Journal of General Virology (1997), **78**, 1319–1326. Printed in Great Britain

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.)

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RT–PCR methods have been applied to the detection and sequencing of the glycoprotein gene of viral haemorrhagic septicaemia virus (VHSV), the rhabdovirus which causes viral haemorrhagic septicaemia (VHS) in farmed salmonid fish. Phylogenetic analysis of a 360 nt region of the glycoprotein gene from a range of marine and fresh water VHSV isolates identified three genogroups, I–III. Significantly, two virus isolates recovered from ulcerated North Sea cod caught off the Shetland Islands, and an isolate recovered from diseased turbot farmed on the island of Gigha, Scotland were assigned to the same genogroup. Moreover, a virus isolated from diseased turbot farmed on the Baltic Sea coast shared 99·4% nucleotide sequence similarity with a virus associated with a VHS outbreak in rainbow trout. This is the first time that a genetic link between a VHS outbreak and natural VHSV infections of marine fish species has been demonstrated.

Viral haemorrhagic septicaemia virus (VHSV), the causative agent of viral haemorrhagic septicaemia (VHS), is a rhabdovirus belonging to the genus *Lyssavirus*. VHS results in devastating losses of farmed rainbow trout (*Oncorhynchus mykiss*) throughout Europe. VHSV infection of non-salmonid species has been demonstrated but natural epizootics are rare. VHSV was isolated in 1988 from adult chinook salmon (*O. tshawytsha*) and coho salmon (*O. kisutch*) returning to rivers in Washington, USA (Brunson *et al.*, 1989; Hopper, 1989) however, VHS has not become established in the large salmon and trout hatcheries in the USA as it has in Europe. This, together with the observation that the North American VHSV isolates are generally avirulent for salmonids, and have been isolated from subsequent salmon stocks (Eaton *et al.*, 1990; Stewart *et al.*, 1990) and several marine fish species (Meyers *et al.*, 1992) including Pacific cod (*Gadus macrocephalus*) and Pacific herring (*Clupea harengus pallasi*), suggests that the virus has been enzootic in the Pacific ocean for some time. The use of T1 fingerprinting (Oshima *et al.*, 1993), Southern blot and sequence analysis (Bernard *et al.*, 1992; Batts *et al.*,1993) has confirmed that the North American isolates are distinct from VHSV found in Europe. Viruses with the genetic characteristics of the typical North American VHSV are not considered to pose the same level of risk to the salmonid farming industry as 'European-type' strains and in America their isolation no longer warrants destruction of entire fish stocks on an infected farm.

In 1979, VHSV was isolated from Atlantic cod (*G. morhua*) caught in Danish waters (Jensen *et al.*, 1979; Jorgensen & Olesen, 1987) and in 1991 from turbot (*Scophthalmus maximus*) farmed on the Baltic Sea coast (Schlotfeldt *et al.*, 1991), raising the question as to whether VHSV is also enzootic in European waters. However, due to a lack of evidence at that time for natural infection of non-salmonid populations in coastal waters of Europe, it was presumed that the marine cases were either the result of contaminated effluent from an infected trout farm in the locality or laboratory contaminants and did not constitute a natural marine infection. In 1994, VHSV was isolated from turbot farmed on the island of Gigha off the west coast of Scotland that were showing signs of a VHS-like disease (Ross *et al.*, 1994). Although, in this case the involvement of a virus-contaminated water supply could not be discounted, infection of the turbot via this route would seem unlikely since the farm is located within a recognized VHSV-free zone. Also, initial challenge experiments suggested that the Gigha isolate has low virulence for rainbow trout (results not shown) and is, therefore, unlikely to have been derived from an outbreak of VHS on a trout farming site. In the same year, VHSV was isolated from ulcerated cod (Smail, 1995) and haddock (*Melanogrammus aeglefinus*) caught off the east coast of Scotland, and more recently from Atlantic herring (*C. harengus harengus*) in the English Channel (P. F. Dixon, S. W.

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The nucleotide sequence reported in this paper has been submitted to the EMBL database and assigned the accession numbers Z93404–Z93431.

