Vol. 47: 81–86, 2001 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published November 8

Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, north east Atlantic Ocean and Irish Sea

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ABSTRACT: A surveillance programme was initiated on the occurrence and distribution of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish. Six research cruises were undertaken in an 18 mo period during 1997 and 1998, covering the North Sea, the Atlantic waters off the north and west coasts of Scotland and the Irish Sea. A total of 19 293 fish were sampled from 23 different species including cod, haddock, Norway pout, herring and sprat. Individual fish lengths were recorded and the fish were checked for lesions, haemorrhaging and other signs of disease. Pools of organ samples were taken for virus assay. The majority of fish sampled did not display clinical signs indicative of viral haemorrhagic septicaemia. A small number of cod were found with skin lesions and haddock with skin haemorrhaging. Of the 2081 organ and skin sample pools collected, 21 tested positive for VHSV by tissue culture and enzyme-linked immunosorbent assay. Seventeen of the isolates originated from Norway pout *Trisopterus esmarkii*, one from cod *Gadus morhua* (skin lesion), one from herring *Clupea harengus*, one from whiting *Merlangius merlangus*, and one from a previously unreported host species, poor cod *Trisopterus minutus*.

KEY WORDS: Viral haemorrhagic septicaemia virus · VHSV · Wild marine fish · North Sea · Atlantic Ocean · Irish Sea

INTRODUCTION

The occurrence of viral haemorrhagic septicaemia virus (VHSV) in the marine environment of continental Europe is well established (Castric & de Kinkelin 1980, Hørlyck et al. 1984, Dixon 1999, Mortensen et al. 1999). Since the first isolation of VHSV from wild Atlantic cod, *Gadus morhua* (Jensen et al. 1979), VHSV has been isolated from a number of wild marine fish species including Atlantic cod (Smail 1995, 2000, Mortensen et al. 1999); Atlantic herring *Clupea harengus* (Dixon et al. 1997, Mortensen et al. 1999); haddock *Melanogramus aeglefinus* (Smail 2000); Norway pout *Trisopterus esmarkii*, sprat *Sprattus sprattus*, rockling *Rhinonemus cimbrius*, whiting *Merlangius merlangus*, blue whiting *Micromesistius poutassou*, and lesser argentine *Argentina sphyraena* (Mortensen et al. 1999). Most of the species that have been shown to harbour VHSV do not display evidence of disease pathology, although there have been isolations from skin lesions on cod (Meyers et al. 1992, Smail 1995, 2000, Mortensen et al. 1999), skin ulcers and subdermal haemorrhage on Pacific herring *Clupea harengus pallasi* (Meyers et al. 1994), and from haddock showing skin haemorrhages (Smail 2000).

VHSV is widespread in the Baltic Sea and of lower prevalence in the North Sea, Skagerrak and Kattegat (Mortensen et al. 1999). Small scale surveys of wild fish in coastal waters around the UK have resulted in isolations of VHSV from herring in the English Channel (Dixon et al. 1997) and cod and haddock from the North Sea, east of Shetland (Smail 1995, 2000).

The susceptibility of cultured marine fish species to VHSV has been highlighted following disease outbreaks in cultured turbot, *Scophthalmus maximus*, in Germany (Schlotfeldt et al. 1991), Scotland (Ross et al.

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1994) and Ireland (J. McArdle unpubl.). Links between isolates from wild fish caught in the North Sea and isolates causing mortality in turbot (Stone et al. 1997, Snow et al. 1999) have raised concerns over the potential impact of the marine reservoir of VHSV on cultured fish.

To further describe the occurrence of VHSV in wild fish populations around Scotland a programme was established to survey fish species of the North Sea and the coastal waters of Scotland for the occurrence of VHSV. This paper describes that survey, which aimed to identify VHSV carrier species and to investigate VHSV prevalence in these geographical areas.

METHODS

Marine fish sampling protocol. Six research cruises were undertaken during the project. The first cruise on FRV 'Scotia' (February and March 1997) was a demersal trawling survey of the grounds off the north and west coasts of Scotland; Cruise 2 (April 1997) was a groundfish survey in the North Sea; Cruise 3 (August 1997) was a demersal trawling survey of the North Sea; Cruise 4 (February and March 1998) was a demersal trawling survey of the grounds off the north and west coasts of Scotland; and Cruise 6 (August 1998) was a demersal trawling survey of the North Sea. Cruise 5 on FRV 'Clupea' (July 1998) was a dedicated survey to determine VHSV occurrence in fish stocks from the coastal waters off western Scotland.

