen N (2005) Genotyping of the fish rhabdovirus, viral haemorrhagic septicaemia virus, by restriction fragment length polymorphisms. Vet Microbiol 106:167-178

Elsayed E, Faisai M, Thomas M, Whelan G, Batts W, Winton J (2006) Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St Clair, Michigan, USA reveals a new sublineage of the North American genotype. J Fish Dis 29:611-619

Fish Pathogens Database (2009) European Reference Laboratory for Fish Diseases. Available at www.fishpathogens.eu/ (accessed 19 Jan 2017)

Gagné N, Mackinnon AM, Boston L, Souter B, Cook-Versloot M, Griffiths S, Oliver G (2007) Isolation of viral haemorrhagic septicaemia virus from mummichog, stickleback, striped bass, and brown trout in eastern Canada. J Fish Dis 30:213-223

Groocock GH, Getchell RG, Wooster GA, Britt KL, Batts WN, Winton JR, Casey RN, Casey JW, Bowser PR (2007) Detection of viral hemorrhagic septicemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. Dis Aquat Org 76:187–192

Hedrick RP, Batts WN, Yun S, Traxler GS, Kaufman J, Winton JR (2003) Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. Dis Aquat Org 55:211–220

Isshiki T, Nishizawa T, Kobayashi T, Nagano T, Miyazaki T (2001) An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder *Paralichthys olivaceus* in Japan. Dis Aquat Org 47:87-99

Ito T, Olesen NJ, Skall HF, Sano M, Kurita J, Nakajima K, Iida T (2010) Development of a Monoclonal Antibody against Viral Haemorrhagic Septicaemia Virus (VHSV) Genotype IVa. Dis Aquat Org 89:17-27

Jensen MH (1965) Research on the virus of Egtved diease. Ann N Y Acad Sci 126:422-426

Jensen NJ, Bloch B, Larsen JL (1979) The ulcus-syndrome in cod (*Gadus morhua*). III. A preliminary virological report. Nord Vet Med 31:436-442

Jørgensen PEV, Einer-Jensen K, Higman KH, Winton JR (1995) Sequence comparison of the central region of the glycoprotein gene of neutralizable, non-neutralizable, and serially passed isolates of viral haemorrhagic septicaemia virus. Dis Aquat Org 23:77–82

Knuesel R, Segner H, Wahli T (2003) A survey of viral diseases in farmed and feral salmonids in Switzerland. J Fish Dis 26:167–182

López-Vázquez C, Raynard RS, Bain N, Snow M, Bandín I, Dopazo CP (2006) Genotyping of marine viral haemorrhagic septicaemia virus isolated from the Flemish Cap by nucleotide sequence analysis and restriction fragment length polymorphism patterns. Dis Aquat Org 73:23-31

Meyers TR, Winton JR (1995) Viral hemorrhagic septicemia virus in North America. Annu Rev Fish Dis 5:3-24

Mortensen HF, Heuer OE, Lorenzen N, Otte L, Olesen NJ (1999) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. Virus Res 63:95-106

Nishizawa T, Iida H, Takano R, Isshiki T, Nakajima K, Muroga K (2002) Genetic relatedness among Japanese, American and European isolates of viral hemorrhagic septicemia virus (VHSV) based on partial G and P genes. Dis Aquat Org 48:143-148

Nordblom B (1998) Report on an Outbreak of Viral Haemorrhagic Septicaemia in Sweden. Report for the Standing Veterinary Committee, Swedish Board of Agriculture, Department for Animal Production and Health, Jönköping.

Nordblom B, Norell AW (2000) Report on an Outbreak of (VHS) Viral Haemorrhagic Septicaemia in Farmed Fish in Sweden. Report for the Standing Veterinary Committee, Swedish Board of Agriculture, Department for Animal Production and Health, Jönköping.