Table 1. The 28 isolates of VHSV used in this study, indicating the country and date of isolation

Virus isolate	Location of isolation	Country of isolation	Host species	Year of isolation
AK'93	Prince William Sound	Alaska, USA	Pacific cod	1993
AK'93#1	Prince William Sound	Alaska, USA	Pacific herring	1993
BC'93	Prince Rupert Sound	British Columbia, Canada	Pacific herring	1993
EB#7	Elliot Bay	Washington, USA	Pacific herring	1993
Elok.	Elokomin River	Washington, USA	Coho salmon	1994
Makah	Makah	Washington, USA	Coho salmon	1988
NA-6	Prince William Sound	Alaska, USA	Pacific cod	1990
NA-7	Prince William Sound	Alaska, USA	Pacific cod	1991
NA-8	Clearwater River	Washington, USA	Coho salmon	1991
NA-5	Bogachiel River	Washington, USA	Coho salmon	1989
H17/5	Coastal waters east of the Shetland Islands	Scotland	Atlantic cod	1993
H19/1	Coastal waters east of the Shetland Islands	Scotland	Atlantic cod	1993
F1	Egtved	Denmark	Rainbow trout	1965
23-75	–	France	Brown trout	1975
02-84	–	France	–	1984
17-91	–	France	–	1991
134.448 (448)	River Maas	Noord-Brabant, Netherlands	Rainbow trout	1992
137.609 (609)	–	Limburg, Netherlands	Rainbow trout	1991
59.670 (670)	Amersfoort	Utrecht, Netherlands	Rainbow trout	1987
13.957 (957)	River Maas	Noord-Brabant, Netherlands	Rainbow trout	1992
Rindsholm (Rinds)	Rindsholm	Denmark	Rainbow trout	1988
Klapmolle (Klap)	Klapmolle	Denmark	Rainbow trout	1988
Grasmuck (Gras)	Grasmuck	France	Rainbow trout	1984
Cod ulcus (Cod'79)	North Sea	Denmark	Atlantic cod	1979
814	Gigha Island	Scotland	Turbot	1994
Hededam (He)	Hededam	Denmark	Rainbow trout	1972
7321		Germany	Turbot	1991
83-53		England*	Rainbow trout	1983

* Isolated in the UK from the viscera of rainbow trout imported from Denmark.

Fiest, E. Kehoe, L. Parry, D. M. Stone & K. Way, CEFAS, UK; unpublished results). There is now an increasing suspicion that herring, which are common prey for salmon and cod, act as a primary reservoir for VHSV and were the most likely source of the viruses isolated from returning salmon in Washington, USA in 1988. It is possible that VHS was originally introduced onto the trout farms in Europe through the use of untreated 'trash' fish in the diet, which was common practice in the early days of the fish-farming industry, particularly in Denmark. Similarly, unpasteurized minced marine fish was also used as feed for the turbot farmed on the island of Gigha.

We have conducted studies to determine the degree of genetic relatedness of the marine VHSV isolates to each other and to the fresh water isolates from classic outbreaks of VHS disease.

By using degenerate PCR primers we have amplified a 360 nt sequence of the glycoprotein gene (nt 361–720, amino acid residues 120–240) of a range of freshwater and marine VHSV isolates. Primers were based on blocks of amino acids conserved between the VHSV and infectious haematopoietic necrosis virus (IHNV) glycoprotein sequences (Thiry *et al.*, 1991; Koener *et al.*, 1987). The 33 nt reverse primer 5′ ACACCT**GAGCTC**TTCTTTGGAGGGCAAACNATY 3′ contained an *Sst*I at its 5′ end for cloning purposes and the 33 nt forward primer 5′ TGCATG**AAGCTT**CAGTCCCCA-GGGATGATGNCC 3′ contained a *Hin*dIII cleavage site. Viral RNA was extracted by proteinase K (100 µg/ml) digestion in the presence of 1·0% SDS at 65 °C for 1 h, followed by a phenol–chloroform extraction and ethanol precipitation. RT–PCR was carried out by following standard procedures. Amplification was done on VHSV isolates from both Europe and North America, and although the signals were generally weaker when amplifying the North American VHSV sequences, all of the isolates tested (Table 1) produced

