Journal of Fish Diseases 2005, **28,** 509–529

Review

Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review

H F Skall[1], N J Olesen[1] and S Mellergaard[2,*]

1 Department of Poultry, Fish and Fur Animals, Danish Institute for Food and Veterinary Research, Århus, Denmark
2 Department for Marine Ecology and Aquaculture, Fish Disease Laboratory, Danish Institute for Fisheries Research, Frederiksberg, Denmark

Abstract

Viral haemorrhagic septicaemia virus (VHSV) has, in recent decades, been isolated from an increasing number of free-living marine fish species. So far, it has been isolated from at least 48 fish species from the northern hemisphere, including North America, Asia and Europe, and fifteen different species including herring, sprat, cod, Norway pout and flatfish from northern European waters. The high number of VHSV isolations from the Baltic Sea, Kattegat, Skagerrak, the North Sea and waters around Scotland indicate that the virus is endemic in these waters. The VHSV isolates originating from wild marine fish show no to low pathogenicity to rainbow trout and Atlantic salmon, although several are pathogenic for turbot. Marine VHSV isolates are so far serologically indistinguishable from freshwater isolates. Genotyping based on VHSV G- and N-genes reveals four groups indicating the geographical origin of the isolates, with one group representing traditional European freshwater isolates and isolates of north European marine origin, a second group of marine isolates from the Baltic Sea, a third group of isolates from the North Sea, and a group representing North American isolates. Examples of possible transfer of virus from free-living marine fish to farmed fish are discussed, as are measures to prevent introduction of VHSV from the marine environment to aquaculture.

Keywords: aquaculture, legislation, marine VHSV isolates, review, viral haemorrhagic septicaemia virus (VHSV), wild marine fish.

Correspondence *Dr Helle Frank Skall, Danish Institute for Food and Veterinary Research, Department of Poultry, Fish and Fur Animals, Hangøvej 2, DK-8200 Århus N, Denmark (e-mail: hfm@dfvf.dk)*
Present address **Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark.*

Introduction

Viral haemorrhagic septicaemia (VHS) is one of the most important viral diseases of salmonid fish in European aquaculture (Olesen 1998), causing estimated losses of £40 million pounds per year in 1991 (Hill 1992). The economic consequences of VHS outbreaks on two Danish fish farms in 2000 producing approximately 165 tonnes rainbow trout, *Oncorhynchus mykiss*, was estimated to be above 211 000€; total mortality on the farms was approximately 50% (Nylin & Olesen 2001).

The causative agent of VHS is a negative-stranded RNA virus called Egtved virus or viral haemorrhagic septicaemia virus (VHSV). The virus belongs to the family Rhabdoviridae and is placed into the newly recognized *Novirhabdovirus* genus (Walker, Benmansour, Dietzgen, Fang, Jackson, Kurath, Leong, Nadin-Davies, Tesh & Tordo 2000). The virus is enveloped and consists of a 11–12 kilobase nucleotide genome encoding five structural proteins.

The most susceptible fish species is the rainbow trout. The disease in farmed rainbow trout is characterized by destruction of the endothelial lining causing clinical signs such as haemorrhages in the meninges, serous surfaces, muscles, internal

organs and in the eyes, exophthalmia, darkening of the body and pale gills. Ascites can occasionally be observed. The affected fish are slow and lethargic. In chronic stages dark discolouration and abnormal swimming behaviour may be observed (Wolf 1988). Mortality depends on the age of the fish but it may be up to 100% in fry, although often less in older fish, typically from 30% to 70%.

The VHSV reservoirs are clinically infected fish and covert carriers among cultured, feral or wild fish. Virulent virus is shed with urine and ovarian fluids. Kidney and spleen are the sites in which virus is most abundant (Wolf 1988) and are also the target organs in rainbow trout (Brudeseth, Castric & Evensen 2002). Once VHSV is established in a farmed stock and, therefore, in a water catchment system, the disease becomes enzootic because of latent virus carrier fish.

The first records of a disease with clinical signs similar to VHS date back to Schäperclaus (1938), when a syndrome called 'Nierenschwellung' (kidney swelling) was described in rainbow trout. Pliszka (1946) described a similar problem occurring in southern Poland. In the early 1950s the syndrome was observed in Denmark (Schäperclaus 1954; Rasmussen 1965) and soon after in France (Besse 1955). In the middle 1950s it became apparent that the syndrome had an infectious background but the viral aetiology was not confirmed until Jensen (1963) made the first virus isolation on trout cell cultures, and proposed the name Egtved virus after the Danish village from which neighbourhood the sample originated (Jensen 1965).

The first VHSV isolation from wild fish in the marine environment were from Atlantic cod, *Gadhus morhua* in the coastal waters south of Zealand in 1979 (Jensen, Bloch & Larsen 1979; Jørgensen & Olesen 1987; J.L. Larsen personal communication), and from haddock, *Melanogrammus aeglefinus*, and cod in Scottish East coast waters in 1993 and 1995 (Smail 1995, 2000).

Viral haemorrhagic septicaemia virus was not found outside Europe until 1988, when it was detected in ovarian fluid from ascending chinook, *O. tshawytscha*, and coho salmon, *O. kisutch*, in the USA (Brunson, True & Yancey 1989; Hopper 1989).

European Community (EC) fish health legislation Council Directive 91/67/EEC (Anonymous 1991) lists VHS in category 2. By this categorization VHS is defined as a disease of serious economic significance for the EC. Coastal and continental areas of the EC, defined as zones, can be classified as approved VHS free if specified criteria can be met.

Viral haemorrhagic septicaemia is widely distributed in those parts of continental Europe with intensive production of rainbow trout in freshwater aquaculture. The whole coastal and inland areas of the UK and Norway, and continental Finland, Ireland and Sweden and parts of Denmark, France, Germany and Spain are EC-recognized VHS-free zones. In these zones there is a comprehensive programme of monitoring farmed freshwater and marine fish. In Spain, the virus has not been diagnosed since 1994 according to the OIE International Database on Aquatic Animal Diseases (http://www.collabcen.net/toWeb/aq2.asp) and it has never been diagnosed in Portugal and Greece (Olesen 1998; Ariel & Olesen 2002). However, there are reports of VHSV isolated from the Iberian Peninsula after 1994 (López-Vázquez, Bain, Olveira, Snow, Raynard, Barja & Dopazo 2003).

The widespread occurrence of VHSV in wild marine fish species in northern Europe raises concerns on coastal zone status for VHS and may have implications for member states having/requesting status of freedom from VHS in coastal zones and adjacent inland water systems. Diadromous fish migrate upstream into freshwaters and can thus act as potential carriers of VHSV into rainbow trout farms.

Currently, VHSV is subtyped into three neutralization patterns according to the 50% plaque neutralization test described by Olesen, Lorenzen & Jørgensen (1993). Variations in virus strain virulence have been recorded in both natural disease cases and infection trials but the subtyping system cannot discriminate between strains of different virulence. Nor can it discriminate between the marine strains non-pathogenic to rainbow trout and rainbow trout pathogenic strains from aquaculture (Skall, Slierendrecht, King & Olesen 2004; authors' observations). The development of diagnostic methods for typing of VHSV isolates according to pathogenicity and geographical origin will thus be crucial for adjustment of the EC disease legislation to account for the unexpected occurrence of VHSV in the marine environment.

The detection of marine VHS strains raises several questions: (i) Can marine VHSV cause infection in rainbow trout farms? (ii) Is marine VHSV a threat to culture of other fish species? (iii) Should all isolates of marine VHSV be treated as VHS or are there differences which could discriminate VHSV virulent for rainbow trout as a definable subgroup?

Definitions

In this review, marine VHSV is defined as a VHSV isolate originating from wild marine fish species. Freshwater VHSV is defined as isolates originating from farmed trout species in Europe. Infected fish are those from which VHSV has been isolated.

VHSV: a global perspective

During the last 15 years VHSV has been isolated from an increasing number of fish species. Thus far, it has been isolated from at least 48 different fish species throughout the northern hemisphere, including the USA, Canada, Japan, Korea and Europe (Fig. 1).

The present host spectrum for VHSV is listed in Table 1. VHSV has been isolated from 18 fish species in the North American Pacific area and three species from the North American Atlantic area, two species in the waters around Japan and 15 species in northern European waters. VHSV has additionally been isolated from five farmed marine fish species and from seven other species from fresh water. A further 11 fish species have been shown to be susceptible to VHSV under experimental conditions. Furthermore, VHSV has been reported from gilt-head sea bream, *Sparus aurata*, sole, *Solea* spp., and Iberian nase, *Chondrostoma polylepis* (López-Vázquez *et al.* 2003). The number of recorded host species is still increasing as a result of increasing monitoring effort. Classical freshwater VHSV isolates pathogenic to rainbow trout have so far only been isolated in Europe.

Marine VHSV isolations

Until 1988, VHSV was recognized as a virus only affecting freshwater fish species. VHSV isolations from farmed rainbow trout kept in marine net pens had occurred in Europe before 1988 (Castric & de Kinkelin 1980; Hørlyck, Mellergaard & Jørgensen 1984). The sources of these infections were not identified with certainty but it was suspected that the virus had been introduced from freshwater fish farms or via insufficiently disinfected transport vehicles.

In 1988, VHSV was isolated for the first time in North America, from ascending chinook (Hopper 1989) and coho salmon (Brunson *et al.* 1989). Subsequently, VHSV was isolated from adult coho salmon returning to hatcheries in the state of Washington on the US Pacific coast in 1989, 1991 and 1994 (Meyers & Winton 1995). In the following years, VHSV was isolated from at least 18 different fish species in North American Pacific waters (Table 1) and as far south as southern California (Cox & Hedrick 2001; Hedrick, Batts, Yun, Traxler, Kaufman & Winton 2003). The

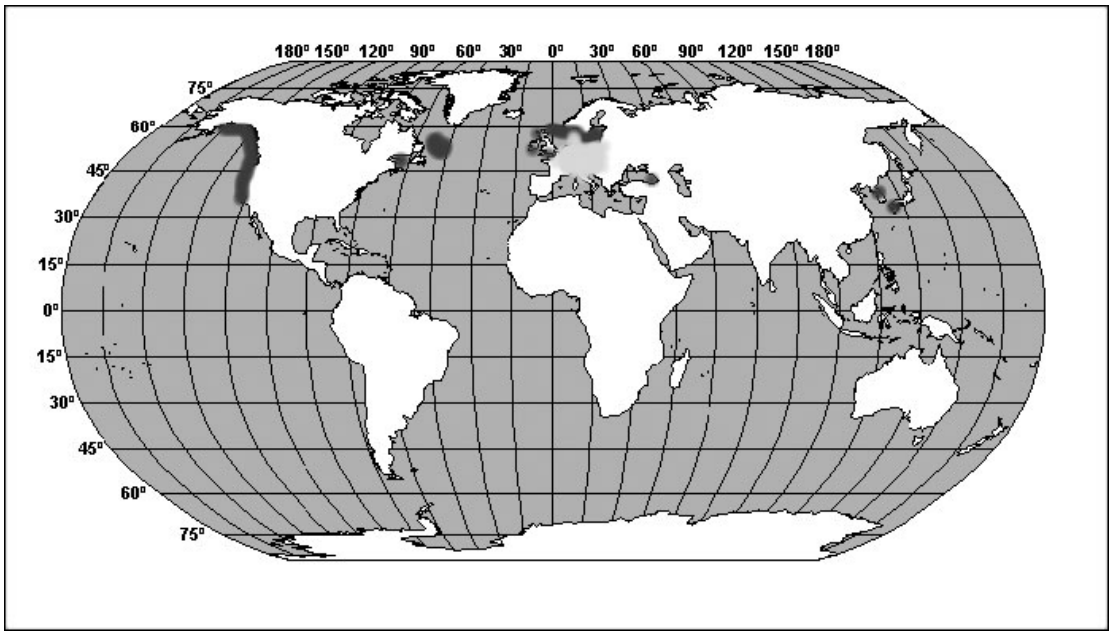

Figure 1 The global distribution of VHSV. Dark colours denote the areas where VHSV has been isolated from marine or anadromous fish species. The light-coloured area denotes the region from which classical freshwater rainbow trout pathogenic isolates occur.

