THE MARINE VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS: DOES IT POSE A THREAT TO FARMED RAINBOW TROUT ONCORHYNCHUS MYKISS?

by D. M. Stone, A. M. Betts, P. F. Dixon and K. Way

CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

During the period 1996-1998 marine fish species were screened for infection with the VHS virus. The virus was only isolated from Atlantic herring caught in Rye Bay in 1996 but was detected by RT-PCR in both Atlantic herring and Atlantic cod caught in several sampling areas around the UK during the 1997 and 1998 surveys. Sequence analysis revealed that there was <2% divergence between the marine and freshwater isolates of VHSV at the amino acid level indicating that the number of amino acid residues influencing the pathogenicity of the virus for rainbow trout was likely to be low. The next phase of this project is to use reverse genetics techniques to determine the virus genes involved in determining virulence for rainbow trout. Also, by gaining a better understanding of the molecular basis of pathogenicity we will establish if the concerns regarding the possible selection of marine strains with increased virulence for salmonids are justified.

Marine Survey

Viral haemorrhagic septicaemia virus (VHS) is generally considered to be a disease of farmed salmonid fish, and as such poses a serious threat to the farmed rainbow trout (Oncorhynchus mykiss) populations in the UK which have not been exposed to it. In recent years however, the VHS virus (VHSV) has been isolated from an increasing number of marine fish species, including Atlantic herring (Clupea harengus harengus), cod (Gadus morhua), haddock (Melanogrammus aeglefinus), sprat (Sprattus sprattus), and rockling species, which suggests that a natural reservoir of the VHSV exists among marine species.

It has been suggested that intensive aquaculture with its high rearing densities and the continuous addition of susceptible fish may exert selective pressures that favour the production of more pathogenic strains, and that the marine strains may become pathogenic for the salmonids if they gained access to the farming environment.

Figure 1. Marine survey research vessel CV Corystes.

There is now increasing suspicion that this has already occurred, and that VHS was originally introduced into the European trout farms through the use of untreated raw marine fish in the diet, a common practice in the early days of industry, particularly in Denmark. It may also help to explain the outbreaks of VHS in turbot farms on Gigha Island, Scotland and Cape Clear, Eire.

During 1996-98 fish were caught using standard trawling methods (Fig 1) in the sampling areas around the United Kingdom (Fig 2) as part of a fish disease monitoring programme.

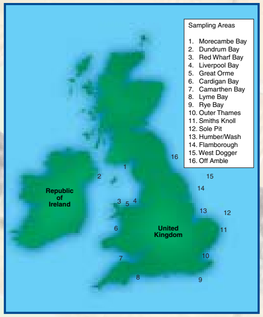


Figure 2. Marine fish survey areas.

Table 1. Fish positive for VHSV by RT-PCR from 1996-1998.

Year	Sampling Area[1]	Fish[2]
1996	9 Rye Bay	8/10 Atlantic herring
1997	3 Red Wharf Bay	3/10 Atlantic herring
	4 Liverpool Bay	3/10 Atlantic herring
	8 Lyme Bay	3/4 Atlantic herring
	12 Sole Pit	2/6 Atlantic cod 1/10 Atlantic herring
	14 Flamborough	1/9 Atlantic cod
	15 West Dogger	1/7 Atlantic cod
1998	3 Red Wharf Bay	5/10 Atlantic cod
	9 Rye Bay	1/10 Atlantic herring

[1] Numbers correspond to those in Figure 1.
[2] Positive samples presented as a fraction of the sample number tested.

Each species at a given sampling area was sampled separately and portions of liver, spleen, heart, kidney and brain were pooled. Tissue samples were screened for VHS virus using a standard tissue culture protocol and the reverse transcription-polymerase chain reaction (RT-PCR) technique (Strømmen and Stone, 1998).

VHSV was detected by RT-PCR in herring and cod samples collected during all three years (Table 1) Samples of turbot, brill, saithe, sprat, scad and whiting were negative by RT-PCR. In 1996 the virus was isolated from Atlantic herring caught in Rye Bay (Dixon *et al.*, 1997). No isolations were made in 1997 or 1998.

The Genetic relationship between marine and freshwater strains of VHSV

Figure 3. DNA sequencing using the ABI 310 genetic analyser.