a PCR product of the expected size (data not shown). The PCR products were digested with *Hin*dIII and *Sst*I, and ligated into *Hin*dIII–*Sst*I-digested pBluescript pSK(-) (Stratagene). The nucleotide sequence of the insert was determined by the dideoxynucleotide chain termination sequencing method (Sanger *et al*., 1977) using the −20 and reverse primers (Stratagene). At least two independent amplification and cloning events were performed for each virus isolate to eliminate errors introduced by the *Taq* polymerase and to identify the consensus sequence within what was likely to be a complex heterogeneous 'quasi-species'. Where nucleotide sequence ambiguities could not be adequately resolved, due to cross-banding or compressions in the sequence data, the appropriate IUPAC codes were used.

Comparison of the 360 nt sequence with the previously published sequence for VHSV 07-71 (Thiry *et al*., 1991) revealed from 0–56 nt differences (0–15·5%) which were distributed evenly throughout the region from nucleotides 361–720 (Fig. 1*a*). Phylogenetic analysis at the nucleotide level identified three main genotypes, I–III (Fig. 2*a*). Some geographical clustering of isolates within the genotypes was evident as genogroup I consisted entirely of North American isolates and groups II and III contained isolates from Europe. There was no correlation between the phylogenetic grouping of viruses and the host species of fish. Isolations made from Atlantic cod were assigned to both genogroup II (H17/5 and H19/1) and genogroup III (Cod'79) sharing between 90·4–98·8% nucleotide sequence similarity. The two turbot isolations (7321 and 814), which were associated with VHS-like disease (Schlotfeldt *et al*., 1991; Ross *et al*., 1994), shared only 90·4% similarity at the nucleotide level and were also assigned to genogroups II and III. The Gigha virus (814) and the two virus isolates from North Sea cod (H17/5 and H19/1) were assigned to the same group (genogroup II) sharing 90·4% nucleotide sequence similarity with the main European VHSV group (genogroup III) and 82% nucleotide sequence similarity with the viruses isolated in North America (genogroup I).

As all three recent marine isolations were obtained in the same laboratory, the possibility of cross-contamination during cultivation of the 814 virus should also be considered. However, since we have shown in this study that 814, H17/5 and H19/1 viruses share only 97·7% nucleotide sequence similarity, cross-contamination seems unlikely. Jorgensen *et al*. (1995) demonstrated that VHSV is remarkably stable, accumulating only a single amino acid substitution in the entire glycoprotein during 500 passages in cell culture. Differences in amino acid sequences between the H19/5 and 814 virus isolates were identified at residues 145 and 171. A further substitution at residue 140 has yet to be confirmed

The glycoprotein gene sequence was shown to be highly conserved between North American VHSV isolates (> 98·6%, this study), and within epizootics of IHN caused by a related fish lyssavirus (Nichol *et al*., 1995). Therefore, it is interesting that whereas nine viruses, including VHSV isolated in France in 1971 (07-71 virus), 1984 (02-84 and Grasmuck virus) and 1991 (17-91 virus), showed a high degree of nucleotide sequence similarity (> 98·5%), the remaining European isolates had a range of 90·4–98·2%. This lower than expected sequence conservation amongst the European isolates may indicate that VHS has been introduced into European trout farms on several occasions from closely related but independent external sources. The feeding of varied sources of 'trash' marine fish to rainbow trout in the early days of the fish-farming industry could explain this.

The majority of the nucleotide substitutions identified in this study do not lead to changes in the deduced amino acid sequence (Fig. 1*b*), and as a result the dendrogram based on the deduced amino acid sequences does not correlate well with that obtained using nucleotide sequence data. The most obvious change is the loss of the separate genogroup containing 814, H17/5 and H19/1 viruses (Fig. 2*b*). These viruses now form part of a larger European group that share 91·7% amino acid sequence similarity with the North American genogroup. This high degree of amino acid conservation demonstrated between the European and North American VHSV is consistent with the observation that amino acid residues 122–246 of the IHNV glycoprotein gene are also well conserved (Nichol *et al*., 1995), and probably reflects the constraints placed on the glycoprotein sequence by protein function (i.e. cell attachment and internalization). Some geographical clustering was still evident in that the North American isolates were assigned to genogroup I and the European isolates were assigned to genogroup II, but there was no correlation between phylogenetic position and serological grouping. This is not unexpected considering that the major neutralizing epitopes have been mapped to two antigenic sites, at amino acid residues 230–231 and 272–276 (Huang, 1993; Kim *et al*., 1994). We have identified no amino acid substitutions over residues 230–231 and residues 272–276 were not covered in this study.