Table 1 Fish species from which VHSV has been isolated, or which have been shown to be susceptible to VHSV by experimental infection

Fish species	Year of first isolation	Reference
Wild caught marine fish species		
North American Pacific area		
Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum)	1988	Hopper (1989)
Coho salmon, *O. kisutch* (Walbaum)	1988	Brunson *et al.* (1989)
Steelhead trout, *O. mykiss* (Walbaum)	1989	Brunson *et al.* (1989)
Pacific cod, *Gadus macrocephalus* Tilesius	1990	Meyers *et al.* (1992)
Pacific herring, *Clupea pallasii* Valenciennes	1993	Meyers *et al.* (1994)
Tube-snout, *Aulorhynchus flavidus* Gill	?	Traxler *et al.* (1995)
Shiner perch, *Cymatogaster aggregata* Gibbons	?	Traxler *et al.* (1995); Kent, Traxler, Kieser, Richard, Dawe, Shaw, Prosperiporta, Ketcheson & Evelyn (1998)
Pacific sandlance, *Ammodytes hexapterus* Pallas	1997	P.K. Hershberger & R.M. Kocan, unpublished data; Hershberger *et al.* (1999); Kocan *et al.* (2001)
Pacific hake, *Merluccius productus* (Ayres)	1998	Meyers *et al.* (1999)
Walleye pollock, *Theragra chalcogramma* (Pallas)	1998	Meyers *et al.* (1999)
Tomcod, *Microgadus proximus* (Girard)	1998	P. Reno, unpublished data; Meyers *et al.* (1999)
Three-spined stickleback, *Gasterosteus aculeatus* L.	?	Kent *et al.* (1998)
Pilchard, *Sardinops sagax* (Jenyns)	1998/99	Traxler *et al.* (1999)
Black cod, *Anoplopoma fimbria* (Pallas)	1998/99	Traxler *et al.* (1999)
English sole, *Parophrys vetulus* Girard	?	Hershberger & Kocan, unpublished data; Hershberger *et al.* (1999)
Eulachon, *Thaleichthys pacificus* (Richardson)	2001	Kaufman & Holt 2001); Hedrick *et al.* (2003)
Pacific mackerel, *Scomber japonicus* Houttuyn	2001	Cox & Hedrick (2001); Hedrick *et al.* (2003)
Surf smelt, *Hypomesus pretiosus* (Girard)	?	Batts & Winton (2002); Hedrick *et al.* (2003)
North American Atlantic area		
Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum)	1994	Bandín *et al.* (1999); Dopazo *et al.* (2002)
Three-spined stickleback, *Gasterosteus aculeatus* L.[a]	2000	Anonymous (2001b); Olivier (2002)
Mummichog, *Fundulus heteroclitus* (L.)[a]	2000	Amos & Olivier (2001); Anonymous (2001b); Olivier (2002)
Japan		
Japanese flounder, *Paralichthys olivaceus* (Temminck & Schlegel)	1999	Takano, Nishizawa, Arimoto & Muroga (2000)
Pacific sand eel, *Ammodytes personatus Girard*	2001	Watanabe, Pakingking, Iida, Nishizawa, Iida, Arimoto & Muroga (2002)
Northern European area		
Cod, *Gadus morhua* L.	1979, 1993	Jensen *et al.* (1979); Jørgensen & Olesen (1987); Smail (1995)
Haddock, *Melanogrammus aeglefinus* (L.)	1995	Smail (2000)
Herring, *Clupea harengus* L.	1996	Dixon *et al.* (1997)
Sprat, *Sprattus sprattus* (L.)	1996	Mortensen *et al.* (1999a)
Four-beard rockling, *Enchelyopus cimbrius* (L.)	1996	Mortensen *et al.* (1999a)
Norway pout, *Trisopterus esmarkii* (Nilsson)	1996	Mortensen *et al.* (1999a)
Whiting, *Merlangius merlangus* (L.)	1997	Mortensen *et al.* (1999a)
Blue whiting, *Micromesistius poutassou* (Risso)	1997	Mortensen *et al.* (1999a)
Lesser argentine, *Argentina sphyriaena* L.	1997	Mortensen *et al.* (1999a)
Poor cod, *Trisopterus minutus* (L.)	1998	King *et al.* (2001b)
Plaice, *Pleuronectes platessa* L.	1998	Mortensen, Olesen & Mellergaard (1999b); Skall *et al.* (2005)
Dab, *Limanda limanda* (L.)	1998	Mortensen *et al.* (1999b); Skall *et al.* (2005)
Flounder, *Platichthys flesus* (L.)	1998	Mortensen *et al.* (1999b); Skall *et al.* (2005)
Sand goby, *Pomatoschistus minutus* (Pallas)	2001	Skall, King, Brudeseth, Mellergaard & Olesen (2002); Skall *et al.* (2005)
Sand eel, *Ammodytes* sp.	2002	Skall *et al.* (2005)
Farmed marine fish species		
Turbot, *Scophthalmus maximus* (L.)	1991	Schlotfeldt *et al.* (1991)
Atlantic salmon, *Salmo salar* L.	1986,1995	Jimenez de la Fuente *et al.* (1988); Traxler *et al.* (1995)
Japanese flounder, *P.olivaceus*	1996?	Isshiki *et al.* (2001); Kim *et al.* (2003)
Rockfish, *Sebastes inermis* Cuvier	?	Isshiki *et al.* (2003)
Pacific sand eel, *A. personatus*	?	Isshiki *et al.* (2003)
Other fish species		
Rainbow trout, *Oncorhynchus mykiss* (Walbaum)	1962	Jensen (1963)

Table 1 (contd)

Fish species	Year of first isolation	Reference
Brown trout, *Salmo trutta* L.	1969	de Kinkelin & le Berre (1977); Jørgensen (1980); Bovo, Zanin & Giorgetti (1982)
Pike, *Esox lucius* L.	1978	Meier & Jørgensen (1979)
Grayling, *Thymallus thymallus* (L.)	1979	Wizigmann, Baath & Hoffmann (1980)
Whitefish, *Coregonus* sp.	1984	Ahne & Thomsen (1985); Meier *et al.* (1986)
European eel, *Anguilla anguilla* (L.)	1987	Castric *et al.* (1992)
Largemouth bass, *Micropterus salmoides* (Lacepéde)	1998	de Kinkelin, Daniel, Hattenberger-Baudouy & Benmansour (1999)
Experimentally susceptible fish species		
Brook trout, *Salvelinus fontinalis* (Mitchill)		Rasmussen (1965)
Golden trout, *Oncorhynchus aguabonita* (Jordan)		Ahne, Negele & Ollenschläger (1976)
Rainbow trout × coho salmon		Ord, Le Berre & de Kinkelin (1976)
European sea bass, *Dicentrarchus labrax* (L.)		Castric & de Kinkelin (1984)
Lake trout, *Salvelinus namaycush* (Walbaum)		Dorson, Chevassus & Torhy (1991)
Atlantic halibut, *Hippoglossus hippoglossus* (L.)		Snow *et al.* (1999a); Bowden (2003)
Black sea bream, *Acanthopagrus schlegelii* (Bleeker)		Isshiki *et al.* (2003)
Red spotted grouper, *Epinephelus akaara* (Temminck & Schlegel)		Isshiki *et al.* (2003)
Schlegel's black rockfish, *Sebastes schlegelii* Hilgendorf		Isshiki *et al.* (2003)
Red sea bream, *Pagrus major* (Temminck & Schlegel)		Ito *et al.* (2004)
Japanese amberjack (Yellowtail), *Seriola quinqueradiata* Temminck & Schlegel		Ito *et al.* (2004)

[a] Caught in fresh water.

geographical area from which VHSV is found in North America was further broadened when VHSV was isolated from the Atlantic waters of North America in Greenland halibut, *Rheinhardtius hippoglossoides* (Bandín, López-Vázquez, Cutrín, Brey, Dopazo & Barja 1999; Dopazo, Bandín, López-Vazquez, Lamas, Noya & Barja 2002), three-spined stickleback, *Gasterosteus aculeatus*, and mummichog, *Fundulus heteroclitus* (Amos & Olivier 2001; Anonymous 2001b; Olivier 2002), the latter two species being caught in a small river.

The first isolations of VHSV in Asia were from farmed Japanese flounder, *Paralichthys olivaceus*, in Japan (Isshiki, Nishizawa, Kobayashi, Nagano & Miyazaki 2001) and Korea (Kim, Lee, Hong, Park & Park 2003). VHSV was subsequently found in wild marine fish in waters around Japan (Table 1). In Europe, American VHSV isolations from cod with skin ulcers (Meyers, Sullivan, Emmenegger, Follett, Short, Batts & Winton 1992) resulted in the organization of fish disease surveys devoted to virological investigations of ulcerated cod, conducted by the Marine Laboratory, Scotland, in 1993 and 1995. The surveys resulted in isolation of VHSV from wild cod (Smail 1995, 2000) and haddock (Smail 2000), in the North Sea.

These cruises confirmed the occurrence of VHSV in the marine environment in Europe, and changed opinions on a rhabdovirus isolate from cod in the Baltic Sea in 1979 (Jensen *et al.* 1979). This isolate was identified as VHSV in 1987 (Jørgensen & Olesen 1987) and was at the time believed to be a laboratory contamination. However, recent studies on the infectivity of this virus to rainbow trout indicated that it did not induce mortality in rainbow trout (Jørgensen 1992; Skall *et al.* 2004), and was thus similar to other tested northern European marine isolates (Skall *et al.* 2004).

These observations led to sampling of wild marine fish for virological investigation by both the Danish Institute for Food and Veterinary Research (DFVF) (Mortensen, Heuer, Lorenzen, Otte & Olesen 1999a) and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), UK in 1996 (Dixon, Feist, Kehoe, Parry, Stone & Way 1997), with isolation of VHSV from Atlantic herring, *Clupea harengus*, sprat, *Sprattus sprattus*, Atlantic cod and four-beard rockling, *Enchelyopus cimbrius*, from the Baltic Sea, and from Atlantic herring in the English Channel.

Presence of marine VHSV in northern Europe – location, species and prevalence

Isolations of VHSV from marine fish, combined with VHS outbreaks in turbot, *Scophthalmus maximus*, farms in Germany in 1991 (Schlotfeldt, Ahne, Jørgensen & Glende 1991), Scotland in 1994 (Ross, McCarthy, Huntly, Wood, Stuart, Rough, Smail & Bruno 1994; Munro 1996) and Ireland in

1997 (J. McArdle, personal communication), created a need to obtain more knowledge on the impact of VHSV in the marine environment, and an EC project (FAIR CT 96-1594. 'Rhabdoviruses in wild marine fish in European coastal waters: characterization and significance for aquaculture') was funded in 1997. This project, which included investigation of the host spectrum and the spatial distribution of marine VHS virus, was conducted in collaboration between Danish, Norwegian and Scottish institutes, and was followed by national projects.

These research programmes covered the coastal waters around Scotland, the North Sea, the waters West and South of Norway, the Skagerrak, Kattegat and the Baltic Sea. In combination with scientific cruises covering the waters around England by CEFAS, a total of at least 54 137 fish representing 69 different species were sampled for VHSV (Mortensen *et al.* 1999a; Smail 2000; King, Snow, Smail & Raynard 2001b; Brudeseth & Evensen 2002; Dixon, Avery, Chambers, Feist, Mandhar, Parry, Stone, Strømmen, Thurlow, Tsin-yee Lui & Way 2003; Skall, Olesen & Mellergaard 2005; B.E. Brudeseth, personal communication).

The data from these surveys of marine fish revealed a significant reservoir of VHSV in at least 15 species in northern European waters (Table 1) with a total of 193 isolations. The lack of isolation of VHSV from many of the fish species examined does not imply that they are refractory to the virus. It may reflect that the number of individuals examined from a species has been insufficient. The research cruises showed that the virus is endemic in the Baltic Sea, Kattegat/Skagerrak, the North Sea and around the British Isles.

Sprat, herring and Norway pout, *Trisopterus esmarkii*, were the species from which VHSV was most frequently isolated. In the Baltic Sea, VHSV was mainly found in herring and sprat, whereas Norway pout was the predominant host in the North Sea. Prevalence calculations indicate that VHSV is common in the Baltic Sea in an area around the island of Bornholm, with prevalence rates of between 0% and 16.7% for herring and 5.6% and 7.8% for sprat (Skall *et al.* 2005). Hedrick *et al.* (2003) reported prevalence rates of 4–8% in apparently healthy sardine, *Sardinops sagax*, mackerel, *Scomber japonicus*, and smelt, *Hypomesus pretiosus*, in waters off California and Oregon, values comparable with those for herring and sprat around Bornholm. Prevalence calculations should be viewed on with caution, as sampling of fish will never be totally random, and comparison can be difficult, as different schools of fish may have different infection levels. Outside certain VHSV 'hot spots' it may be necessary to sample many fish to isolate VHSV.