Raja-Halli M, Vehmas TK, Rimaila-Pärnänen E, Sainmaa S, Skall HF, Olesen NJ, Tapiovaara H (2006) Viral haemorrhagic septicaemia (VHS) outbreaks in Finnish rainbow trout farms. Dis Aquat Org 72:201-211

Reschova S, Pokorova D, Hulova J, Kulich P, Vesely T (2008) Surveillance of viral fish diseases in the Czech Republic over the period January 1999 – December 2006. Vet Med– Czech 53:86-92

Ross K, McCarthy U, Huntly PJ, Wood BP, Stuart D, Rough EI, Smail DA, Bruno DW (1994) A outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. Bull Eur Assoc Fish Pathol 14:213–214

Schlotfeldt HJ, Ahne W, Jørgensen PEV, Glende W (1991) Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – a natural outbreak. Bull Eur Ass Fish Pathol 11:105-107

Snow M, Cunningham CO, Melvin WT, Kurath G (1999) Analysis of the nucleoprotein gene identifies distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. Virus Res 63:35-44

Snow M, Bain N, Black J, Taupin V, Cunningham CO, King JS, Skall HF, Raynard RS (2004) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). Dis Aqua Org 61:11-21

Stone DM, Way K, Dixon PF (1997) Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas: a link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). J Gen Virol 78:1319–1326

Takano R, Nishizawa, T, Aritmoto M, Muroga K (2000) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, *Paralichthys olivaceus*. Bull Eur Assoc Fish Pathol 20:186–192

Thiéry R, de Boisséson C, Jeffroy J, Castric J, De Kinkelin P, Benmansour A (2002) Phylogenetic analysis of viral haemorrhagic septicaemia virus (VHSV) isolates from France. Dis Aquat Org 52:29–37

Traxler G, Kieser D, Evelyn TPT (1995) Isolation of North American strain of VHS virus from farmed Atlantic salmon. In Aquaculture Update No. 72, Edited by Margolis, L. Aquaculture Division, Pacific Biological Station, Nanaimo, BC

Table S2. Various piscine rhabdoviruses used for cross reaction test

Virus	Abbreviation	Isolates	Reference
Members and probable members of the genus Novirhabdovirus			
Infectious haematopoetic necrosis virus	IHNV	RBH	LaPatra et al. 1993
	IHNV	32/87	Laurencin 1987
	IHNV	Coleman	Amend et al. 1969
	IHNV	4008	Bovo et al. 1987
	IHNV	OSV	Wingfield et al. 1969
	IHNV	TR	LaPatra et al. 1993
	IHNV	ER	LaPatra et al. 1993
	IHNV	HAG	LaPatra et al. 1993
	IHNV	Austria	DTU.Vet
Hirame rhabdovirus	HIRRV	8401	Kimura et al. 1986
Capione brown trout rhabdovirus	Carpione	583	Bovo et al. 1995
Snakehead rhabdovirus	SHRV		Ahne et al. 1988
Eel virus	EEV	B12	Castric et al. 1984
Vesiculovirus-like viruses			
Spring viremia of carp virus (Rhabdovirus carpio)	SVCV	56/70	Fijan et al. 1971
Pike fry rhabdovirus	PFRV	S64	De Kinkelin et al. 1973
Tench rhabdovirus	Tench RV	Italian IZSV	Ahne et al. 1982
Perch rhabdovirus	Perch RV		Dorson et al. 1984
Eel virus European X	EVEX		Sano et al. 1977

DTU Vet; National Veterinary Institute, Technical University of Denmark

References

Ahne W, Mahnel H, Steinhagen P (1982) Isolation of pike fry rhabdovirus from tench, *Tinca tinca* L., and white bream, *Blicca bjoerkna* (L.). J Fish Dis 5:535–537

Ahne W, Jorgensen PEV, Olesen NJ, Wattanavijarn W (1988) Serological examination of a rhabdovirus isolated from snakehead (*Ophicephalus striatus*) in Thailand with ulcerative syndrome. J Appl Ichthyology 4:194–196

Amend DF, Yasutake WT, Mead RW (1969) A haematopoietic virus disease of rainbow trout and sockeye salmon. Trans Am Fish Soc 98:796–804