During each cruise, hauls between 30 and 60 min in duration were carried out at specified positions in each statistical square charted for sampling. The gear used was Grande Ouverture Verticale (GOV) trawl (BT137) for Cruises 1, 2, 4 and 6; Aberdeen trawl (75 mm mesh) for Cruise 3; and Rockhopper trawl for Cruise 5.

Species of marine fish targeted for sampling included cod, haddock, whiting, Norway pout, herring and sprat. Cod were specifically targeted for sampling to record the occurrence of lesions or ulcers. For each haul, 30 fish of each species selected for sampling were graded by size and split into 3 groups of 10. Each fish was swabbed with 70% ethanol, the body cavity was opened and a fresh sterile scalpel was used to sample kidney, spleen, and heart or brain. The organs for each group of 10 fish were pooled (total tissue mass of approximately 1 g) and stored in liquid nitrogen. The dissecting areas were disinfected between each pool of 10 fish using 70% ethanol and gloves were changed. Individual fish lengths were recorded and the fish were checked for the presence of skin lesions and subdermal haemorrhage, which were noted along with any other gross pathologies. Lesion-positive fish were sampled for VHSV occurrence in both organs and skin.

Tissue testing protocol for rhabdovirus. The testing procedure for the monitoring of VHSV in the fish tissue samples followed the methods outlined in European Commission decision 96/240/EC (sampling and testing procedures for viral haemorrhagic septicaemia and infectious haematopoietic necrosis [IHN] monitoring). Briefly, the tissue samples were taken from liquid nitrogen storage, thawed rapidly and weighed. Each sample was then diluted 1:10 (w/v) in transport medium (Glasgow Minimum Essential Medium [Gibco] plus 10% calf serum [Sigma]) containing antibiotics and antimycotics (1 mg ml^{-1} gentamycin, 200 U ml^{-1} polymixin-B and 12.5 µg ml^{-1} fungizone [Gibco]); homogenised in a glass tube using a PTFE pestle attached to a motor; incubated at 4°C overnight and then centrifuged at 1000 × *g* for 20 min at 4°C. BF-2 cells were seeded onto 24-well tissue culture plates (Nunc) to reach a confluency of 60 to 70% after 24 h incubation at 23°C. These were then inoculated with 100 µl of the fish tissue supernatants at dilutions of 1:100, 1:1000 and 1:10 000. The inoculated plates were incubated at 15°C and monitored for signs of viral growth and replication (cytopathic effect [CPE]) and read after 7 d. If no CPE had developed after the primary incubation, pooled supernatants from the primary culture were sub-cultivated onto homologous cell cultures, undiluted and diluted 1:10, and again incubated at 15°C and monitored for viral activity for a further 7 to 10 d.

The detection limit of this assay, assuming the presence of 1 VHSV infected fish in a pool of 10, would be approximately 10 000 infectious particles per gram of tissue in the infected fish.

Cell cultures showing evidence of CPE were tested by enzyme-linked immunosorbent assay using a commercial diagnostic kit, as per the manufacturer's instructions (Test-Line Limited Clinical Diagnostics, Brno, Czech Republic), to confirm identification of virus as VHSV. Medium from each CPE-positive well was centrifuged at 30 000 × *g* for 10 min at 4°C to remove cell debris. The resulting supernatant was added to a microtitre plate precoated with purified polyclonal anti-VHSV antibody. Positive and negative controls and blanks supplied in the kit were also added. VHSV was detected by the binding of rabbit anti-VHSV antibody, followed by peroxidase labelled antibody to rabbit immunoglobulin and finally the chromogenic substrate tetramethyl-benzidine.

RESULTS

The fish species and the total numbers sampled during each of the 6 cruises are summarised in Table 1. The predominant species were haddock, herring, whiting, Norway pout and sprat.

Table 1. Numbers of marine fish species sampled (F) and numbers of tissue pools tested for rhabdovirus (P) with resulting VHSV isolations (V) for each of the 6 research cruises, with total numbers for individual fish species for all 6 cruises and total numbers of all fish species for individual cruises