It is interesting that herring, sprat and Norway pout are shoaling fish. The close proximity of fish in schools may enhance the transmission of virus via water and fish-to-fish contact. Eating infected prey may transfer the infection to carnivorous fish that feed on species such as herring and sprat. Flatfish may become infected by eating VHSV-infected fish that die and sink to the bottom.

None of the fish sampled during the Danish, Norwegian, Scottish and English cruises showed pathology typical of VHS in rainbow trout (Mortensen *et al.* 1999a; King *et al.* 2001b; Brudeseth & Evensen 2002; Dixon *et al.* 2003; Skall *et al.* 2005). Most fish showed no pathology. Some fish showed parasite infestations, and severe ulcers and bleedings were seen in cod caught in the Baltic Sea. These infestations were not associated with the finding of VHSV (Mortensen *et al.* 1999a; authors' observations). Almost all fish tested were 1+ or older due to sampling bias of the trawl nets used.

In virulence assessments using 0+ turbot many isolates were found to be virulent, i.e. producing typical VHS pathology (King, Snow, Skall & Raynard 2001a). If this finding is also true for young fish of other marine fish species, VHSV may contribute to the 'natural' mortality in juvenile marine fish. However, the effect of this on natural fish stocks may be difficult to establish as predation on the very young life stages of most species are extremely high.

The finding of VHSV in 1+ and older fish with no pathology gives no clues on how the fish were infected, at what age the infection took place, or whether they survived VHS to become carriers, or became exposed *per aqua* or *per os* and succumbed as older fish to a low-grade infection. The present data do not allow distinction between latent carriers and fish undergoing an active infection. However, many of the examined samples showed cytopathic effect (CPE) only after subculture, or only weak CPE in the primary inoculation on cells, indicating that the quantity of infective virus particles in the wild marine fish was around the detection limit, supporting the carrier hypothesis. At DFVF, 101 of 172 VHSV-positive samples (59%) did not develop CPE in the primary inoculation and another 27

isolates (16%) were only faintly positive in the primary inoculation. When examining samples from clinically infected fish, CPE is observed 2–4 days after primary inoculation.

Method of isolation and identification of VHSV from wild marine fish

In order to enhance sensitivity, many of the Danish samples have been inoculated into two wells at the lowest dilution (Skall *et al.* 2005) in contrast to the normal one well inoculation at the lowest dilution used for surveillance of fish farms according to EC Commission Decision 2001/183/EEC (Anonymous 2001a). In addition, many of the samples have been examined in pools consisting of material from less than 10 fish (single fish or five-fish pools), which also increases the sensitivity.

The cell line used to isolate VHSV is also important for sensitivity. The most commonly applied method in northern Europe for isolation of VHS virus from wild marine fish has been inoculation of fish tissue onto BF-2 cell cultures (Dixon *et al.* 1997; Mortensen *et al.* 1999a; King *et al.* 2001b; Brudeseth & Evensen 2002). Occasionally, EPC, FHM and CHSE-214 cells have also been used (Dixon *et al.* 1997, 2003; Smail 2000). Subsequent identification of virus was carried out by enzyme-linked immunosorbent assay and/or immunofluorescence.

In North America, the preferred cell line for VHSV isolation is EPC, often pretreated with polyethylene glycol (Brunson *et al.* 1989; Hopper 1989; Meyers *et al.* 1992; Meyers, Short, Lipson, Batts, Winton, Wilcock & Brown 1994; Marty, Freiberg, Meyers, Wilcock, Farver & Hinton 1998; Hershberger, Kocan, Elder, Meyers & Winton 1999; Meyers, Short & Lipson 1999; Traxler, Kieser & Richard 1999; Hedrick *et al.* 2003). CHSE-214 and/or BF-2 cells have also been used (Hopper 1989; Meyers *et al.* 1994, 1999; Hedrick *et al.* 2003).

The applicability of cell lines probably depends on the performance and sensitivity of a given cell line in a particular laboratory. There may also be differences in cell line preference of the individual isolates. However, previous published work has showed that BF-2 cells in general are superior for VHSV isolation (Olesen & Vestergård Jørgensen 1992; Lorenzen, Carstensen & Olesen 1999).

Due to the limitations of cell culture test sensitivity, it is likely that the number of infected fish was higher in the samples tested than was revealed by cell culture. Furthermore, Dixon *et al.* (2003) reported on 19 samples from herring and cod that tested VHSV positive by reverse transcriptase-polymerase chain reaction (RT-PCR) and from which it was impossible to isolate the virus by cell culture. If these RT-PCR bands are true indicators of VHSV, then virus prevalence in northern European waters may be higher than existing studies indicate.

Mass mortalities in wild marine fish stocks associated with VHSV

Meyers & Winton (1995) summarized the VHSV isolations in North America until 1995. Many of the isolations were from herring sampled due to morbidity and mortality and/or reduced stocks. The authors concluded, however, that it is unlikely that VHSV alone was responsible for the herring decline seen in Prince William Sound, Alaska, in the early 1990s.

Since the review by Meyers & Winton (1995) mass mortalities in marine fish have been reported from the North American Pacific area. In August 1998, mass mortalities in Pacific herring, *Clupea pallasii*, Pacific hake, *Merluccius productus*, and walleye pollock, *Theragra chalcogramma*, were reported in Lisianski Inlet, Alaska. VHSV was isolated from samples of dead fish of all three species (Meyers *et al.* 1999). None of the affected fish showed any gross external or internal lesions and histology revealed non-specific changes some of which could have been due to autolysis.

From November 1998 to February 1999, another mass mortality occurred a little further south in Queen Charlotte Strait, on the north-east coast of Vancouver Island. In particular pilchards, but also Pacific herring, blackcod, *Anoplopoma fimbria*, ratfish, *Hydrolagus colliei*, and shiner perch were reported floating moribund or dead on the surface. On this occasion marine VHSV was isolated from pilchard, Pacific herring and blackcod (Traxler *et al.* 1999). Mass mortality has not been reported in European wild fish stocks from which VHSV has been isolated.

Epidemiology

Viral haemorrhagic septicaemia virus in Pacific herring appears to be an opportunistic pathogen triggered by stress (Meyers *et al.* 1994). The VHS

epizootics in Pacific herring have involved a series of stressors such as exceptionally strong year classes of fish, infection by other diseases such as viral erythocytic necrosis, the rigour of spawning in shallow water, pursuit and capture in pond net fishery, harassment by predators, colder than normal seawater temperatures, nutritional deprivation, and acute exposure to pollutants (hydrocarbons) (Meyers & Winton 1995). Most of these stressors were abundant in the Prince William Sound area (Meyers *et al.* 1994). For the pilchard and herring mortality in Queen Charlotte Strait, low water temperatures have been suggested as the triggering factor for the VHS infection (Traxler *et al.* 1999).

Carls, Marty, Meyers, Thomas & Rice (1998) showed that exposure to toxicants may be able to activate subclinical VHSV infection in Pacific herring. Adult Pacific herring with unknown VHSV status were exposed to weathered crude oil for 16–18 days and the prevalence of VHSV and total polynuclear aromatic carbon load in the fish were significantly correlated.

Stress may increase the prevalence of VHSV and enhance the spread of the disease, especially if the stock density is high. Such a case was observed in the spawn-on-kelp fishery in Prince William Sound where mature herring are caught and kept in net pens in order to collect their roe on kelp (*Microcystis* leaves). Under these circumstances the VHSV prevalence increased significantly in the herring in the enclosures compared with the free-living stock and the release of virus reached levels where it could be isolated from the water (Hershberger *et al.* 1999).

Many of the other species which have been shown to harbour VHSV, e.g. salmonid species, Pacific cod and Pacific hake, are fish that prey on herring. The VHSV seems to be enzootic in the North American Pacific herring stock and the infection is probably transferred to other species through predation.

Pathogenicity of marine VHSV to different fish species

Rainbow trout

In cases of isolation of VHSV in seawater-reared rainbow trout in France and Denmark (Castric & de Kinkelin 1980; Hørlyck *et al.* 1984), the affected fish showed all the classical clinical signs of VHS; exophthalmia, haemorrhages in the skin, eyes, gills, muscles, swim bladder, liver, gonads and abdominal adipose tissue, as well as high mortality (15–50%). As these cases probably originated from fresh water, the observed lesions were almost certainly directly associated with the VHSV.

Several infection trials with rainbow trout using North European marine and North American VHSV isolates have been conducted (Winton, Batts, Deering, Brunson, Hopper, Nishizawa & Stehr 1991; Meyers *et al.* 1994; Dixon *et al.* 1997; Follett, Meyers, Burton & Geesin 1997; Dixon & Avery 1998; Skall *et al.* 2004). These trials show that the North American and North European marine VHSV isolates are non- to low pathogenic for rainbow trout by immersion, causing either no mortality in the case of the North European marine VHSV or chronic mortality of 20% over a 30-day period for North American VHSV. This level should be compared with mortalities of 50–100%, which are normally observed in rainbow trout during infection trials with the classical freshwater VHSV (Skall *et al.* 2004).

Using intraperitoneal (i.p.) injection the marine VHSV was shown to be non- to medium pathogenic. It is concluded that classical freshwater isolates are pathogenic to rainbow trout by immersion, whereas isolates from wild marine fish are non-pathogenic under similar conditions.

Chinook, coho, sockeye and pink salmon

The North American VHSV strain was originally isolated from apparently healthy chinook and coho salmon (Brunson *et al.* 1989; Hopper 1989) and seems to be non- to low pathogenic for chinook, coho, sockeye, *O. nerka*, and pink salmon, *O. gorbuscha*, although the results of only a few infection trials have been published.

All four species were refractory by immersion infection to a VHSV isolate from Pacific cod (Follett *et al.* 1997). This was supported by Traxler *et al.* (1999) who tested chinook and sockeye salmon by injection and immersion, using isolates originating from mass mortalities of pilchard and Pacific herring. Waterborne virus challenges in coho salmon fingerlings resulted in mortalities below 10%, using VHSV isolates originating from chinook and coho salmon (Winton *et al.* 1991).

Atlantic salmon

Viral haemorrhagic septicaemia virus has not been isolated from Atlantic salmon, *Salmo salar*, in

European waters during routine monitoring for VHS and infectious haematopoietic necrosis (IHN) conducted according to Council Directive 91/67/EEC (Anonymous 1991) and Commission Decision 2001/183/EC (Anonymous 2001a), including thousands of samples collected in Norway, the UK and Ireland. VHSV has been isolated from Atlantic salmon in Spain (Jimenez de la Fuente, Marcotegui, San Juan & Basurco 1988; López-Vázquez *et al.* 2003) and the North American strain of VHSV was isolated from farmed Atlantic salmon in 1995, 1998, 1999 and 2002 in British Columbia, Canada. The virus isolations usually occurred in the winter/spring period, often coinciding with herring spawning in the area close to the farm site. Losses were in one case chronic, eventually reaching 10%, and in another were 2% per week (G.S. Traxler, Pacific Biological Station, Nanaimo, Canada, personal communication). VHSV has also been isolated from a single farmed Atlantic salmon from a marine net pen in Puget Sound, Washington. The fish had ceased feeding and clinical signs in some moribund fish suggested an infectious agent. Abnormal mortality levels were not observed (Amos & Thomas 2002).

Infection trials indicate that, in general, all types of VHSV are non- to low pathogenic for Atlantic salmon by immersion infection (de Kinkelin & Castric 1982; Traxler, Kieser & Evelyn 1995; Traxler & Keiser, in Meyers & Winton 1995; Dixon & Avery 1998; Traxler *et al.* 1999; King *et al.* 2001a). VHSV has shown to be pathogenic for Atlantic salmon when administered by injection with up to 78% mortalities (de Kinkelin & Castric 1982; Traxler *et al.* 1995, 1999; Traxler & Keiser, in Meyers & Winton 1995).

The results of infection trials, combined with the fact that VHSV has been isolated from Atlantic salmon in North America and the Iberian Peninsula, suggest that VHSV may be a potential problem in Atlantic salmon farming.