Bovo G, Giorgetti G, Jørgensen PEV, Olesen NJ (1987) Infectious haematopoietic necrosis: first detection in Italy. Bull Eur Assoc Fish Pathol 7:24

Bovo G, Olesen NJ, Jørgensen PEV, Ahne W, Winton JR (1995) Characterization of a rhabdovirus isolated from carpione *Salmo trutta carpio* in Italy. Dis Aquat Organ 21:115–122

Castric J, Rasschaert D, Bernard J (1984) Evidence of lyssaviruses among rhabdovirus isolates from the European eel *Anguilla anguilla*. Ann Virol 135E:35–55

De Kinkelin P, Galimard B, Bootsma P (1973) Isolation and identification of the causative agent of 'red disease' of pike (*Esox lucius* L. 1766). Nature 241:465–467

Dorson M, Torchy C, Chilmonczyk S, de Kinkelin P, Michel C (1984) A rhabdovirus pathogenic for perch, *Perca fluviatilis* L.: isolation and preliminary study. J Fish Dis 7:241–245

Fijan N, Petrinec Z, Sulimanovic D, Zwillenberg LO (1971) Isolation of the viral causative agent from the acute form of infectious dropsy of carp. Vet Arh 41:125–138

Kimura T, Yoshimizu M, Gorie S (1986) A new rhabdovirus isolated in Japan from cultured hirame (Japanese flounder) *Paralichthys olivaceus* and ayu *Plecoglossus altivelis*. Dis Aquat Organ 1:209–217

LaPatra SE, Fryer JL, Rohovec JS (1993) Virulence comparison of different electropherotypes of infectious hematopoietic necrosis virus. Dis Aquat Organ 16:115–120

Laurencin FB (1987) IHN in France. Bull Eur Assoc Fish Pathol 7:104

Sano T, Nishimura T, Okamoto N, Fukuda H (1977) Studies on viral diseases of Japanese fishes. VII A rhabdovirus isolated from European eel, *Anguilla anguilla*. Bull Jpn Soc Sci Fish 43:491–495

Wingfield WH, Fryer JL, Pilcher KS (1969) Properties of the sockeye salmon virus (Oregon strain). Proc Soc Exp Biol Med 130:1055–1059

Table S3. Primers used for complete genomic sequencing of VHSV Ib isolates in this study.

Primer	Sequence
VH common FRONT term2 new	CDTAAGTRGCAAAAAGTTTTCAAGTTTG
VN N mid F1	TYTTGTCCACMGAGTACTTG
VH N mid F2	GTGGACAARATGATCAAGTAC
VH N mid R1	CAAGTACTCKGTGGACAARA
VH N mid R2	GTACTTGATCATYTTGTCCAC
VH N rear F	GCCCGCAACTTCAGGAGC
VH N REV 216	TCAACCTCACCAGGTACAAGCAC
VH N REV NEST 170	CGATCCTGATGTCATTCAAAC
VH P front R	CYTTRGGGGCGTTGTCTAG
VH P mid F	GGAAGAAGACCGACAACATAC
VH P mid R	AGTATGTTGTCGGTCTTCTTC
VH P rear F	CATYGCCATGAAGAAGTTCAAG
VH M front R	ATGGTKGAGACACGGTCCTC
VH M midrear F	AGACATGGGAGTGTGACTTA
VH M midrear R	TAAGTCACACTCCCATGTCT
VH G front R	TTCATCCARATGCAGGARGGTTC
VH G front F	GAACCYTCCTGCATYTGGATGAA
VH G midrear F	TTTCTCCTMTCAAAGTTTCGTCC
VH G midrear R	GGACGAAACTTTGAKAGGAGAAA
VH G rear F	TCTCCAACACATCYGATCTYTC
VH G rear R	GARAGATCRGATGTGTTGGAGA
VH NV front F	GACCCAAGYAACTACCTCAAC
VH NV front R	GTTGAGGTAGTTRCTTGGGTC
VH L front F	GTACCAGCTGGTGCACCTCAG
VH L front R	ATCCTAACTCATTGCTCTGTGTC
VH L front F-2	ATTTAAAGAGGGGGTGGTGG
VH L front R-2	GAGTCCAGGATCTGTCAGCC
VH L 2R-2	GATGATTTTGAAGAGGGCCA
VH L 2F	GCTCTCTTCCAATATTTTGGACTG
VH L 2R	ATGAGTCGCTTGGTTTGTCACTG
VH L 2F-2	TGGCCACTTAGTGACACCCT
VH L 5R-2	TTTCTGTGGTGTCTCTTCCTGA
VH L 3R	GACTTTTACTGATGTGGATGTATC
VH L 2F-3	AACGCCAGTTCAACTCCTTG
VH L 5R-3	ATCCGTCCACAATCCATGTT
VH L 2F-4	TGAGGAGTGGCCTGACTCTT
VH L 5R-4	AGCAAGGGTAAGGTGCTGTG
VH L 5R-5	TGAGTAACGTAGTCTCCCACACC
VH L 4F	CGAAAACCATGCGAATGGTTGC
VH L 4F-2	GGGAGACCATGATTAAGGCA
VH 5RACES1-2	CTTGTGCTTGAAGTGAGGGG
VH L 5R	CGTTTCCTTTGGGATGTCCAAG
VH 5RACES1-3	AGGACCAAGGTCACAGGATG
VH 5RACES1-4	TCTTCCCAGCTTTCTTGTCC
VH L8.5comf F	GTCAAGACCAAATCTCTGTTC
VH 5RACES1-5	AACAGAGGTGAGGTTGCACA
VH 5RACERT5P	GCTCATCACTCTTCTTGAAGAAG
VH 5RACES1	TTCTCGGTGATGGCATCCGAG
VH 5endR	GGCGATTCCATCACTGTTCT