Fish species	Cruise number																		Total no. for individual fish species		
	1			2			3			4			5			6					
	F	P	V	F	P	V	F	P	V	F	P	V	F	P	V	F	P	V	F	P	V
Cod *Gadus morhua*	3	6	0	95	31	0	39	14	0	208	34	1	1	2	0	160	16	0	506	103	1
Poor cod *Trisopterus minutus*										480	48	1	376	38	0	30	3	0	886	89	1
Haddock *Melanogrammus aeglefinus*	210	21	0	296	35	0	869	89	0	1036	105	0	392	40	0	1979	198	0	4782	488	0
Norway pout *Trisopterus esmarkii*							120	12	0	830	83	6	535	54	0	1050	105	11	2535	254	17
Whiting *Merlangius merlangus*	180	20	0	241	26	0	30	3	0	1058	106	0	684	69	0	1231	126	1	3424	350	1
Blue whiting *Micromesistius poutassou*										82	8	0	155	16	0	60	6	0	297	30	0
Herring *Clupea harengus*	270	27	0	930	96	0	476	48	0	856	86	0	200	20	0	979	98	1	3711	375	1
Sprat *Sprattus sprattus*	210	21	0	570	63	0	30	3	0	684	68	0	150	15	0	60	6	0	1704	176	0
Hake *Merluccius merluccius*										135	14	0	195	22	0				330	36	0
Mackerel *Scomber scombrus*										30	3	0	30	3	0	120	12	0	180	18	0
Horse mackerel *Trachurus trachurus*										30	3	0							30	3	0
Four-bearded rockling *Rhinonemus cimbrius*				21	43	0	11	11	0	8	5	0	81	9	0				121	68	0
Grey gurnard *Eutriglia gurnardus*				10	2	0							10	1	0	30	3	0	50	6	0
Red gurnard *Aspitriglia cuculus*													30	3	0				30	3	0
Saithe *Pollachius virens*										30	3	0				60	6	0	90	9	0
Lesser argentine *Argentina sphyraena*										127	14	0							127	14	0
Flounder *Platichthys flesus*										10	1	0							10	1	0
Dab *Limanda limanda*													59	6	0	90	9	0	149	15	0
Long rough dab *Hippoglossoides platessoides*													50	5	0	27	3	0	77	8	0
Turbot *Scophthalmus maximus*										2	2	0	1	1	0				3	3	0
Plaice *Pleuronectes platessa*										20	2	0	75	8	0				95	10	0
Greater sandeel *Hyperoplus lanceolatus*				78	13	0							17	2	0				95	15	0
Raitt's sandeel *Ammodytes marinus*				61	7	0													61	7	0
Total numbers for individual cruises	873	95	0	2302	316	0	1575	180	0	5626	585	8	3041	314	0	5876	591	13	19293	2081	21

Occurrence of VHSV carrier marine fish species

The total number of fish sampled during the 6 cruises and the resulting number of tissue pools collected are shown in Table 1. From the 591 tissue pools sampled from Cruises 1 to 3 during 1997 and the 314 tissue pools sampled from Cruise 5 during 1998, no VHSV was isolated. However, of the 585 tissue pools sampled from Cruise 4, 8 were positive for VHSV with 6 of the isolates originating from Norway pout, one from cod from a pool of skin lesions and one from poor cod, which was the first reported isolation from this species (Table 2). Of the 591 tissue pools sampled from Cruise 6, there were a further 13 VHSV isolations, the majority taken from Norway pout (11 isolates), with one from herring and one from whiting (Table 2). The geographic catchment locations of fish from which VHSV was isolated are shown in Fig. 1.

Of the fish sampled, few showed any gross signs indicative of viral haemorrhagic septicaemia pathology either externally or internally, including those fish that later tested positive for VHSV.

Occurrence of VHSV associated with lesions in marine fish species

Table 3 shows the number of cod and haddock sampled for virology and of those fish sampled, the number exhibiting skin lesions or skin haemorrhage. Of the 506 cod sampled, 23 were found with lesions. One sample of skin lesions from a pool of fish tested positive for VHSV; however, the corresponding pool of organs tested negative. A small number of the haddock sampled (16 out of 4782) showed skin haemorrhage somewhat similar to that seen previously on haddock from which VHSV was isolated (Smail 2000); however, all tested negative for VHSV.