As a general conclusion, Salmoninae species do not appear to be very susceptible to marine and North American VHSV isolates by immersion, whereas injection may induce high mortality. As it is possible to isolate VHSV from naturally infected Atlantic salmon, this species may act as a vector. It appears that the North American isolates are able to cause clinical infection with low mortality in sea-farmed Atlantic salmon. Spread of these viruses may pose a serious risk for the large European and South American Atlantic salmon industry.

Pacific cod

Viral haemorrhagic septicaemia virus was isolated from ulcerative skin lesions in Pacific cod resembling those described from the ulcus syndrome in Atlantic cod (Meyers *et al.* 1992, 1994). The organs from the cod sampled in 1992 were negative for VHSV, whereas those from cod sampled in 1994 were positive.

Atlantic cod

The first isolate of marine VHSV was made from one pool of seven Atlantic cod displaying stage I of the ulcus syndrome (the papulo-vesicular stage: Jensen & Larsen 1979). The samples taken for virus cultivation were papules with underlying tissue (Jensen *et al.* 1979). After this observation, isolation of VHSV from skin lesions has been attempted on numerous occasions, with varying success. Smail (2000) also examined Atlantic cod with ulcus syndrome-like lesions and found that a small proportion of skin samples from the lesion-positive fish were VHSV positive.

Similarly, Mortensen *et al.* (1999a) reported on VHSV isolations from cod skin. Two of the isolates were from skin with vesicles and one from normal skin, VHSV was not found from five other skin samples from cod with vesicles, examined as single fish. VHSV was also isolated from a pooled sample of skin tissue from five cod with vesicles. Four other samples of skin tissue from cod with vesicles examined in pools of two to five fish (in total 17 fish) were negative for VHSV.

Of 21 cod with skin lesions from the North Sea, VHSV was isolated from one lesion but not from viscera (King *et al.* 2001b). No virus was isolated from 16 haddock with haemorrhages similar to those from which Smail (2000) isolated VHSV.

Only a few infection trials have been performed using cod. Snow, Cunningham & Bricknell (2000) reported on an infection trial in juvenile Atlantic cod using a North European marine VHSV isolate from cod. No VHSV-associated mortality was demonstrated following immersion infection and no VHSV was recovered. In excess of 80% of the cod died when virus was administered by i.p. injection, and VHSV was recovered from the brain and viscera from all the dead fish. The moribund fish did not exhibit classical VHSV, as seen in rainbow trout, but limited exophthalmia and ascites was observed. VHSV was isolated from a subcuta-

neous lesion but not from the organs of a surviving i.p.-infected cod and VHSV was also recovered from haemorrhaged fin tissue sampled from a cod which died after i.p. injection.

Based on published and unpublished work, and supported by studies performed by the authors involved in the first VHSV isolation in cod (Jensen & Larsen 1982; Larsen & Jensen 1988), and corresponding with the conclusions of Mortensen *et al.* (1999a), it may be concluded that ulcers are not a prominent feature of VHSV infection in cod, and may be an incidental finding.

Pacific herring

Viral haemorrhagic septicaemia virus has been isolated from Pacific herring, *Clupea pallasii*, caught along the North American and Canadian Pacific coast, where it has apparently caused severe disease outbreaks and been a significant factor in the herring stock regulation, especially in Prince William Sound, Alaska (Kocan, Bradley, Elder, Meyers, Batts & Winton 1997; Traxler *et al.* 1999).

Meyers *et al.* (1994) isolated VHSV from viscera of Pacific herring displaying skin ulcers or subdermal haemorrhages, without being able to detect other pathogens in the affected fish. Skin samples were not examined. This finding was reproduced when Marty *et al.* (1998) found that fin base reddening and skin reddening was significantly associated with VHSV. Meyers & Winton (1995) reported on several isolations of VHSV from herring with skin haemorrhages. Virus was often isolated only from the lesions and not the viscera. Meyers *et al.* (1994) also reported on histopathologic features of subdermal and kidney haemorrhages, kidney tubule degeneration and active reticulo-endothelial foci in the liver and kidney suggesting viraemia due to a weakly pathogenic virus. The minor extent of these lesions was not typical of classical freshwater VHS.

Cutaneous ulcers were not observed during VHS infection in wild Pacific herring and Pacific sandlance, *Ammodytes hexapterus*, after transportation to the laboratory (Kocan, Hershberger, Elder & Winton 2001), or among experimentally infected specific pathogen-free (SPF) herring (Kocan *et al.* 1997).

The prevalence of VHSV increased in wild herring after confinement in net cages for the production of spawn-on-kelp (Hershberger *et al.* 1999) and confinement of apparently healthy Pacific herring in laboratory tanks often led to active VHSV infections after 3–7 days (Kocan, Landolt & Winton 1996; Kocan *et al.* 2001).

Kocan *et al.* (1997) fulfilled Koch's postulates by infecting SPF-reared Pacific herring with North American VHSV isolates by bath challenge. It was found that these isolates were highly pathogenic by the immersion route with mortality approaching 100%. Mortality began 4–6 days after exposure and moribund fish displayed petechial haemorrhages on the lower jaw, isthmus and eyes. Histopathological examination of tissues from moribund fish revealed multifocal coagulative necrosis of hepatocytes, diffuse necrosis of interstitial haematopoietic tissues in the kidney, diffuse necrosis of the spleen, epidermis, and subcutis, and occasional necrosis of pancreatic acinar cells.

Experimental work and findings from natural outbreaks show that the North American VHSV strain is highly pathogenic for Pacific herring. As in cod, cutaneous ulcers appear to be an incidental finding, whereas skin haemorrhages are a prominent feature.

Atlantic herring

No infection trials have been conducted in Atlantic herring and mass mortality of wild Atlantic herring with isolation of VHSV has not been observed. Whether similar conclusions can be drawn for Atlantic herring as for Pacific herring awaits further investigations.

Turbot

In association with the isolation of VHSV from farmed turbot (Schlotfeldt *et al.* 1991; Ross *et al.* 1994; Munro 1996), high mortality as well as pathology such as exophthalmia, haemorrhages in the skin, eyes, musculature and on the serous surfaces, resembling the classical signs in rainbow trout, was observed. These changes were regarded as being products of the viral infection. The isolate responsible for the outbreak among turbot in Scotland was tested in infection trials and found to induce high mortality in turbot (Snow & Smail 1999; King *et al.* 2001a).

After experimental infection of turbot, the fish displayed classical gross signs of VHS except that there were no haemorrhages in the muscles (Castric & de Kinkelin 1984). The histopathological findings were necrosis of the spleen and the

interstitial tissue of the kidney and haemorrhages in the different intestinal organs. These gross signs, including darkening of the body, haemorrhages on the head and fins, and ascitic fluid in the body cavity were also reproduced by experimental infection of turbot by King *et al.* (2001a).

These authors showed that several, but not all, of the marine VHSV isolates are pathogenic to turbot by immersion under experimental conditions, with mortalities ranging from 0% to 68%. Both Dixon & Avery (1998) and King *et al.* (2001a) showed no or low mortality with the Danish freshwater isolate DK-3592B (Lorenzen, Olesen & Jørgensen 1993) when turbot were tested by immersion. This isolate is highly pathogenic to rainbow trout causing mortalities up to 100% (Skall *et al.* 2004). Other VHSV freshwater strains, however, were shown to be pathogenic for turbot (Castric & de Kinkelin 1984).

Natural VHS outbreaks in turbot have occurred with virus strains that resemble both freshwater (Schlotfeldt *et al.* 1991) and marine isolates (Ross *et al.* 1994; J. McArdle, unpublished data). The American VHSV strain has not been tested for pathogenicity in turbot. Both experimental and natural outbreaks show that turbot is a VHSV-susceptible species, and both freshwater as well as marine isolates are able to produce mortality.

Japanese flounder

The North American type of VHSV is virulent to Japanese flounder. During VHSV outbreaks in Japanese flounder, the cumulative mortality reached 50–70%. Individual diseased fish showed dark body discoloration, clear ascitic fluid in the peritoneal and pericardial cavities, congested liver, splenomegaly, swollen kidney and occasionally haemorrhages in the lateral musculature (Isshiki *et al.* 2001). North American type VHSV has also been isolated from cultured, diseased Japanese flounders in Korea (Kim *et al.* 2003).

Several infection experiments using the Japanese VHSV isolates have been conducted, using mainly the isolates Obama25 or JF00Ehi1 (North American type) or KRRV-9601 (European type), by immersion, i.p. injection or cohabitation (Isshiki *et al.* 2001; Takano, Mori, Nishizawa, Arimoto & Muroga 2001; Isshiki, Nagano & Miyazaki 2002, 2003; Mori, Iida, Nishizawa, Arimoto, Nakajima & Muroga 2002; Iida, Mori, Nishizawa, Arimoto & Muroga 2003; Ito, Mori, Arimoto & Nakajima 2004; Muroga, Iida, Nishizawa & Arimoto 2004). The experiments have proved that VHSV is pathogenic for Japanese flounder, producing a disease similar to that observed during natural outbreaks among farmed Japanese flounder, and with up to 100% mortality.

Halibut

Studies on VHSV susceptibility in halibut, *Hippoglossus hippoglossus*, are rather preliminary. Snow, Bowden & Bricknell (1999a), using a turbot isolate suspected to be of marine origin, showed it to be of low pathogenicity (up to 20%) to halibut by immersion, with moribund fish displaying ascites and darkening of the skin. Up to 80% of halibut infected by the i.p. route died. These results were confirmed by Bowden (2003) who, using the same isolate, showed a cumulative mortality of 2% by immersion, 19% by cohabitation and 28% by i.p. injection.

Other fish species

Infection trial by immersion demonstrated that shiner perch, *Cymatogaster aggregata*, was susceptible to Canadian herring VHSV isolates with a cumulative mortality of 38% (Traxler *et al.* 1995; Traxler & Keiser, in Meyers & Winton 1995). Infection trials with tube snouts, *Aulorhynchus flavidus*, by both waterborne and i.p. exposure also produced mortality (Traxler *et al.* 1995).

The susceptibility of sea bass, *Dicentrarchus labrax*, to freshwater VHSV has been tested experimentally by Castric & de Kinkelin (1984). Sea bass appeared to be susceptible to VHSV by both immersion and i.p. injection. At temperatures below 15 °C, up to 50% of the sea bass infected by immersion died and diseased fish displayed classical signs of VHS.

VHS in marine aquaculture

Farmed rainbow trout

Most VHS outbreaks have occurred in rainbow trout freshwater farms, but outbreaks have also occurred in marine farms (Castric & de Kinkelin 1980). Since 1982 Danish mariculture has experienced several VHS outbreaks, some of which were most probably because of transmission by fish in the incubation period of the disease, and some to

further waterborne spread to adjacent marine farms (Hørlyck *et al.* 1984; Jørgensen 1992).

Transfer of infection in the freshwater environment between free-living and farmed fish has been suspected in many cases during the 40-year history of VHS control in Denmark, as well as in Germany. Free-living, mainly feral fish in watercourses testing VHSV positive by either virus isolation or serology have been identified on several occasions (Jørgensen 1982; Olesen & Jørgensen 1983; Meier, Ahne & Jørgensen 1986; Enzmann, Konrad, Parey & Wetzlar 1987; Enzmann, Konrad & Rapp 1992; Ahne & Jørgensen 1993; Enzmann, Konrad & Parey 1993). Whether such feral fish were already infected when released or became infected subsequently and then transferred the infection to farmed fish is difficult to ascertain. Enzmann *et al.* (1992) suggest that feral fish transferred VHSV to fish farms as they were able to detect antibodies in fish located upstream and separated from the upper fish farm on a water course by a weir. Even though fish were not able to pass the weir, herons, other animals or humans may have transferred fish from one side of the weir to the other and the infection may have passed in the other direction. The same problem has been encountered in Denmark, where VHSV has been detected in feral rainbow trout upstream from fish farms undergoing VHS outbreaks (Olesen & Jørgensen 1983), making it difficult to determine the route of infection.

Evidence of transfer of infection between wild fish and farmed rainbow trout in the marine environment is found in the outbreaks of VHS in net pen-reared rainbow trout off Gothenburg, Sweden, in 1998 (Nordblom 1998) and 2000 (Nordblom & Norell 2000). Sweden was considered free of VHS and rainbow trout from a Swedish freshwater farm was used to stock the marine farm. It has not been possible to isolate VHSV from the delivering farm. The net pens were situated in the Kattegat close to an area where VHS virus previously had been isolated from wild marine fish.