The PCR products generated during the marine survey were ligated into the pGEM-T sequencing vector. Both DNA strands were sequenced using the M13 universal primers and the ABI PRISM™ dye terminator cycle sequencing system (Perkin Elmer) and analysed on an ABI 310 genetic analyzer (Fig 3). A phenogram (Figure 4) was generated using the CLUSTAL V algorithm within MEGALIGN (DNASTAR inc.).

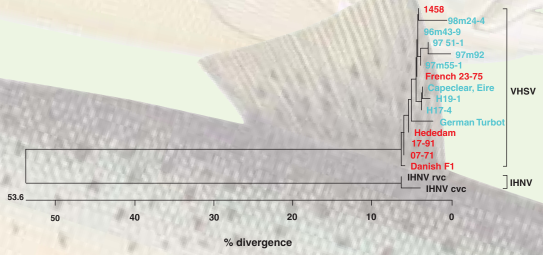


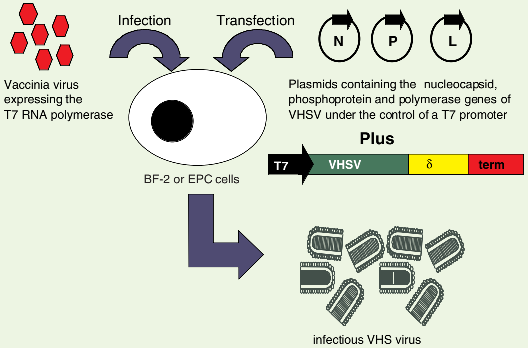
Figure 4. Phenogram showing the genetic relationship of the marine VHSV isolates (blue) to those associated with VHS outbreaks (red) The tree is based on the deduced amino acid sequence (residues 120-240) of the glycoprotein gene and the analysis was done with the Clustal analysis package within MEGALIGN (DNAstar inc.). This figure includes data taken from Stone et al., (1997).

The amino acid comparisons (Fig 4) suggest a strong genetic link between the viruses identified in marine fish species and those associated with disease outbreaks in rainbow trout. However, as the analysis is based on <4% of the genome sequence (360 nucleotides) and does not necessarily reflect the similarity of the viruses as a whole, we have sequenced the complete coding region (10,845 nucleotides) of representatives of both the marine and freshwater strains. The results presented in Table 2 how closely related the marine strains are to the freshwater isolates, sharing up to 99% amino acid sequence identity.

Table 2. Comparison of the nucleotide and deduced amino acid sequences of the coding regions of the pathogenic (14-58 and Hededam) and non-pathogenic marine (96-43 and Cod ulcus) strains of VHSV. Adapted from Betts and Stone (2000).

		% nucleotide identity			
	Virus strains	**96-43**	**1458**	**Cod ulcus**	**Hededam**
% amino acid identity	**96-43**	-	97.3	99.4	98.3
	1458	98.6	-	97.6	97.7
	Cod ulcus	99.6	98.9	-	98.6
	Hededam	98.9	98.8	99.0	-

Genetic manipulation



Infection
Transfection
Vaccinia virus expressing the T7 RNA polymerase
N
P
L
Plasmids containing the nucleocapsid, phosphoprotein and polymerase genes of VHSV under the control of a T7 promoter
Plus
T7
VHSV
δ
term
BF-2 or EPC cells
infectious VHS virus

Figure 5. Recovery of Infectious Virus from cDNA. Transfection of fish cell lines with plasmids encoding the N, P and L genes of VHSV together with a full length cDNA of VHSV will give rise to fully infectious virus particles.

By gaining a better understanding of the molecular basis for the pathogenicity of VHS viruses this project seeks to establish if the concerns regarding the adaptation and a possible increase in the pathogenicity of marine VHSV strains for cultivated fish species are justified.

Genetic manipulation of RNA viruses requires the ability to recover virus from a cDNA copy of the RNA genome. Exploiting the procedure outlined in figure 5 we hope to generate infectious virus from a cDNA copy of the 96-43 strain. Using recombinant DNA technology it will be possible to identify the VHSV gene(s) that determine the pathogenicity of the virus and, by employing site directed mutagenesis, establish the number of amino acid substitutions required to increase the pathogenicity of the 96-43 strain for rainbow trout. Essentially, the greater the number of substitutions required to increase the pathogenicity of the virus the less likely it is that selection of pathogenic strains will occur on a farm site.

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The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK http://www.cefas.co.uk