Table S4. Experimental design, and total length and body weight of fish in each group. IP: intraperitoneal

Experiment (Location)	Group	Infection route (dose)		Number of fish	Number of replicates	Average total length (cm)	Average body weight (g)
1	DK-3592B (Positive control)	IP injection	($10^{6.3}$ $TCID_{50}$ $fish^{-1}$)	10	1	9.41	11.4
(NRIA)	SE-SVA-14-3D	"	($10^{6.5}$ $TCID_{50}$ $fish^{-1}$)	10	1	9.89	10.5
	SE-SVA-14-5G	"	($10^{6.2}$ $TCID_{50}$ $fish^{-1}$)	10	1	9.42	10.1
	SE-SVA-1033-3F	"	($10^{6.1}$ $TCID_{50}$ $fish^{-1}$)	10	1	9.53	10.9
	SE-SVA-1033-9C	"	($10^{6.3}$ $TCID_{50}$ $fish^{-1}$)	10	1	10.2	11.6
	Negative control	"	(MEM 0.1 ml $fish^{-1}$)	10	1	10.9	9.68
2	SE-SVA-14 wild-type	IP injection	(10^{5} $TCID_{50}$ $fish^{-1}$)	96	3	-	-
(DTU Vet)	SE-SVA-14-3D	"		112	3	-	-
	SE-SVA-14-5G	"		90	3	-	-
	SE-SVA-1033 wild-type	"		110	3	-	-
	SE-SVA-1033-3F	"		106	3	-	-
	SE-SVA-1033-9C	"		108	3	-	-
	Negative control	"	(MEM 0.05 ml $fish^{-1}$)	76	2	-	2.34±0.75
	DK-3592B (Positive control)	Immersion	(10^{5} $TCID_{50}$ ml^{-1} 2 hour)	69	2	-	-
	SE-SVA-14 wild-type	"		102	3	-	-
	SE-SVA-14 3D	"		109	3	-	-
	SE-SVA-14 5G	"		106	3	-	-
	SE-SVA-1033 wild-type	"		108	3	-	-
	SE-SVA-1033 3F	"		115	3	-	
	SE-SVA-1033 9C	"		110	3	-	-
	Negative control	"	(treatment by MEM)	72	2	-	1.71 ±0.56

Table S5. Results of IFAT of MAb VHS-4.20 against various non-VHSV piscine rhabdovirus isolates. +: positive; -: negative. IFAT was performed according to Ito et al. (2012).