In Finland, two outbreaks of VHS occurred in 2000; one in the Finnish archipelago at the island of Kumlinge, one of the Åland Islands, and another in the south-eastern coastal area of Finland in Pyhtää (Husu-Kallio & Suokko 2000). In 2001, an additional four outbreaks occurred. A further 12 new farms were infected in 2002 around the Åland Islands, 10 owned by the same company. This brought the total number of infected farms to 21, with 17 at the Åland Islands. The size of the infected fish varied between 50 g and 2 kg. Mortality was typically 10–20%, but in one case as high as 50%. There were no clinical signs on many of the affected farms, and the infection was only detected by screening. It has not been possible to explain these latest outbreaks of VHS in rainbow trout marine net pens by transfer of VHSV from the freshwater environment as Finland was considered free of VHS. Raw fish were earlier used as feed for farmed rainbow trout in Finland, but this has not happened since the end of the 1990s. On the first affected farm in Pyhtää, the owner had stopped using raw fish as feed 1–2 years prior to the outbreak (Sainmaa and Rimaila-Pärnärnen, National Veterinary and Food Research Institute, Finland, personal communication).

In infection studies using immersion both the Swedish and the Finnish isolates caused mortality in rainbow trout fingerlings of approximately 20% and 40%, respectively, unlike the isolates from marine fish species (unpublished results in Skall *et al.* 2004). The mortalities, however, were less than those mortalities observed by infection with VHSV isolates from recent outbreaks in freshwater farms (isolates from 1995 and subsequently) which showed a cumulative mortality of 60% or more (Skall *et al.* 2005).

Genetic studies have shown that the Swedish isolates group together with marine isolates (Genotype Ib) and the Finnish isolates (Genotype Id) are located close to the ancestral source (Einer-Jensen, Ahrens, Forsberg & Lorenzen 2004). These isolates may thus represent new introductions in rainbow trout.

Farmed turbot

During the past 16 years, there have been three VHS outbreaks in turbot in land-based marine aquaculture facilities in northern Europe; in Germany in 1989 (Schlotfeldt *et al.* 1991), the UK in 1994 (Ross *et al.* 1994) and Ireland in 1997 (J. McArdle, personal communication), confirming earlier experimental work by Castric & de Kinkelin (1984) that turbot is susceptible to VHS.

The German turbot farm was situated on the Baltic coast and it is not known whether the virus was introduced into the fish farm by farmed or wild infected fish, or by contaminated water from rainbow trout farms, which were situated 25–35 km away. The isolate falls genetically within a genogroup containing both freshwater and Baltic

Sea marine isolates, in the freshwater subclade (Snow, Cunningham, Melvin & Kurath 1999b). The isolate proved pathogenic for rainbow trout with a cumulative mortality of 95% at day 22 (authors' unpublished results), similar to other freshwater VHSV isolates (Skall *et al.* 2004). Duplicate tanks with 50 fish in each were infected using the German turbot isolate 5927 at a concentration of 5×10^5 $TCID_{50}$ mL^{-1} water for 2 h. The freshwater isolate DK-3592B was used as a positive control and induced 96% cumulative mortality. No mortality was recorded in the negative control fish. The phylogenetic analysis and the results from the experimental infections strongly suggest this isolate to be of freshwater origin.

The outbreaks in Scottish and Irish turbot farms may be other examples of transfer of infection from wild to farmed fish. Both countries are free of VHS so infection through transfer of pathogens from already infected farmed fish is not likely. The Scottish farm used sea water pumped directly from the sea and frozen raw minced marine fish, mainly haddock, were used for feed (Munro 1996).

Both the Scottish (Stone, Way & Dixon 1997) and Irish turbot isolates fall into the marine group according to sequencing data (Snow *et al.* 1999b; Einer-Jensen *et al.* 2004). The pathogenicity of the Scottish and Irish turbot farm isolates to rainbow trout is very low as are other isolates originating from wild marine fish (Skall *et al.* 2004). It is therefore plausible that the farmed turbot in Scotland and Ireland became infected indirectly from wild fish.

Differences between European freshwater, European marine and North American VHSV

Serological analyses have been unable to distinguish the North American VHSV isolates from the typical European reference strains (Winton, Batts & Nishizawa 1989), nor has serological analysis been able to distinguish the North European marine isolates from the European freshwater isolates (authors' own observation).

It is not possible to assign VHSV isolates to the freshwater or marine groups using currently available monoclonal antibodies. Preliminary work at DFVF however, indicates that a panel of newly developed monoclonal antibodies may be able to differentiate between freshwater and non-Baltic marine isolates and Baltic marine isolates (N. Lorenzen, unpublished data). More isolates should be analysed before any final conclusions can be made regarding the efficacy of this assay.

Both the North American VHSV isolates and the northern European marine isolates are less pathogenic to rainbow trout than the European freshwater strains (Meyers *et al.* 1994; Follett *et al.* 1997; Skall *et al.* 2004).

Genetic characterization

DNA hybridization techniques made it possible to detect a nucleotide sequence of the N-gene of the Makah VHSV isolate that was absent in the European VHSV isolate 07–7. By using a DNA-probe complementary to this it was possible to distinguish the European freshwater isolates from the North American VHSV isolates (Batts, Arakawa, Bernard & Winton 1993).

Based on the knowledge that the North American Makah VHSV isolate appeared to have a unique 20 nucleotide sequence in close proximity to the N-gene, which was not present in the European VHSV isolates, a PCR technique was developed. By using a specific primer set for the amplification of this specific nucleotide fragment it was possible to separate the North American and European VHSV isolates within hours (Einer-Jensen, Olesen, Lorenzen & Jørgensen 1995).

Genetic comparison of four North American VHSV isolates recovered from chinook and coho salmon and one from Pacific cod, and four European isolates including one from cod, was made by using T1 ribonuclease fingerprinting. The result revealed two distinct groups comprising the North American and the European isolates (Oshima, Higman, Arakawa, de Kinkelin, Jørgensen, Meyers & Winton 1993). The results of these studies indicate that the North American VHSV isolates are not of European origin.

In order to detect nucleotide sequence variation within the nucleoprotein gene of 39 VHSV isolates of European marine origin by applying a ribonuclease (RNase) protection assay it was possible to define 10 different groups (Snow *et al.* 1999b). RNase protection assay is a fast method to initially characterize many isolates in a short time. However, it is not as discriminatory as sequencing.

Sequence comparisons of many VHSV isolates by several laboratories have shown that genetic differences appear to be more related to geographic

location than to year of isolation or host species (Bernard, Bremont & Winton 1992; Benmansour, Paubert, Bernard & de Kinkelin 1994; Basurco, Vende, Monnier, Winton, de Kinkelin & Benmansour 1995; Benmansour, Basurco, Monnier, Vende, Winton & de Kinkelin 1997; Stone *et al.* 1997; Snow *et al.* 1999b; Thiéry, de Boisséson, Jeffroy, Castric, de Kinkelin & Benmansour 2002; Einer-Jensen *et al.* 2004; Snow, Bain, Black, Taupin, Cunningham, King, Skall & Raynard 2004). Four major genotypes have been identified, based on sequencing of the N-gene (Snow *et al.* 1999b):

Genotype I: European freshwater VHSV isolates and a group of marine isolates from the Baltic Sea;
Genotype II: A group of marine isolates from the Baltic Sea;
Genotype III: Isolates from the North Sea, Skagerrak and Kattegat;
Genotype IV: North American isolates.

Phylogenetic analysis, based on sequencing of the G-gene, supported the four major genotypes (Einer-Jensen *et al.* 2004) broadening genotype I to include not just marine isolates from the Baltic Sea but also from the Kattegat, Skagerrak, the North Sea and the English Channel. This was confirmed by sequencing of the N-gene (Snow *et al.* 2004).

As one of these groups comprised VHSV isolated from wild marine fish as well as isolates causing mortality in rainbow trout from continental Europe, a relationship between freshwater and marine types was suggested (Snow *et al.* 1999b), concurring with the results of Stone *et al.* (1997) and Betts & Stone (2000).

All the Japanese isolates but one fall into the North American genotype. The remaining isolate falls into the traditional European genogroup (Nishizawa, Iida, Takano, Isshiki, Nakajima & Muroga 2002) and is considered to have been introduced from outside Japan.

Restriction fragment length polymorphism (RFLP) is a promising method for genetic differentiation of VHSV isolates (Einer-Jensen, Winton & Lorenzen 2005; K. Einer-Jensen, personal communication). This molecular typing system is based on enzymatic digestion of RT-PCR-amplified G-gene products and subsequent grouping of the obtained restriction patterns. The RFLP method has been developed on the basis of sequence data from more than 60 isolates, representing a broad geographical range. The method has allowed grouping of VHS virus isolates into the major geographical areas given above as well as detailed geno-subgrouping according to the phylogenetic analysis. It is not possible to assign virulence of an isolate using this method.

The origin of VHSV

Several authors have discussed the origin of VHSV (Meyers & Winton 1995; Stone *et al.* 1997; Dixon 1999; Einer-Jensen *et al.* 2004; Snow *et al.* 2004). They conclude that it is likely that VHSV had its origin in the marine environment and may constitute a potential risk for mariculture. It may have been transferred to the freshwater environment by marine fish species, e.g. herring, sprat, sand eel, which are still used as fresh fish feed in some countries. After introduction to the freshwater environment VHSV may, through mutations, have reached the high level of virulence against rainbow trout which is characteristic for the freshwater types of VHSV. Only a limited number of amino acid residues may be involved in the determination of VHSV virulence for salmonids and this further highlights the potential risk that marine strains may pose to freshwater aquaculture (Betts & Stone 2000). An increase in virulence of non-pathogenic marine isolates into rainbow trout pathogenic isolates has never been proved experimentally but is hypothetically possible. Snow & Cunningham (2000) observed an increase in virulence of the farmed turbot isolate 860/94 following five passages in rainbow trout, though this increased virulence was not accompanied by a difference in the consensus sequence in the glycoprotein.

The genetic similarities between VHSV with different neutralization patterns (Olesen *et al.* 1993) are high and only a few amino acid substitutions can change the neutralization pattern from type I to III (determined by sequencing of isolates after neutralization inhibition tests, i.e. culturing type I isolates in the presence of type I neutralizing antibodies can change the neutralization pattern from I to III; N. Lorenzen, personal communication). Bearzotti, Monnier, Vende, Grosclaude, de Kinkelin & Benmansour (1995) similarly reported that a single point mutation in the G-gene was able to transform a VHSV isolate usually neutralizable by a monoclonal antibody, into a non-neutralizable form.

Given the examples from Finland, Sweden and UK, and the sequencing work performed by different research teams, there is circumstantial evidence that support the hypothesis that non-virulent VHSV isolates can become virulent. Recombinant technology may provide a definitive answer as it has been demonstrated for infectious haematopoietic necrosis virus (IHNV), a virus related to VHSV, that NV-knockout recombinant IHNV does not induce mortality in trout (Thoulouze, Bouguyon, Carpentier & Brémont 2004).

In order to eradicate VHS in freshwater farms, an eradication programme was implemented in Denmark in 1965 (Jørgensen 1992; Olesen 1998). The programme has resulted in a dramatic reduction in the number of infected farms from approximately 400 to 30 by 2004. At the end of 1999 the number of VHSV-infected farms in Denmark was as low as seven. Unfortunately, it has been difficult to eradicate the virus from the remaining infected farms due to relapse of infection after eradication, and thus the number of infected farms has risen again. It was noteworthy that during the eradication period, the number of infected farms levelled out at approximately 100 from 1975 to 1983 (Fig. 2). At that time fresh fish were still used as feed for rainbow trout in Denmark. When that practice was prohibited in 1985 the number of infected farms declined further (Olesen 1998). Thus one of the reasons for the successful control of VHS in Denmark might be that a constant pressure of infection was stopped in the 1980s by using pelleted feed instead of fresh minced marine fish. In relation to these observations, it is important to keep in mind that it is still common practice in some countries to feed farmed fish, especially marine species, with fresh fish.