Table 2. Viral haemorrhagic septicaemia virus (VHSV) isolated from samples collected during all 6 research cruises, listing isolate, host fish species, fish length, date fish sampled, catchment location of fish and ICES statistical square

Isolate	Host species	Fish length (mean ± SD)	Date sampled (d.mo.yr)	Location	ICES square
MLA98/4PT1	Norway pout	12.0 ± 1.1, n = 8	18.02.1998	N Atlantic, W Barra	42E1
MLA98/4PT2	Norway pout	13.7 ± 1.5, n = 10	02.03.1998	N Atlantic, Kildas	43E1
MLA98/4PT3	Norway pout	14.4 ± 0.8, n = 10	02.03.1998	N Atlantic, Kildas	44E1
MLA98/4PT4	Norway pout	14.5 ± 1.4, n = 10	03.03.1998	N Atlantic, W of Sula	47E3
MLA98/4PT5	Norway pout	14.1 ± 0.3, n = 10	04.03.1998	N Atlantic, W of Orkneys	47E5
MLA98/4PT6	Norway pout	14.0 ± 0.9, n = 10	04.03.1998	N Atlantic, W of Orkneys	47E5
MLA98/4PC1	Poor cod	16.1 ± 1.4, n = 10	03.03.1998	N Atlantic, W of Sula	47E3
MLA98/4CO1	Cod	80.0 ± 15.4, n = 9	04.03.1998	N Atlantic, NE Rona	47E4
MLA98/6HE1	Herring	27.4 ± 1.9, n = 10	20.08.1998	North Sea, Balta	50E9
MLA98/6PT7	Norway pout	15.4 ± 0.7, n = 10	20.08.1998	North Sea, Balta	50E9
MLA98/6WH1	Whiting	11.3 ± 1.1, n = 10	17.08.1998	North Sea, East Bank	44F3
MLA98/6PT8	Norway pout	18.0 ± 1.2, n = 10	18.08.1998	North Sea	47F3
MLA98/6PT9	Norway pout	16.1 ± 1.2, n = 10	18.08.1998	North Sea, Patch	47F2
MLA98/6PT10	Norway pout	15.9 ± 0.9, n = 10	19.08.1998	North Sea, Bergen Ground	47F1
MLA98/6PT11	Norway pout	16.5 ± 0.7, n = 10	19.08.1998	North Sea, Beryl	48F1
MLA98/6PT12	Norway pout	15.7 ± 1.2, n = 10	19.08.1998	North Sea, Beryl	48F1
MLA98/6PT13	Norway pout	15.2 ± 1.6, n = 10	19.08.1998	North Sea	50F2
MLA98/6PT14	Norway pout	17.1 ± 1.5, n = 10	20.08.1998	North Sea	50F1
MLA98/6PT15	Norway pout	16.3 ± 1.3, n = 10	20.08.1998	North Sea	51F1
MLA98/6PT16	Norway pout	15.2 ± 1.2, n = 10	21.08.1998	North Sea	49F0
MLA98/6PT17	Norway pout	17.1 ± 1.1, n = 10	25.08.1998	North Sea, Bergen Bank	46F0

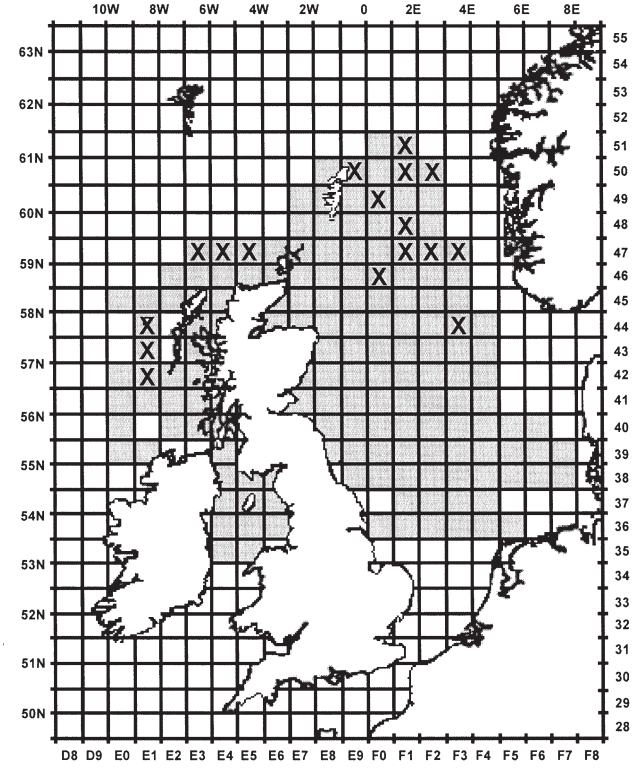

Fig. 1. Map of the ICES statistical squares charted from which fish were sampled for viral haemorrhagic septicaemia virus (VHSV) (shaded squares), showing those squares from which VHSV was isolated (X)

DISCUSSION

The survey identified 5 species of fish from the North Sea and the coastal waters of Scotland that were carriers of VHSV. These were Norway pout, cod, herring, whiting and poor cod. The isolation of VHSV from cod supports previous findings by Smail (1995, 2000), who described VHSV isolations from cod caught in Scottish seas. Poor cod has not previously been reported as a host species for VHSV, while herring, whiting and Norway pout have previously been described as carriers (Dixon et al. 1997, Mortensen et al. 1999).