Virus genera	Abbreviation	Isolates	VHS-4.20	Positive control (used antibody)
Novirhabdovirus	IHNV	RBH	-	+ (mAb 136-3[a])
	IHNV	32/87	-	+ (mAb 136-3)
	IHNV	Coleman	-	+ (mAb 136-3)
	IHNV	4008	-	+ (mAb 136-3)
	IHNV	OSV	-	+ (mAb 136-3)
	IHNV	TR	-	+ (mAb 136-3)
	IHNV	ER	-	+ (mAb 136-3)
	IHNV	HAG	-	+ (mAb 136-3)
	IHNV	Austria	-	+ (mAb 136-3)
	HIRRV	8401	-	+ (anti rabbit serum)
	Carpione	583	-	+ (mAb IP5B11)
	SHRV		-	+ (anti rabbit serum K3401)
	EEV	B12	-	+ (anti rabbit serum)
Vesiculovirus	SVCV	56/70	-	+ (mAb 2E1[b])
	PFRV	S64	-	+ (anti rabbit serum F30)
	Tench RV	Italian IZSV	-	+ (anti rabbit serum F55)
	Perch RV		-	+ (anti rabbit serum F28)
	EVEX		-	+ (anti rabbit serum K12)

[a] Fregeneda-Grandes JM, Skall HF, Olesen NJ (2009) Antibody response of rainbow trout with single or double infections involving viral haemorrhagic septicaemia virus and infectious haematopoietic necrosis virus. Dis Aquat Organ 83 :23–29

[b] Reschova S, Pokorova D, Nevorankova Z, Hulova J, Vesely T (2007) Detection of spring viraemia of carp virus (SVCV) with monoclonal antibodies. Vet Med Czech 52 :308–316

Table S6. Comparative analysis of the amino acid substitutions among the positive control DK-3592b, the 4p37 isolate and the Swedish VHSV Ib clones (SE-SVA-14-3D, SE-SVA-14-5G, SE-SVA-1033-3F and SE-SVA-1033-9C) with different virulence properties to rainbow trout once IP injected (Skall et al. 2004 or in this study). High virulence: less than or equal to 40% of survival rate by IP injection; Moderate: less than 70% in survival rate by IP injection; Low: more than or equal to 70% of mortality rate by IP injection.

Protein	Substitution Position (AA)	Isolates or variant clones (Virulence to rainbow trout, genotype)					
		DK-3592B (High, Ia)	4p37 (Low, Ib)	SE-SVA-14-3D (Low/moderate, Ib)	SE-SVA-14-5G (Low, Ib)	SE-SVA-1033-3F (Moderate/high, Ib)	SE-SVA-1033-9C (Low, Ib)
N	31	Asp	Glu	Glu	Glu	Glu	Glu
	37	Val	Tyr	Tyr	Tyr	Tyr	Tyr
	43	Glu	Glu	Lys	Lys	Glu	Glu
	46	**Arg**	**Gly**	**Gly**	**Gly**	**Arg**	**Gly**
	49	Ile	Ile	Ile	Ile	Ile	Thr
	82	Glu	Gly	Gly	Gly	Gly	Gly
	83	Thr	Met	Met	Met	Met	Met
	99	Val	Ala	Ala	Ala	Ala	Ala
	113	Arg	Arg	Arg	Gly	Arg	Arg
	168	**His**	**Tyr**	**His**	**Tyr**	**Tyr**	**Tyr**
	358	Lys	Arg	Arg	Arg	Arg	Arg
	371	Lys	Arg	Arg	Arg	Arg	Arg
	392	Gly	Glu	Glu	Glu	Glu	Glu
	393	Glu	Gly	Gly	Gly	Gly	Gly
	401	Glu	Gly	Gly	Gly	Gly	Gly
P	23	Arg	Lys	Lys	Lys	Lys	Lys
	39	Thr	Pro	Pro	Pro	Pro	Pro
	41	Gly	Glu	Glu	Glu	Glu	Glu
	211	Ile	Val	Val	Val	Val	Val