Phylogenetic studies indicate that VHSV may have been present in marine fish species in Europe for centuries (Benmansour *et al.* 1997) and these authors suggested that the genogroups became separated a long time before fish farming was established in Europe and North America. However, no isolates from wild marine fish were included in this study. Work on molecular clocks supports this hypothesis and showed that a marine clock without positive selection and a freshwater clock with positive selection exist (Einer-Jensen *et al.* 2004). It is estimated that the North American and European VHSV types diverged around 1500, and that the European freshwater and marine isolates diverged around 1950 (Einer-Jensen *et al.* 2004). This validates the statement by Benmansour *et al.* (1994) that the European and North American VHSV strains derive from a common ancestor. Even though a disease with clinical signs compatible with VHSV was observed as early as the 1930s a divergence around 1950 may very well be correct. The early reports of VHS-like diseases may have different causes. In Denmark, a disease with signs

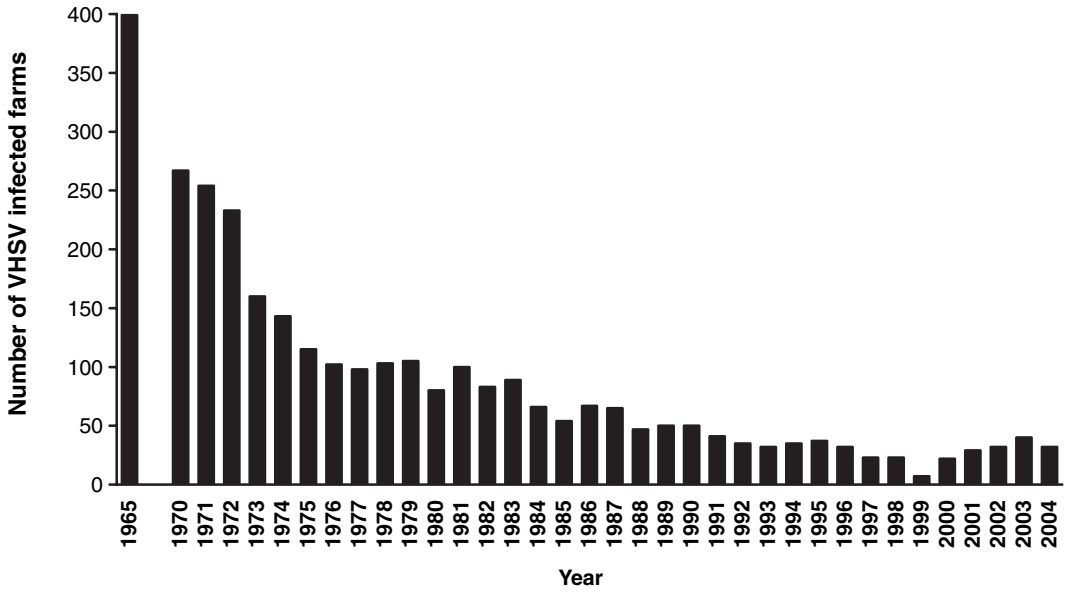

Figure 2 Number of VHSV-infected rainbow trout farms in Denmark since 1965, when the VHSV eradication programme started.

similar to VHS was observed for the first time at the beginning of the 1950s (Rasmussen 1965). Schäperclaus visited Denmark in 1953 in order to assist Danish fish farmers in elucidating the aetiological background for this disease. During the 4 years prior to this it had become common practice to feed rainbow trout with minced herring (Schäperclaus 1954). Schäperclaus noted that '…the correlation that feeding with herring may have for the disease, does not mean that the virus is present in the herring, and can be transferred at feeding'. Though not believed at that time, this may have been the case.

What threat does marine VHSV pose for fish farming?

The knowledge that VHSV is enzootic in the marine environment should not lead to decisions to abandon control of VHS in the EC. Although VHSV has apparently been present for a very long time in North European marine waters, the UK and Ireland have never experienced the disease in farmed rainbow trout. The few outbreaks in Norway in the 1960s and 1970s may be attributed to importation of infected fish or eyed eggs (presumably not disinfected) (Håstein, Holt & Krogsrud 1968; T. Håstein, Norway, personal communication). In Denmark, it has also been possible to maintain and expand VHS-free zones despite the fact that some river systems were heavily infected. This leads to the conclusion that VHS is primarily spread by transport of infected farmed fish and that the existing trade regulations and surveillance programmes are successful in maintaining freedom of VHS in approved free zones. However, it must not be forgotten that VHSV infection from the marine environment is a constant threat to the control programmes and that the latter must be organized accordingly, i.e. by introducing regulations minimizing the risk of transfer between wild marine fish and farmed fish in sea water and fresh water. Diadromous fish migrating upstream are also a potential risk for transfer of VHSV from the marine to the freshwater environment. In France, VHSV was isolated from an eel caught in the River Loire estuary (Castric, Jeffroy, Bearzotti & de Kinkelin 1992). This isolate belonged to the genogroup comprising marine isolates (Thiéry *et al.* 2002).

The use of fresh fish as feed in farms in EU-approved zones/VHS-free areas should be prohibited. Such a prohibition should not pose any problems for most farms except for some newly introduced fish species for which fresh fish or live feed from sea water is the only alternative. In such cases dispensation may be granted on condition that rainbow trout are not co-cultivated.

Co-cultivation of flatfish and rainbow trout in mariculture in EU-approved zones/VHS-free areas should be avoided. The co-cultivation of salmonids and flatfish, e.g. rainbow trout and turbot, poses an increased risk for rainbow trout to contract marine VHSV, which might become virulent. In view of the considerable efforts which are put into the domestication of wild marine fish species for aquaculture purposes the protection of already existing aquaculture species should be kept in mind.

The introduction of farmed fish from sea water into fresh water (except for non-susceptible species) is not recommended. Once cultured salmonids have been exposed to sea water in North Europe they are at risk of contracting VHSV. Therefore, the transfer of susceptible fish back to fresh water should be prohibited in approved VHS-free zones/areas. This requirement will pose some problems for rainbow trout broodstock produced in sea water which is common in some countries, e.g. Finland. In these cases production should be modified so that fish are stripped in sea water and only fertilized eggs are transferred to freshwater conditions after proper disinfection.

Approved status of freedom of coastal zones from VHS should not be lifted as a consequence of finding VHS in wild marine fish, as free trade between coastal areas and non-approved continental areas could lead to an unacceptable spread of VHS in the EC. The major cause of VHS infection is still transfer of infected fish. Likewise, free trade, should not be accepted from approved coastal to approved continental zones, for the reasons given above. Therefore, approved coastal zones, already divided from continental zones in the present EU legislation, should have an intermediate status between approved continental zones and non-approved zones.

In recent years, the development of specific diagnostic techniques to discriminate between rainbow trout pathogenic VHSV isolates and isolates of marine origin that do not cause clinical infection in rainbow trout (but are pathogenic, e.g. turbot) have been attempted. According to present EC legislation, the finding of marine VHSV in farmed marine fish species in approved coastal and

continental zones leads to withdrawal of the approved status. Such measures are not appropriate and spoil the considerable efforts invested in achieving and maintaining approval. It is therefore necessary to discriminate between the two types of virus, and to accept the presence of marine VHSV-infected non-salmonid fish even in the approved zones of the EC. At present, marine VHSV can only be differentiated from freshwater isolates by experimental infection studies in rainbow trout (Skall *et al.* 2004). However, if marine VHSV in the future is treated differently from freshwater VHSV, an easily applicable discriminatory test needs to be developed. Such tests discriminating between the marine and freshwater isolates are under development. If the tests are found applicable they may justify a difference in the legislative treatment of marine and freshwater VHS.

Acknowledgement

The research network SCOFDA (Sustainable Control of Fish Diseases in Aquaculture) is thanked for financial support.

References

Ahne W. & Jørgensen P.E.V. (1993) Prevalence of neutralising antibodies to IHNV and VHSV in free-living and cultured rainbow trout in Germany. *Bulletin of the European Association of Fish Pathologists* **13,** 7–9.

Ahne W. & Thomsen I. (1985) Occurrence of VHS virus in wild white fish (*Coregonus* sp.). *Zentralblatt fur Veterinarmedizin* **32,** 73–75.

Ahne W., Negele R.D. & Ollenschläger B. (1976) Vergleichende Infektionsversuche mit Egtved-Viren (Stamm F1) bei Regenbogenforellen (*Salmo gairdneri*) und Goldforellen (*Salmo aguabonita*). *Berliner und Münchener Tierärztliche Wochenschrift* **89,** 161–164.

Amos K. & Olivier G. (2001) Disease interactions between wild and cultured fish – east meets west in Dublin. *10th International Conference of the EAFP on Diseases of Fish and Shellfish*, Dublin, Eire, Abstract Book, O-081, 9–14 September, 2001.

Amos K. & Thomas J. (2002) Disease interactions between wild and cultured fish: observations and lessons learned in the Pacific Northwest. *Bulletin of the European Association of Fish Pathologists* **22,** 95–102.

Anonymous (1991) Council Directive 91/67/EEC of 28 January 1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products. *Official Journal of the European Communities* **L046,** 0001–0018.

Anonymous (2001a) 2001/183/EC: Commission Decision of 22 February 2001 laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases and repealing Decision 92/532/EEC. *Official Journal of the European Communities* **L067,** 0065–0076.

Anonymous (2001b) *Report of the Working Group on Pathology and Diseases of Marine Organisms*. Santiago de Compostela, 13–17 March 2001, ICES CM2001/F:02.

Ariel E. & Olesen N.J. (2002) Finfish in aquaculture and their diseases – a retrospective view on the European Community. *Bulletin of the European Association of Fish Pathologists* **22,** 72–85.

Bandín I., López-Vázquez C., Cutrín J.M., Brey I.R., Dopazo C.P. & Barja J.L. (1999) VHS in wild Greenland halibut. *9th International Conference of the EAFP on Diseases of Fish and Shellfish*, Rhodes, Greece, Abstract P-175, 19–24 September, 1999.

Basurco B., Vende P., Monnier A.F., Winton J.R., de Kinkelin P. & Benmansour A. (1995) Genetic diversity and phylogenetic classification of viral hemorrhagic septicemia virus (VHSV). *Veterinary Research* **26,** 460–463.

Batts B. & Winton J. (2002) Expanded host and geographic ranges for North American VHSV: genetic analysis of new isolates. *Fifth International Symposium for Viruses of Lower Vertebrates*, Seattle, Washington, Abstract Book, P-43, 27–30 August, 2002.

Batts W.N., Arakawa C.K., Bernard J. & Winton J.R. (1993) Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. *Diseases of Aquatic Organisms* **17,** 67–71.

Bearzotti M., Monnier A.F., Vende P., Grosclaude J., de Kinkelin P. & Benmansour A. (1995) The glycoprotein of viral hemorrhagic septicemia virus (VHSV): antigenicity and role in virulence. *Veterinary Research* **26,** 413–422.

Benmansour A., Paubert G., Bernard J. & de Kinkelin P. (1994) The polymerase-associated protein (M1) and the matrix protein (M2) from a virulent and an avirulent strain of viral hemorrhagic septicemia virus (VHSV), a fish rhabdovirus. *Virology* **198,** 602–612.

Benmansour A., Basurco B., Monnier A.F., Vende P., Winton J.R. & 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. *Journal of General Virology* **78,** 2837–2846.

Bernard J., Bremont M. & Winton J. (1992) Nucleocapsid gene sequence of a North American isolate of viral haemorrhagic septicaemia virus, a fish rhabdovirus. *Journal of General Virology* **73,** 1011–1014.

Besse P. (1955) Recherche sur l'étiologie de l'anèmie infectieuse de la Truite. *Bulletin de l'Acadèmie Vétérinaire de France* **5,** 194–198.

Betts A.M. & Stone D.M. (2000) Nucleotide sequence analysis of the entire coding regions of virulent and avirulent strains of viral haemorrhagic septicaemia virus. *Virus Genes* **20,** 259–262.

Bovo G., Zanin E. & Giorgetti G. (1982) Grave episodio di setticemia emorragica virale (SEV) in avannotti di trota fario (*Salmo trutta*) d'allevamento. *Atti della Societá Italiana delle Scienze Veterinarie* **36,** 631–634.

Bowden T.J. (2003) A study of the susceptibility of Atlantic halibut, *Hippoglossus hippoglossus* (L.), to viral haemorrhagic

septicaemia virus isolated from turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* **26,** 207–212.

Brudeseth B.E. & Evensen Ø. (2002) Occurrence of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish species in the coastal regions of Norway. *Diseases of Aquatic Organisms* **52,** 21–28.