It is known that the glycoprotein gene can play a role in the virulence of VHSV. Béarzotti *et al*. (1995) demonstrated that as few as two concomitant amino acid substitutions in antigenic regions at residues 140 and 430 of the glycoprotein are sufficient to reduce the virulence of the virus for fish and increase the frequency of chronic nervous system involvement. Also, Kim *et al*. (1994) proposed that substitutions in IHNV escape mutants at residues 78 and 218 were responsible for an altered tissue tropism and loss of virulence. However, in both cases viruses were selected using monoclonal antibodies and it is not clear whether a parallel situation could occur within natural epizootics of VHS. The North American isolates sequenced as part of this study have amino acid substitutions at residues 139 (Asp/Asn) and 222 (Glu/Lys) and at least six additional substitutions compared to strain 07-71 that could influence the level of pathogenicity for salmonids. Both the H17/5 and H19/1 strains have a threonine to alanine change at residue 135, and H19/1 has an additional lysine to arginine

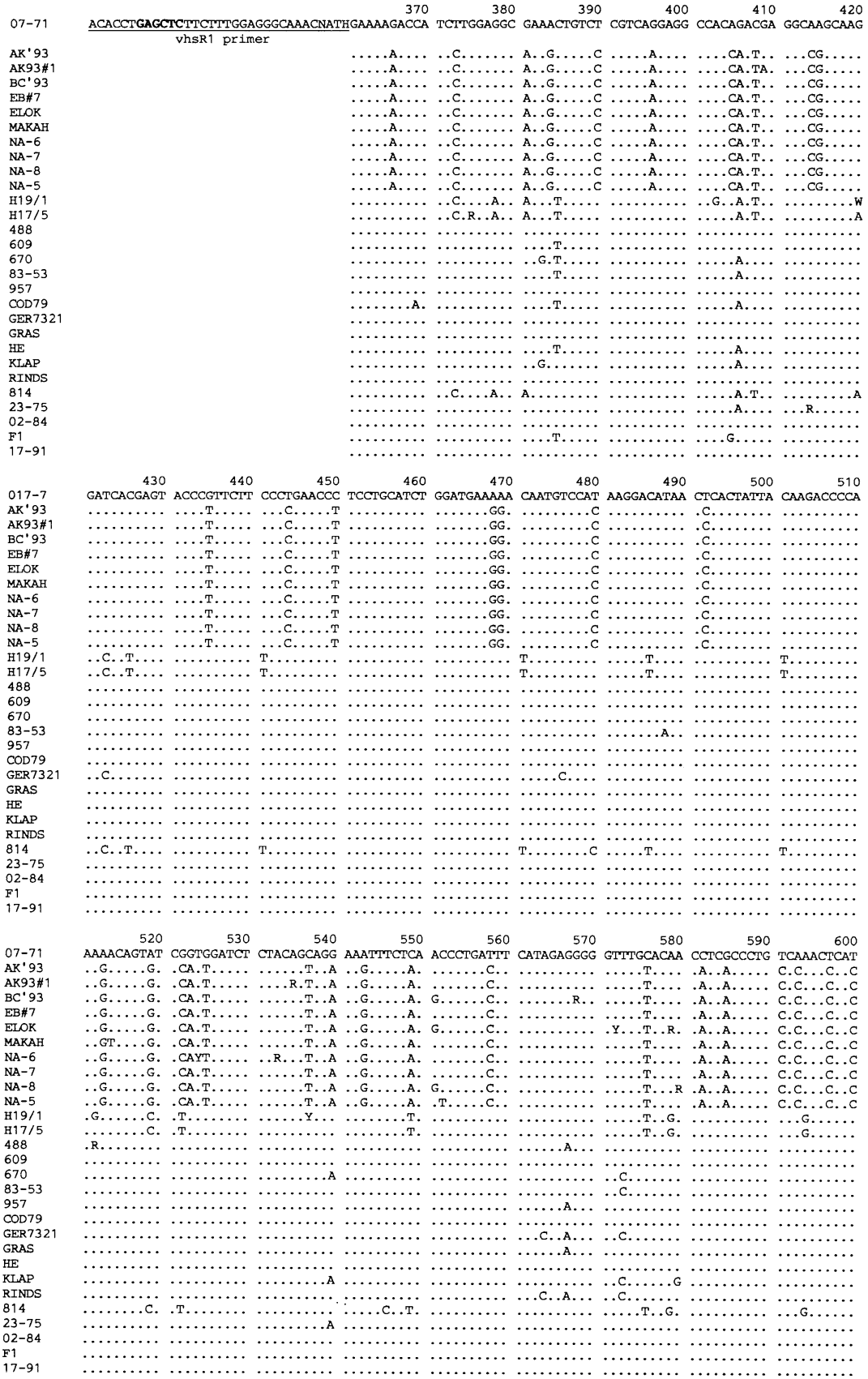

Fig. 1 (*a*). For legend see facing page.

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                610        620        630        640        650        660        670        680        690
07-71     TGGCAGGGAG TCTATTGGGT CGGTGCCACA CCTAAAGCCC ATTGCCCCAC GTCGGAAACA CTAGAAGGAC ACCTGTTCAC CAGGACCCAT

AK'93     .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
AK93#1    .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
BC'93     .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
EB#7      .....A.... ....C...A. ...C..T... ...C.G.... .G.....T.. C..A.....G ..TA.G..G. .T..... ......A...
ELOK      .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A..... ..TA.G..G. .T..... ......A...
MAKAH     .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
NA-6      .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
NA-7      .....A.... ....C...AY ...Y..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