There is a wide geographical distribution of infected fish within the sea areas sampled, with virus isolations from the North Sea, east of Shetland and west of Orkney, and the northeast Atlantic, west of the outer Hebrides.

VHSV was most prevalent in Norway pout with a total of 17 virus isolations from the 254 tissue pools taken from 2535 fish. Other species of fish having been reported to be carriers of VHSV, such as herring and sprat (Mortensen et al. 1999), were also sampled in high numbers. However, only 1 isolation was made from herring from 375 pools taken from 3711 fish and no isolations were made from the 176 pools taken from 1704 sprat. Poor cod, the novel host species identified, had a relatively high VHSV prevalence rate with 1 isolation from the 89 pools taken from 886 fish.

The range of fish species and numbers of fish sampled on each of the 6 surveys varied. In 1997, fish spe-

Table 3. Total numbers of cod and haddock sampled from the 3 research cruises during 1997 and the 3 research cruises during 1998, showing numbers of fish exhibiting skin lesions or skin haemorrhage and the resulting number of VHSV isolations

Species	Year	Total no. of fish sampled	No. of lesion/ haemorrhage-positive fish	No. of VHSV isolations
Cod	1997	137	16	0
	1998	369	7	1 (lesion pool)
Haddock	1997	1375	10	0
	1998	3407	6	0

cies that had previously been reported as carriers of VHSV within European waters, such as cod, haddock, sprat, herring and whiting (Smail 1995, 2000, Dixon et al. 1997, Mortensen et al. 1999), were mainly targeted. However, this sampling strategy failed to identify infected fish even though the numbers tested would have been sufficient to detect infection at prevalence levels comparable with those of previous studies that did isolate VHSV (Smail 1995, 2000, Dixon et al. 1997, Mortensen et al. 1999). Therefore, during the surveys of 1998 the range of species tested was expanded to include the more abundant species caught, such as Norway pout and poor cod. Additionally, 3 times as many animals were tested in 1998 as in 1997.

Although no virus was isolated in 1997, compared with the 21 isolations in 1998, it is difficult to compare prevalence between the 2 yr, as 17 of the isolates were from Norway pout and 1 isolate from poor cod in 1998 and these species were not targeted in 1997. Single isolations were made from each of the species cod, herring and whiting in 1998, but the number of these species tested was at least double that of 1997. It is, therefore, not statistically valid to compare prevalence between these years.

Results from this survey together with the earlier work of Smail (1995, 2000) show that VHSV has been persistent in wild fish populations over a period of more than 5 yr and is consistent with the virus being enzootic within populations of several species of marine fish in waters around Scotland.

Almost all of the infected fish identified showed no gross pathology consistent with that reported for marine species with viral haemorrhagic septicaemia (Meyers et al. 1994, Ross et al. 1994, Smail 1995, 2000), which suggests that virus may have been present at low concentrations in tissues of these fish that can be classed as asymptomatic carriers. Herring and haddock were often noted to have cutaneous haemorrhage caused by capture methods and it was not easy to differentiate between damage caused by capture and potential pathologies. The exception was for cod, where it was possible to recognise the skin lesions that have been previously described (Jensen et al. 1979). Indeed, during our study VHSV was isolated from a pool of skin lesions from cod. Interestingly, VHSV was not isolated from the corresponding pool of organs, indicating that skin is a primary site of infection. The original marine VHSV isolation was from an Atlantic cod showing the ulcus syndrome (Jensen et al. 1979). This syndrome was observed widely in cod during fish disease surveys carried out in the North Sea by Smail (1995, 2000) and VHSV was isolated from lesion-positive fish. VHSV has been isolated from lesions on cod from the Baltic Sea; however, the virus was also isolated from a normal skin specimen (Mortensen et al. 1999). Frequency analysis data showed no correlation between the presence of lesions and VHSV infection (Mortensen et al. 1999). Experimentally, VHSV was recovered from a sub-cutaneous lesion on a cod infected intra-peritoneally with an isolate from a wild cod, H16/7/95 (Smail 2000), although virus was not recovered from the organs (Snow et al. 2000). The North American strain of VHSV has been isolated from skin lesions, but not organs, of Pacific cod *Gadus macrocephalus* and Pacific herring *Clupea harengus pallasi*, where it was postulated that skin and fin tissues may be initial sites of replication leading to a viraemia and infection of kidney and spleen (Meyers & Winton 1995).