Brudeseth B.E., Castric J. & Evensen Ø. (2002) Studies on pathogenesis following single and double infection with viral hemorrhagic septicemia virus and infectious hematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*). *Veterinary Pathology* **39,** 180–189.

Brunson R., True K. & Yancey J. (1989) VHS virus isolated at Makah National Fish Hatchery. *American Fisheries Society Fish Health Section Newsletter* **17,** 3–4.

Carls M.G., Marty G.D., Meyers T.R., Thomas R.E. & Rice S.D. (1998) Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. *Canadian Journal of Fisheries and Aquatic Sciences* **55,** 2300–2309.

Castric J. & de Kinkelin P. (1980) Occurrence of viral haemorrhagic septicaemia in rainbow trout *Salmo gairdneri* Richardson reared in sea-water. *Journal of Fish Diseases* **3,** 21–27.

Castric J. & de Kinkelin P. (1984) Experimental study of the susceptibility of two marine fish species, sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*), to viral haemorrhagic septicaemia. *Aquaculture* **41,** 203–212.

Castric J., Jeffroy J., Bearzotti M. & de Kinkelin P. (1992) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild elvers *Anguilla anguilla*. *Bulletin of the European Association of Fish Pathologists* **12,** 21–23.

Cox B. & Hedrick R. (2001) VHS virus in Pacific sardines and mackerel. *American Fisheries Society Fish Health Section Newsletter* **29,** 6.

Dixon P.F. (1999) VHSV came from the marine environment: clues from the literature, or just red herrings? *Bulletin of the European Association of Fish Pathologists* **19,** 60–65.

Dixon P.F. & Avery S. (1998) Susceptibility of cultivated fish species to viral haemorrhagic septicaemia virus isolates from marine hosts. Weymouth, UK, Poster presented at the *Fourth International Symposium on Viruses of Lower Vertebrates*, 12–15 May 1998.

Dixon P.F., Feist S., Kehoe E., Parry L., Stone D.M. & Way K. (1997) Isolation of viral haemorrhagic septicaemia virus from Atlantic herring *Clupea harengus* from the English Channel. *Diseases of Aquatic Organisms* **30,** 81–89.

Dixon P.F., Avery S., Chambers E., Feist S., Mandhar H., Parry L., Stone D.M., Strømmen H.K., Thurlow J.K., Tsin-yee Lui C. & Way K. (2003) Four years of monitoring for viral haemorrhagic septicaemia virus in marine waters around the United Kingdom. *Diseases of Aquatic Organisms* **54,** 175–186.

Dopazo C.P., Bandín I., López-Vazquez C., Lamas J., Noya M. & Barja J.L. (2002) Isolation of viral hemorrhagic septicemia virus from Greenland halibut *Reinhardtius hippoglossoides* caught at the Flemish Cap. *Diseases of Aquatic Organisms* **50,** 171–179.

Dorson M., Chevassus B. & Torhy C. (1991) Comparative susceptibility of three species of char and of rainbow trout × char triploid hybrids to several pathogenic salmonid viruses. *Diseases of Aquatic Organisms* **11,** 217–224.

Einer-Jensen K., Olesen N.J., Lorenzen N. & Jørgensen P.E.V. (1995) Use of the polymerase chain reaction (PCR) to differentiate serologically similar viral haemorrhagic septicaemia (VHS) virus isolates from Europe and America. *Veterinary Research* **26,** 464–469.

Einer-Jensen K., Winton J. & Lorenzen N. (2005) Genotyping of the fish rhabdovirus, viral haemorrhagic septicaemia virus by restriction fragment length polymorphism. *Veterinary Microbiology* **106,** 167–178.

Einer-Jensen K., Ahrens P., Forsberg R. & Lorenzen N. (2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* **85,** 1167–1179.

Enzmann P.-J., Konrad M., Parey K. & Wetzlar H. (1987) Natürliches wirtsspektrum des virus der viralen hämorrhagischen septikämie der regenbogenforelle. *Tierarztliche Umschau* **42,** 228–230.

Enzmann P.J., Konrad M. & Rapp J. (1992) Epizootiological studies on viral haemorrhagic septicaemia in brown trout *Salmo trutta fario*. *Diseases of Aquatic Organisms* **12,** 143–146.

Enzmann P.-J., Konrad M. & Parey K. (1993) VHS in wild living fish and experimental transmission of the virus. *Fisheries Research* **17,** 153–161.

Follett J.E., Meyers T.R., Burton T.O. & Geesin J.L. (1997) Comparative susceptibilities of salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). *Journal of Aquatic Animal Health* **9,** 34–40.

Håstein T., Holt G. & Krogsrud J. (1968) Hemorrhagisk virusseptikemi (Egtvedsyke) hos regnbueørret i Norge. *Nordisk Veterinærmedicin* **20,** 708–711.

Hørlyck V., Mellergaard S. & Jørgensen P.E.V. (1984) Occurrence of VHS in Danish maricultured rainbow trout. *Bulletin of the European Association of Fish Pathologists* **4,** 11–13.

Hedrick R.P., Batts W.N., Yun S., Traxler G.S., Kaufman J. & Winton J.R. (2003) Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. *Diseases of Aquatic Organisms* **55,** 211–220.

Hershberger P.K., Kocan R.M., Elder N.E., Meyers T.R. & Winton J.R. (1999) Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Prince William Sound, Alaska, USA. *Diseases of Aquatic Organisms* **37,** 23–31.

Hill B. (1992) Impact of viral diseases on salmonid fish in Europe. In: *Proceedings of the OJI International Symposium on Salmonid Diseases* (ed. by T. Kimura), Hokkaido University Press, Sapporo, Japan.

Hopper K. (1989) The isolation of VHSV from chinook salmon at Glenwood Springs, Orcas Island, Washington. *American Fisheries Society Fish Health Section Newsletter* **17,** 1.

Husu-Kallio J. & Suokko M. (2000) *Two Outbreaks of VHS (Viral Hemorrhagic Septicaemia) in Finland*. Report for the Standing Veterinary Committee, Ministry of Agriculture and Forestry, Veterinary and Food Department.

Iida H., Mori K.-I., Nishizawa T., Arimoto M. & Muroga K. (2003) Fate of viral hemorrhagic septicemia virus in Japanese flounder *Paralichthys olivaceus* challenged by immersion. *Fish Pathology* **38,** 87–91.

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. *Diseases of Aquatic Organisms* **47,** 87–99.

Isshiki T., Nagano T. & Miyazaki T. (2002) Effect of water temperature on pathological states of Japanese flounder experimentally infected with haemorrhagic septicemia virus, a flounder isolate KRRV-9601. *Fish Pathology* **37,** 95–97.

Isshiki T., Nagano T. & Miyazaki T. (2003) Susceptibility of various marine fish species to viral hemorrhagic septicemia virus isolated from Japanese flounder. *Fish Pathology* **38,** 113–115.

Ito T., Mori K.-I., Arimoto M. & Nakajima K. (2004) Virulence of viral hemorrhagic septicemia virus (VHSV) isolates from Japanese flounder *Paralichthys olivaceus* in rainbow trout and several species of marine fish. *Fish Pathology* **39,** 103–104.

Jørgensen P.E.V. (1980) Egtved virus: the susceptibility of brown trout and rainbow trout to eight virus isolates and the significance of the findings for the VHS control. In: *Fish Diseases. Third COPRAQ-Session* (ed. by W. Ahne), pp. 1–7. Springer-Verlag, Berlin, Heidelberg.

Jørgensen P.E.V. (1982) Egtved virus: occurrence of inapparent infections with virulent virus in free-living rainbow trout, *Salmo gairdneri* Richardson, at low temperature. *Journal of Fish Diseases* **5,** 251–255.

Jørgensen P.E.V. (1992) Recent advances in surveillance and control of viral haemorrhagic septicaemia (VHS) of trout. In: *Proceedings of the OJI International Symposium on Salmonid Diseases* (ed. by T. Kimura), pp. 60–71. Hokkaido University Press, Sapporo, Japan.

Jørgensen P.E.V. & Olesen N.J. (1987) Cod ulcus syndrome rhabdovirus is indistinguishable from the Egtved (VHS) virus. *Bulletin of the European Association of Fish Pathologists* **7,** 73–74.

Jensen M.H. (1963) Preparation of fish tissue cultures for virus research. *Bulletin de l'Office International des Epizooties* **59,** 131–134.

Jensen M.H. (1965) Research on the virus of Egtved disease. *Annals of the New York Academy of Sciences* **126,** 422–426.

Jensen N.J. & Larsen J.L. (1979) The ulcus-syndrome in cod (*Gadus morhua*) I. A pathological and histopathological study. *Nordisk Veterinærmedicin* **31,** 222–228.

Jensen N.J. & Larsen J.L. (1982) The ulcus-syndrome in cod (*Gadus morhua*) IV. Transmission experiments with two viruses isolated from cod and *Vibrio anguillarum*. *Nordisk Veterinærmedicin* **34,** 136–142.

Jensen N.J., Bloch B. & Larsen J.L. (1979) The ulcus-syndrome in cod (*Gadus morhua*) III. A preliminary virological report. *Nordisk Veterinærmedicin* **31,** 436–442.

Jimenez de la Fuente J., Marcotegui M.A., San Juan M.L. & Basurco B. (1988) Diagnosis of viral diseases in salmonid farms in Spain. *Bulletin of the European Association of Fish Pathologists* **8,** 1–2.

Kaufman J. & Holt R.A. (2001) Isolation of North American viral hemorrhagic septicemia virus (VHSV) from Columbia River smelt (*Thaleichthys pacificus*). *American Fisheries Society Fish Health Section Newsletter* **29,** 1–3.

Kent M.L., Traxler G.S., Kieser D., Richard J., Dawe S.C., Shaw R.W., Prosperiporta G., Ketcheson J. & Evelyn T.P.T. (1998) Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. *Journal of Aquatic Animal Health* **10,** 211–219.

Kim S.-U., Lee J.-I., Hong M.-J., Park H.-S. & Park S.-I. (2003) Genetic relationship of the VHSV (viral hemorrhagic septicemia virus) isolated from cultured olive flounder, *Paralichthys olivaceus* in Korea. *Fish Pathology* **16,** 1–12.

King J.A., Snow M., Skall H.F. & Raynard R.S. (2001a) Experimental susceptibility of Atlantic salmon *Salmo salar* and turbot *Scopththalmus maximus* to European freshwater and marine isolates of viral haemorrhagic septicaemia virus. *Diseases of Aquatic Organisms* **47,** 25–31.

King J.A., Snow M., Smail D.A. & Raynard R.S. (2001b) Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, North East Atlantic Ocean and Irish Sea. *Diseases of Aquatic Organisms* **47,** 81–86.

de Kinkelin P. & le Berre M. (1977) Isolement d'un Rhabdovirus pathogéne de la Truite Fario (*Salmo trutta* L., 1766). *Comptes Rendus de l'Academie des Sciences, Paris* **284,** 101–104.

de Kinkelin P. & Castric J. (1982) An experimental study of the susceptibility of Atlantic salmon fry, *Salmo salar* L., to viral haemorrhagic septicaemia. *Journal of Fish Diseases* **5,** 57–65.

de Kinkelin P., Daniel P., Hattenberger-Baudouy A.M. & Benmansour A. (1999) The large-mouth bass (*Micropterus salmoides*): a novel host for viral haemorrhagic septicaemia virus (VHSV). *9th International Conference of the EAFP on Diseases of Fish and Shellfish*, Rhodes, Greece, Abstract Book, P-174, 19–24 September, 1999.

Kocan R.M., Landolt M.L. & Winton J.R. (1996) Laboratory challenge of pacific herring with and without stressors. Section II in: Marty G.D., Hinton D.E., Kocan R.M., Landolt M.L., Winton J.R., Kennedy C.J. & Farrell A.P. Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound. Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95320S), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska. 20 pages.

Kocan R., Bradley M., Elder N., Meyers T.R., Batts W.N. & Winton J.R. (1997) North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. *Journal of Aquatic Animal Health* **9,** 279–290.

Kocan R.M., Hershberger P.K., Elder N.E. & Winton J.R. (2001) Epidemiology of viral hemorrhagic septicemia among juvenile Pacific herring and Pacific sand lances in Puget Sound, Washington. *Journal of Aquatic Animal Health* **13,** 77–85.

Larsen J.L. & Jensen N.J. (1988) Dear Editor. *Bulletin of the European Association of Fish Pathologists* **8,** 93.