NA-8      .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. AT..... ......A...
NA-5      .....A.... ....C...A. ...C..T... ...C.G.... .......T.. C..A.....G ..TA.G..G. .T..... ......A...
H19/1     .......... .......... .......... ..C.C..... ....T..... T..A..... .....G..G. .......... ...A..T...
H17/5     .......... .......... .......... ..C.C..... ....T..... T..A..... .....G..G. .......... ...A..T...
488       .......... .......... .......... .......... .......... .......... .......... .......... ..........
609       .......... .......... .....A.... ..A....... .......... .......... .......... .......... ..........
670       .......... ....C..... .......... ..C.C..... .......... .........G .......... .......... ..........
83-53     .......... .......... .......... ....C..... .........GA .......... .......... .......... ..........
957       .......... .......... .......... .......... .......... .......... .......... .......... ..........
COD79     .......... .......... .......... ....C..... .......... .......... .......... .......... ..........
GER7321   .......... .......... ...C...... ..C..T.... .......... .......... .......... .......... ..........
GRAS      .......... .......... .......... ..C....... .......... .......... .......... .......... ..........
HE        .......... .......... .......... .......... .......... .......... .....G.... .......... ..........
KLAP      .......... ....C..... .......... ..C.C..... C...T..... .......... .......... .......... .C........
RINDS     .......... .......... ...C...... ..C..T.... .......... .......... .......... .......... ..........
814       .......... .......... .......... ..C.C..... ....T..... .......... .......... .......... ...A..T...
23-75     .......... .......... .........C ....C..... .......... .......... .......... .......... ..........
02-84     .......... .......... .......... .....G.... .......... ...A...... .......... .......... ..........
F1        .......... .T........ .......... ..C....... .......... .......... .......... .......... ..........
17-91     .......... .......... .......... .......... .......... .......... .......... .......... ..........