Viral pathogenicity of VHSV isolates obtained during this study for the host species of fish is unknown. Possible explanations for the lack of gross pathology observed in infected fish is that although isolates were virulent, the fish were low level carriers having recovered from a disease condition or the fish had an early primary infection. Alternatively, the virus may be of low virulence for the host species. McVicar (1997) discusses the difficulty of observing disease conditions in wild fish primarily because lack of fitness results in death. Host population size and density are conditions that contribute to selective pressure for pathogenicity (Frank 1996). Numbers of wild fish populations fluctuate between years and commercially caught fish such as cod are on the point of collapse in the areas studied (Cook et al. 1997). Lower host densities may be expected to result in lower pathogenicity. However, the relationship between host population size and viral virulence is not known; neither is the effect of VHSV infection on fish growth and survival.

Genetic analyses have shown that isolates of VHSV from wild fish in Scottish and other northern European coastal waters are related to isolates from cultured diseased turbot and that the marine isolates of VHSV are distinct from the isolates from freshwater species

(Stone et al. 1997, Snow et al. 1999). Experimental bath challenges with VHSV have shown turbot to be highly susceptible to many of the marine isolates (King et al. 2001), but that rainbow trout (H. F. Skall pers. comm.) and Atlantic salmon (King et al. 2001) are not susceptible. However, current European Union harmonised diagnostic tests do not differentiate between marine and freshwater isolates. The existence of wild fish acting as a reservoir of VHSV in coastal waters of Scotland requires a re-evaluation of the EC legislation as it applies to marine species in the coastal zone. Future work by our laboratory will address this issue, particularly with regard to whether diagnostic tests can be developed to distinguish marine and freshwater isolates of VHSV.

Acknowledgements. Thanks go to Ken Coull, Andrew Newton and staff of the Fisheries Management Programme of FRS Marine Laboratory Aberdeen for allowing us to participate in cruises and for their expertise and assistance. Many thanks to Ian Bricknell for assistance with sampling and to Victoria Lopez-Doriga for her invaluable help with processing. Thanks also go to the crews of FRV 'Scotia' and FRV 'Clupea'. This work was carried out under the EC project Rhabdoviruses in Wild Marine Fish in European Coastal Waters: Characterisation and Significance for Aquaculture. FAIR CT 96.1594.

LITERATURE CITED

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

Cook RM, Sinclair A, Stefansson G (1997) Potential collapse of North Sea cod stocks. Nature 385:521–522

Dixon PF (1999) VHSV came from the marine environment: clues from the literature, or just red herrings? Bull Eur Assoc Fish Pathol 19:60–65

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

Frank SA (1996) Models of parasite virulence. Q Rev Biol 71: 37–78

Hørlyck V, Mellergard S, Dalsgaard I, Jørgensen PEV (1984) Occurrence of VHS in Danish maricultured rainbow trout. Bull Eur Assoc Fish Pathol 4:11–13

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

King JA, Snow M, Skall HF, Raynard RS (2001) Experimental susceptibility of Atlantic salmon *Salmo salar* and turbot *Scophthalmus maximus* to European freshwater and marine isolates of viral haemorrhagic septicaemia virus. Dis Aquat Org 47:25–31

McVicar AH (1997) The development of marine environmental monitoring using fish diseases. Parassitologia 39: 177–181

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

Meyers TR, Sullivan J, Emmenegger E, Follett J, Short S, Batts WN, Winton JR (1992) Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, USA. Dis Aquat Org 12:167–175

Meyers TR, Short S, Lipson K, Batts WN, Winton JR, 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. Dis Aquat Org 19:27–37

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

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

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

Smail DA (1995) Isolation and identification of viral haemorrhagic septicaemia (VHS) virus from North Sea cod (*Gadus morhua* L.). ICES Maricult Comm CM 1995/F:15

Smail DA (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. Dis Aquat Org 41:231–235

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

Snow M, Cunningham CO, Bricknell IR (2000) Susceptibility of juvenile Atlantic cod *Gadus morhua* to viral haemorrhagic septicaemia virus isolated from wild-caught Atlantic cod. Dis Aquat Org 41:225–229

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

Editorial responsibility: Jo-Ann Leong, Corvallis, Oregon, USA

Submitted: June 19, 2001; Accepted: September 11, 2001
Proofs received from author(s): October 30, 2001