López-Vázquez C., Bain N., Olveira J.G., Snow M., Raynard R.S., Barja J.L. & Dopazo C.P. (2003) Characterization of VHSV isolates from Iberian origin and from the Flemish Cap by sequencing. *11th International Conference of the EAFP on Diseases of Fish and Shellfish*, St. Julians, Malta, Abstract Book, P-138, 21–26 September, 2002.

Lorenzen N., Olesen N.J. & Jørgensen P.E.V. (1993) Antibody response to VHS virus proteins in rainbow trout. *Fish and Shellfish Immunology* **3,** 461–473.

Lorenzen E., Carstensen B. & Olesen N.J. (1999) Inter-laboratory comparison of cell lines for susceptibility to three viruses: VHSV, IHNV and IPNV. *Diseases of Aquatic Organisms* **37,** 81–88.

Marty G.D., Freiberg E.F., Meyers T.R., Wilcock J., Farver T.B. & Hinton D.E. (1998) Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. *Diseases of Aquatic Organisms* **32,** 15–40.

Meier W. & Jørgensen P.E.V. (1979) Egtved virus: characteristics of a virus strain isolated from pike fry (*Esox lucius* L.). *Nordisk Veterinærmedicin* **31,** 484–485.

Meier W., Ahne W. & Jørgensen P.E.V. (1986) Fish viruses: viral haemorrhagic septicaemia in white fish (*Coregonus* sp.). *Journal of Applied Ichthyology* **2,** 181–186.

Meyers T.R. & Winton J.R. (1995) Viral hemorrhagic septicemia virus in North America. *Annual Review of Fish Diseases* **5,** 3–24.

Meyers T.R., Sullivan J., Emmenegger E., Follett J., Short S., Batts W.N. & Winton J.R. (1992) Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, USA. *Diseases of Aquatic Organisms* **12,** 167–175.

Meyers T.R., Short S., Lipson K., Batts W.N., Winton J.R., Wilcock J. & Brown E. (1994) Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. *Diseases of Aquatic Organisms* **19,** 27–37.

Meyers T.R., Short S. & Lipson K. (1999) Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fish. *Diseases of Aquatic Organisms* **38,** 81–86.

Mori K.-I., Iida H., Nishizawa T., Arimoto M., Nakajima K. & Muroga K. (2002) Properties of viral hemorrhagic septicemia virus (VHSV) isolated from Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* **37,** 169–174.

Mortensen H.F., Heuer O.E., Lorenzen N., Otte L. & Olesen N.J. (1999a) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Research* **63,** 95–106.

Mortensen H.F., Olesen N.J. & Mellergaard S. (1999b) Isolation of viral haemorrhagic septicaemia virus (VHSV) in the marine environment. *9th International Conference of the EAFP on Diseases of Fish and Shellfish*, Rhodes, Greece, Abstract Book, O-012, 19–24 September, 1999.

Munro A.L.S. (1996) Report of the first recorded outbreak of viral haemorrhagic septicaemia (VHS) in GB and subsequent actions to contain, eradicate and investigate the origins of the infection. *Scottish Aquaculture Research Report* **3,** 1–12.

Muroga K., Iida H., Nishizawa T. & Arimoto M. (2004) Experimental horizontal transmission of viral hemorrhagic septicemia virus (VHSV) in Japanese flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* **58,** 111–115.

Nishizawa T., Iida H., Takano R., Isshiki T., Nakajima K. & Muroga K. (2002) Genetic relatedness among Japanese, American and European isolates of viral haemorrhagic septicaemia virus (VHSV) based on partial G and P genes. *Diseases of Aquatic Organisms* **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.

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

Nylin B. & Olesen N.J. (2001) Egtvedsyge i tre dambrug. En beskrivelse af dødeligheden og de økonomiske konsekvenser af udbrud af Egvedsyge i vinteren 2000. *Ferskvandsfiskeribladet* **99,** 126–132.

Olesen N.J. (1998) Sanitation of viral haemorrhagic septicaemia (VHS). *Journal of Applied Ichthyology* **14,** 173–177.

Olesen N.J. & Jørgensen P.E.V. (1983) Egtvedsyge (VHS) i fritlevende regnbueørreder. *Ferskvandsfiskeribladet* **81,** 130–132.

Olesen N.J. & Vestergård Jørgensen P.E. (1992) Comparative susceptibility of three fish cell lines to Egtved virus, the virus of viral haemorrhagic septicaemia (VHS). *Diseases of Aquatic Organisms* **12,** 235–237.

Olesen N.J., Lorenzen N. & Jørgensen P.E.V. (1993) Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralizing monoclonal and polyclonal antibodies. *Diseases of Aquatic Organisms* **16,** 163–170.

Olivier G. (2002) Disease interactions between wild and cultured fish – perspectives from the American Northeast (Atlantic Provinces). *Bulletin of the European Association of Fish Pathologists* **22,** 103–109.

Ord W.M., Le Berre M. & de Kinkelin P. (1976) Viral haemorrhagic septicaemia: comparative susceptibility of rainbow trout (*Salmo gairdneri*) and hybrids (*S. gairdneri* × *Oncorhynchus kisutch*) to experimental infection. *Journal of the Fisheries Research Board of Canada* **33,** 1205–1208.

Oshima K.H., Higman K.H., Arakawa C.K., de Kinkelin P., Jørgensen P.E.V., Meyers T.R. & Winton J.R. (1993) Genetic comparison of viral hemorrhagic septicemia virus isolates from North America and Europe. *Diseases of Aquatic Organisms* **17,** 73–80.

Pliszka F. (1946) Anamia zarazliwa pstragow (Anemia infectiosa truttae). *Medycyna Weterynaryjna* **2,** 133–140.

Rasmussen C.J. (1965) A biological study of the Egtved disease (INUL). *Annals of the New York Academy of Sciences* **126,** 427–460.

Ross K., McCarthy U., Huntly P.J., Wood B.P., Stuart D., Rough E.I., Smail D.A. & Bruno D.W. (1994) An outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. *Bulletin of the European Association of Fish Pathologists* **14,** 213–214.

Schäperclaus W. (1938) Die schädigungen der deutschen fischerei durch fischparasiten und fischkrankheiten. *Fischerei-Zeitung* **22,** 1–20.

Schäperclaus W. (1954) Undersøgelse af sygdom hos ørrederne i danske ørreddambrug og forslag til bekæmpelse heraf. *Ferskvandsfiskeribladet* **52,** 145–149, 161–166.

Schlotfeldt H.-J., Ahne W., Jørgensen P.E.V. & Glende W. (1991) Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – a natural outbreak. *Bulletin of the European Association of Fish Pathologists* **11,** 105–107.

Skall H.F., King J., Brudeseth B., Mellergaard S. & Olesen N.J. (2002) Viral haemorrhagic septicaemia virus in the marine environment in Northern Europe. *Fifth International Symposium for Viruses of Lower Vertebrates*, Abstract Book, 11.

Skall H.F., Slierendrecht W.J., King J.A. & Olesen N.J. (2004) Experimental infection of rainbow trout *Oncorhynchus mykiss* with viral haemorrhagic septicaemia virus isolates from European marine and farmed fishes. *Diseases of Aquatic Organisms* **58,** 99–110.

Skall H.F., Olesen N.J. & Mellergaard S. (2005) Prevalence of viral haemorrhagic septicaemia virus in Danish marine fishes and its occurrence in new host species. *Diseases of Aquatic Organisms*, in press.

Smail D.A. (1995) *Isolation and Identification of Viral Haemorrhagic Septicaemia (VHS) Virus from North Sea Cod (Gadus morhua* L.). ICES CM 1995/F:15.

Smail D.A. (2000) Isolation and identification of viral haemorrhagic septicaemia (VHS) viruses from cod *Gadus morhua* with the ulcus syndrome and from haddock *Melanogrammus aeglefinus* having skin haemorrhages in the North Sea. *Diseases of Aquatic Organisms* **41,** 231–235.

Snow M. & Cunningham C.O. (2000) Virulence and nucleotide sequence analysis of marine viral haemorrhagic septicaemia virus following *in vivo* passage in rainbow trout *Onchorhynchus mykiss*. *Diseases of Aquatic Organisms* **42,** 17–26.

Snow M. & Smail D.A. (1999) Experimental susceptibility of turbot *Scophthalmus maximus* to viral haemorrhagic septicaemia virus isolated from cultivated turbot. *Diseases of Aquatic Organisms* **38,** 163–168.

Snow M., Bowden T.J. & Bricknell I.R. (1999a) Susceptibility of Atlantic halibut (*Hippoglossus hippoglossus*) to viral haemorrhagic septicaemia virus. *9th International Conference of the EAFP on Diseases of Fish and Shellfish*, Abstract Book, P-176.

Snow M., Cunningham C.O., Melvin W.T. & Kurath G. (1999b) Analysis of the nucleoprotein gene identifies distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. *Virus Research* **63,** 35–44.

Snow M., Cunningham C.O. & Bricknell I.R. (2000) Susceptibility of juvenile Atlantic cod *Gadus morhua* to viral haemorrhagic septicaemia virus isolated from wild-caught Atlantic cod. *Diseases of Aquatic Organisms* **41,** 225–229.

Snow M., Bain N., Black J., Taupin V., Cunningham C.O., King J.A., Skall H.F. & Raynard R.S. (2004) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* **61,** 11–21.

Stone D.M., Way K. & Dixon P.F. (1997) Nucloetide 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.). *Journal of General Virology* **78,** 1319–1326.

Takano R., Nishizawa T., Arimoto M. & Muroga K. (2000) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, *Paralichthys olivaceus*. *Bulletin of the European Association of Fish Pathologists* **20,** 186–192.

Takano R., Mori K., Nishizawa T., Arimoto M. & Muroga K. (2001) Isolation of viruses from wild Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* **36,** 153–160.

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 (1971–1999). *Diseases of Aquatic Organisms* **52,** 29–37.

Thoulouze M.-I., Bouguyon E., Carpentier C. & Brémont M. (2004) Essential role of the NV protein of *Novirhabdovirus* for pathogenicity in rainbow trout. *Journal of General Virology* **78,** 4098–4107.

Traxler G., Kieser D. & Evelyn T.P.T. (1995) Isolation of North American strain of VHS virus from farmed Atlantic salmon. In: *Aquaculture Update No. 72* (ed. by L. Margolis), Aquaculture Divison, Pacific Biological Station, Nanaimo, BC.

Traxler G.S., Kieser D. & Richard J. (1999) Mass mortality of pilchard and herring associated with viral hemorrhagic septicemia virus in British Columbia, Canada. *American Fisheries Society Fish Health Section Newsletter* **27,** 3–4.

Walker P.J., Benmansour A., Dietzgen R., Fang R.-X., Jackson A.O., Kurath G., Leong J.C., Nadin-Davies S., Tesh R.B. & Tordo N. (2000) Family Rhabdoviridae. In: *Virus Taxonomy. Classification and Nomenclature of Viruses* (ed. by M.H.V. Van Regenmortel, C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle & R.B. Wickner), pp. 563–583. Academic Press, San Diego, CA.

Watanabe L., Pakingking R.J., Iida H., Nishizawa T., Iida Y., Arimoto M. & Muroga K. (2002) Isolation of aquabirnavirus and viral hemorrhagic septicemia virus (VHSV) from wild marine fishes. *Fish Pathology* **37,** 189–191.

Winton J.R., Batts W.N. & Nishizawa T. (1989) Characterization of the first North American isolates of viral hemorrhagic septicemia virus. *American Fisheries Society Fish Health Section Newsletter* **17,** 2–3.

Winton J.R., Batts W.N., Deering R.E., Brunson R., Hopper K., Nishizawa T. & Stehr C. (1991) Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. *Proceedings of the Second International Symposium on Viruses of Lower Vertebrates*, 43–50.

Wizigmann G., Baath C. & Hoffmann R. (1980) Isolierung des Virus der viralen hamorrhagischen Septikamie (VHS) aus Regenbogenforellen-, Hecht- und Aschenbrut. *Zentralblatt fur Veterinarmedizin* **27B,** 79–81.

Wolf K. (1988) Viral hemorrhagic septicemia. In: *Fish Viruses and Fish Viral Diseases* (ed. by K. Wolf), pp. 217–249. Comstock Publishing Associates, Cornell University Press, Ithaca and London.

Received: 13 October 2003
Revision received: 14 June 2005
Accepted: 16 June 2005