                700        710        720
07-71     GATCACAGGG TGGTCAAGGC AATTGTGGCAGGNCATCATCCCTGGGGACTGAAGCTTCATGCA
                                                    vhsF1 primer
AK'93     .......... .......... ...C..T..G
AK93#1    .......... .......... ...C..T..G
BC'93     .......... .......... ...C..T..G
EB#7      .......... .......... ...C..T..G
ELOK      .......... .......... ...C..T..G
MAKAH     .......... .......... ...C..T..G
NA-6      .......... .......... ...C..T..G
NA-7      .......... .......... ...C..T..G
NA-8      .......... .......... ...C..T..G
NA-5      .......... .......... ...C..T..G
H19/1     ........A. .......... .........G
H17/5     ........A. .......... .........G
488       .......... .T........ ..........
609       .......... .......... ..........
670       .......... ....T..... ......A...
83-53     .......... .......... ..........
957       .......... .T........ ..........
COD79     .......... .......... ..........
GER7321   .......... ........A. ..........
GRAS      .......... .......... ..........
HE        .......... .......... ..........
KLAP      .......... ....T..... .........G
RINDS     .......... .......... ..........
814       ........A. .......... .........G
23-75     .......... .......... ..........
02-84     .......... .......... ..........
F1        .......... .......... .G........
17-91     .......... .......... ..........
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Fig. 1. (*a*) Alignment of nucleotides 361–720 of the glycoprotein gene of the 28 VHSV isolates described in Table 1. (.) indicates the positions of sequence identity compared to the published sequence for the 07-71 strain (Thiry *et al.*, 1991). The positions of the reverse and forward primers used in RT–PCR are underlined, and the restriction endonuclease sites are shown in bold. Multiple alignments were done with the DNAsis program (Hitachi software) which is based on the Higgins–Sharp algorithm (Higgins & Sharp, 1988).

substitution at residue 171. In contrast, the Gigha isolate (814) showed 100% amino acid sequence identity with both the original cod ulcus virus (Cod'79) and another virulent French isolate (23-75) over residues 121–240. The significance of 100% amino acid conservation between 814 and 23-75 in this region is not yet known, but it serves to illustrate that the overall number of amino acid differences between the highly pathogenic and non-pathogenic European strains may be minimal. We are currently sequencing the entire glycoprotein gene of a range of VHSV to determine the extent of amino acid conservation, with the aim of establishing which amino acids play a key role in the pathogenicity of the virus for salmonids.

We have shown for the first time a genetic link between VHSV isolated from fish living in the European coastal waters and viruses causing VHS in a farmed fish species. The data demonstrate a strong epidemiological link between the VHS outbreak on the island of Gigha and viruses infecting the cod from the coastal waters around Scotland. This highlights a potential threat to the salmonid farming industry from marine fish reservoirs of VHSV, and in particular the use of untreated 'trash' fish as feed. Since several marine fish including the Atlantic species of cod, haddock, herring and turbot, as well as Pacific species of herring, cod and salmon are proven carriers of VHSV, perhaps all marine fish species should be considered as potential carriers of the virus. Moreover, the majority of the North American isolations were made from apparently healthy fish emphasizing that the lack of any obvious signs of infection cannot be taken as an indicator of virus absence. In future studies, it will be of interest to apply highly sensitive detection methodologies such as RT–PCR to screen the marine and fresh

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                130         140         150         160         170         180         190         200         210         220
07-71    EKTILEAKLS RQEATDEASK DHEYPFFPEP SCIWMKNNVH KDITHYYKTP KTVSVDLYSR KFLNPDFIEG VCTTSPCQTH WQGVYWVGAT PKAHCPTSET