Table S6. (Continued)

Gene	Substitution Position (AA)	Isolates or variant clones (Virulence to trout, genotype)					
		DK-3592B (High, Ia)	4p37 (Low, Ib)	SE-SVA-14-3D (Low/moderate, Ib)	SE-SVA-14-5G (Low, Ib)	SE-SVA-1033-3F (Moderate/high, Ib)	SE-SVA-1033-9C (Low, Ib)
M	2	Thr	Ala	Ala	Ala	Ala	Ala
	165	Glu	Gly	Gly	Gly	Gly	Gly
	182	Ile	Thr	Thr	Thr	Thr	Thr
	201	Trp	Arg	Arg	Arg	Arg	Arg
G	51	Asp	Glu	Glu	Glu	Glu	Glu
	91	Lys	Arg	Arg	Arg	Arg	Arg
	113	Ser	Gly	Gly	Gly	Ser	Ser
	136	Asp	Asn	Asn	Asn	Asn	Asn
	212	Lys	Thr	Thr	Thr	Thr	Thr
	230	His	Asn	Asn	Asn	Asn	His
	258	Ala	Thr	Thr	Thr	Thr	Thr
	277	Thr	Ala	Ala	Ala	Ala	Ala
	278	Gly	Arg	Arg	Arg	Arg	Arg
	288	Ala	Thr	Thr	Thr	Thr	Thr
	292	Met	Met	Met	Met	Met	Thr
	371	Ser	Ser	Ser	Ser	Arg	Ser
	388	Asp	Asn	Asn	Asn	Asn	Asn
	433	Thr	Ile	Ile	Ile	Ile	Ile
	459	His	Asn	Asn	Asn	Asn	Asn
	462	Ile	Leu	Leu	Leu	Leu	Leu
	506	Thr	Met	Met	Met	Met	Met

Table S6. (Continued)

Gene	Substitution Position (AA)	Isolates or variant clones (Virulence to trout, genotype)					
		DK-3592B (High, Ia)	4p37 (Low, Ib)	SE-SVA-14-3D (Low/moderate, Ib)	SE-SVA-14-5G (Low, Ib)	SE-SVA-1033-3F (Low/moderate/high, Ib)	SE-SVA-1033-9C (Low, Ib)
Nv	12	Phe	Phe	Phe	Phe	Leu	Phe
	43	Pro	Thr	Thr	Thr	Thr	Thr
	44	Val	Met	Met	Met	Met	Met
	67	His	Tyr	Tyr	Tyr	Tyr	Tyr
	80	Arg	Lys	Lys	Lys	Lys	Lys
	102	Glu	Gly	Gly	Gly	Gly	Gly
	104	Val	Ile	Ile	Ile	Ile	Ile
	116	Arg	Ser	Ser	Ser	Ser	Ser
	121	Pro	Thr	Thr	Thr	Thr	Thr
L	56	Ala	Ala	Ala	Ala	Ser	Ala
	149	Glu	Gly	Gly	Gly	Gly	Gly
	232	Ile	Val	Val	Val	Val	Val
	298	Lys	Glu	Glu	Glu	Glu	Glu
	302	Lys	Arg	Arg	Arg	Arg	Arg
	365	Ile	Val	Val	Val	Val	Val
	411	Phe	Tyr	Tyr	Tyr	Tyr	Tyr
	474	Ile	Ile	Ile	Val	Ile	Ile
	511	Arg	Lys	Lys	Lys	Lys	Lys
	849	Gln	Gln	Gln	Gln	Gln	His
	1012	Phe	Ile	Ile	Ile	Ile	Ile
	1281	Ala	Asp	Asp	Asp	Asp	Asp
	1313	Met	Thr	Thr	Thr	Thr	Thr
	1317	Asn	Asn	Asn	Asn	Asn	Lys
	1742	Ala	Thr	Thr	Thr	Thr	Th