AK'93    .......... .....N..G. .......... ......D... .......... ....I..... .......... .......P.. ......I... .Q........
AK93#1   .......... .....NK.G. .......... ......D... .......... ....I...X. .......... .......P.. ......I... .Q........
BC'93    .......... .....N..G. .......... ......D... .......... ....I..... ...S.....X .......P.. ......I... .Q........
EB#7     .......... .....N..G. .......... ......D... .......... ....I..... .......... .......P.. ......I... .Q........
ELOK     .......... .....N..G. .......... ......D... .......... ....I..... ...S...... X......P.. ......I... .Q........
MAKAH    .......... .....N..G. .......... ......D... .......... ....I..... .......... .......P.. ......I... .Q........
NA-6     .......X.. .....N..G. .......... ......D... .......... ....X..X.. .......... .......P.. ......I... .Q........
NA-7     .......... .....N..G. .......... ......D... .......... ....I..... .......... .......P.. ......X... .Q........
NA-8     .......... .....N..G. .......... ......D... .......... ....I..... ...S...... ..TX...P.. ......I... .Q........
NA-5     .......... .....N..G. .......... ......D... .......... ....I..... .......... .......P.. ......I... .Q........
H19/1    .......... ....AN...X .......... .......... .......... R......... .......... .......... .......... .T........
H17/5    .......... ....AN.... .......... .......... .......... .......... .......... .......... .......... .T........
488      .......... .......... .......... .......... .......... X......... .......... .......... .......... ..........
609      .......... .......... .......... .......... .......... .......... .......... .......... ........D. ..........
670      .......R.. .....N.... .......... .......... .......... .......... .......... .......... .......... .T........
83-53    .......... .....N.... .......... .......... ..K....... .......... .......... .......... .......... .T...E...
957      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
COD'79   .......... .....N.... .......... .......... .......... .......... .......... .......... .......... .T........
GER7321  .......... .......... .......... ........A. .......... .......... .......... .......... .......... .N........
GRAS     .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
HE       .......... .....N.... .......... .......... .......... .......... .......... .......... .......... ..........
KLAP     .......R.. .....N.... .......... .......... .......... .......... .......... ...A...... .......... .T.P......
RINDS    .......... .......... .......... .......... .......... .......... .......... .......... .......... .N........
814      .......... .....N.... .......... .......... .......... .......... .......... .......... .......... .T........
23-75    .......... .....N.... .......... .......... .......... .......... .......... .......... .......... .T........
02-84    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
F1       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
17-91    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........


                230         240
07-71    LEGHLFTRTH DHRVVKAIVA

AK'93    .K........ ..........
AK93#1   .K........ ..........
BC'93    .K........ ..........
EB#7     .K........ ..........
ELOK     .K........ ..........
MAKAH    .K........ ..........
NA-6     .K........ ..........
NA-7     .K........ ..........
NA-8     .K.X...... ..........
NA-5     .K........ ..........
H19/1    .......... ..........
H17/5    .......... ..........
488      .......... ..........
609      .......... ..........
670      .......... ..........
83-53    .......... ..........
957      .......... ..........
COD'79   .......... ..........
GER7321  .......... ......T...
GRAS     .......... ..........
HE       .......... ..........
KLAP     .......... ..........
RINDS    .......... ..........
814      .......... ..........
23-75    .......... ..........
02-84    .......... ..........
F1       .......... .......V..
17-91    .......... ..........
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Fig. 1. (*b*) Alignment of the deduced amino acids of the glycoprotein gene (residues 121–240) of 29 VHSV isolates. (x) indicates unassigned amino acids and (.) indicates positions of amino acid identity compared to the published amino acid sequence for the 07-71 strain of VHSV (Thiry *et al.*, 1991).

water fish stocks in the wild, particularly the returning salmon and sea trout.

The data also demonstrate an epidemiological link between the earlier cod and turbot isolations (Jensen *et al.*, 1979; Schlotfeldt *et al.*, 1991) and strains which have been associated with disease outbreaks. At present, it is unclear whether virus transmission in these cases was from the marine environment to fresh water or vice versa. However, since it has been established that numerous marine and fresh water species are susceptible to VHSV infection, and will develop the clinical signs of disease (Meier *et al.*, 1994), the impact of VHSV contaminated discharges and escapes from infected farming sites on marine and fresh water fish stocks in the wild must be of concern.

Initial indications are that the recent marine isolates are less pathogenic for salmonids than the classical fresh water strains and as such pose little or no serious threat to the salmonid farming industry. However, other well-studied virus systems have demonstrated how readily an avirulent phenotype determined by only a few nucleotide mutations can be lost or suppressed during virus replication *in vivo* (Evans *et al.*, 1985; Macadam *et al.*, 1989). Considering the frequency with which mutations can occur in RNA viruses, and lack of detailed knowledge of the molecular basis of VHSV pathogenicity, it would be unwise to ignore the potential threat marine strains of VHSV could pose to the fish-farming industry if provided with the opportunity to adapt under intensive farming conditions.

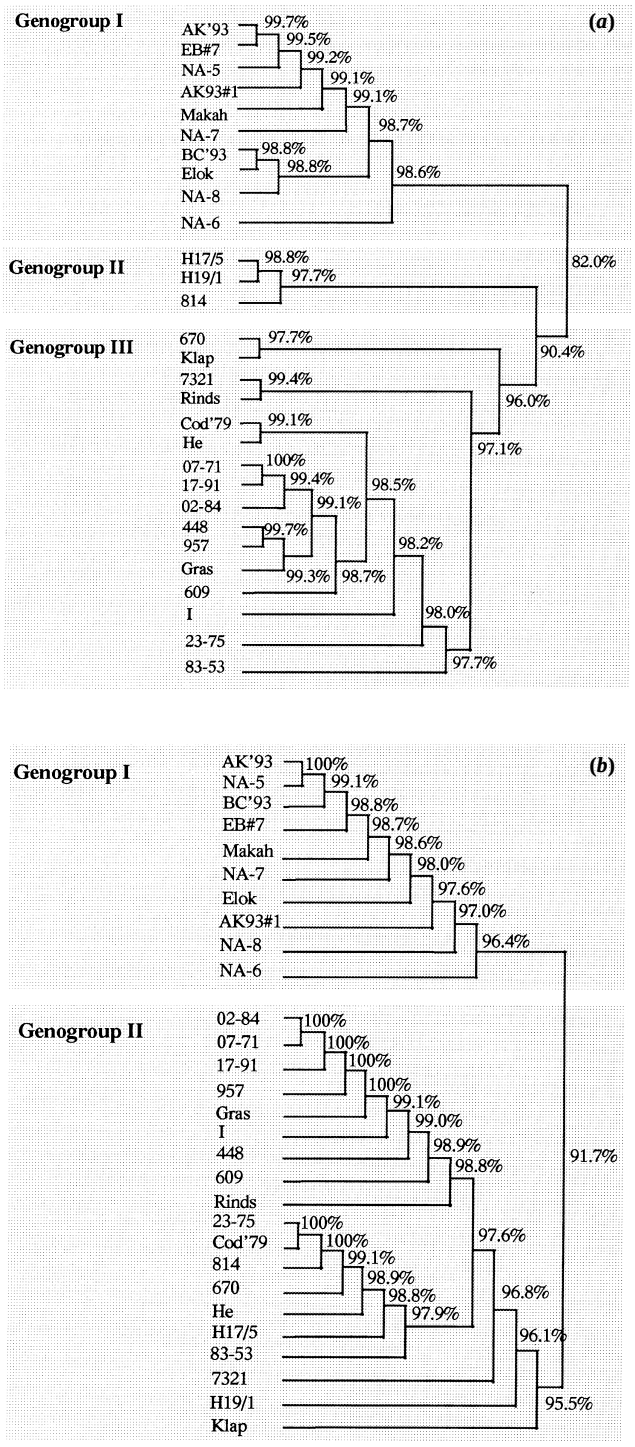

Fig. 2. Dendrogram showing the genetic relationships of 29 isolates of VHSV based on their glycoprotein nucleotide sequences (*a*) and deduced amino acid sequence (*b*). The trees are based on nucleotides 361–720 and amino acid residues 121–240, respectively. The percentage similarity is indicated at each branching point. Computer analysis was done with the DNAsis program (Hitachi software) which is based on the Higgins–Sharp algorithm (Higgins & Sharp, 1988).

We wish to thank J. R. Winton, O. Haenen, N. Olesen, P. de Kinkelin, H. Schlotfeldt and D. A. Smail for providing many of the VHS virus isolates used in this study, and we are grateful to L. Richens and J. P. Crossman for their technical assistance.

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Received 27 September 1996; Accepted 4 February 1997