International Council for the Exploration of the Sea

CM 1995/F:15
Mariculture Committee

ISOLATION AND IDENTIFICATION OF VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) VIRUS FROM NORTH SEA COD (*Gadus morhua* L.)

D A Smail

SOAFD Marine Laboratory
PO Box 101, Victoria Road
Aberdeen, AB9 8DB
Scotland, UK

SUMMARY

During a research cruise of the FRS *Scotia* in June 1993 in the North Sea, cod were inspected for skin ulcers previously described as the cod ulcus-syndrome from Danish waters. Ulcers were observed in 13.7% of cod at stations ranging from near St Abbs Head, South Eastern Scotland to the Halibut bank North East of Shetland. Ulcers were observed most commonly in older/larger cod. Ulcers were processed for virus isolation on Epithelioma Papulosum of Carp (EPC) cells and a reproducible cytopathic effect was observed from 2/19 ulcers processed for virology. The virus isolates from two cod at two separate stations east and northeast of Shetland were both identified as VHS virus by an immunofluorescent antibody test. This study identifies cod as a definite host of VHS virus and emphasises the close association of the virus to the skin ulcers in cod.

INTRODUCTION

The ulcus lesion in cod was well described by Jensen and Larsen (1979) in fish from Danish coastal waters. They reported on the external appearance of the lesions, the stages of infection seen and the histology. It is basically a papular skin lesion which progresses to a bleeding ulcer which later heals. From fish with this lesion the same authors made two virus isolations of a rhabdovirus and an iridovirus (Jensen and Larsen, 1979). Interestingly, the rhabdovirus isolate was identified as identical to VHS virus by Vestergard-Jorgensen and Olesen (1987) which cast doubt on the authenticity of the isolate from cod. These authors suggested cod probably did not represent a new host for the virus, but that the rhabdovirus isolation more likely resulted from contamination of a cod surface or a cell culture with VHS virus of fresh water origin. However, in 1992 Meyers *et al.* reported lesions in Pacific cod (*Gadus macrocephalus*) in Alaskan coastal waters associated with a strain of VHS virus. The Alaskan report in particular stimulated interest to examine North Sea cod for the ulcus-type skin lesion and perform virus isolations for VHS virus. This approach seemed particularly worthwhile to confirm the earlier report of Jensen and Larsen (1979) that the ulcus skin lesion in cod may have a viral aetiology.

MATERIALS AND METHODS

As part of a fish disease survey (McVicar, Bruno and Fraser, 1988) in eastern coastal waters off eastern Scotland in 1993, cod were caught using BT101 fishing gear (48' Aberdeen trawl) with tickler chain and small mesh cod-end. Twenty-three trawls were carried out by the FRS *Scotia* from 5-9 June 1993, and 328 cod inspected for skin ulcus lesions. The haul stations showing cod with the typical lesions ranged from off St Abbs Head in the Firth of Forth to 50 miles east of Shetland (Table 1 and Fig. 1).

The cod were washed and the skin carefully inspected for the small papular lesions as described by Jensen and Larsen (1979). Lesions could also be staged from I-III according to the size of the lesion and the degree of associated skin bleeding as previously described by Jensen and Larsen (1979) as stages I-V. Early stages in the development of the lesion were most commonly seen from stage I to stage III. The data on the lengths of cod suggested that it was largely the older fish of age 2-5+ that displayed the ulcus lesions. Internal organs were not sampled on this survey.

Ulcers were dissected from washed and alcohol-swabbed skin using a sterile scalpel. Each lesion was stored at 4°C in 9 ml of Eagles MEM transport medium with high antibiotics (gentamycin, kanamycin and fungizone) and foetal calf serum at 10%. It was cut with two scalpels and homogenised in the original transport medium using a Stomacher 80 lab-blender (Seward Medical). To the homogenate polyethylene glycol (PEG 20,000 MW) at 70% w/v in Tris MEM with 2% serum was added to give a final concentration of 7% at a 10x dilution after the method of Batts and Winton (1989). The homogenate was sedimented at 2,000 rpm for 20 minutes at 4°C. 0.5 ml of the supernatant was passed through a Millipore low-protein binding HV (0.45 µm) filter and duplicate wells of EPC cells (Fijan *et al.*, 1983) were inoculated with 0.5 ml volumes of undiluted and 1/10 diluted filtrate. Inoculum was adsorbed onto cells for 1 h at 15°C and removed before the addition of 2 ml per well of MEM-10 buffered with 26 mM tris buffer and sodium bicarbonate to give pH 7.6. The plate was sealed in a plastic bag and incubated at 15°C. After 10 days incubation, the plates were read microscopically for cytopathic effect (CPE). Passage was then carried out at 1/10 dilution to fresh 80% confluent EPC cells on 12 well plates. The supernatant from the two positive CPE wells was passed through a Millipore HV filter. The plates were then incubated at 15°C and read at 17 days post passage. Two positive CPE wells were recorded and an aliquot of medium was stored at -70°C for identification later.

The virus isolates were identified by immunofluorescence as follows. Monolayers of EPC cells on 13 mm diameter glass cover slips (Merck Ltd) at 80% confluence were infected with the two virus isolates H19/1 and H17/5 at 1/10 and 1/100 dilutions by direct inoculation and incubated at 15°C. Cell controls were also included. After 23 hours, the cell monolayers on cover slips were fixed in 80% acetone in water and stored at -20°C. The coverslip cultures were then thawed and incubated overnight at 4°C in 1/50 and 1/100 dilutions of a monoclonal antibody to VHS virus (IP5B11, a gift of N Lorenzen, Danish Veterinary Laboratory, Aarhus) diluted in PBS. Cover slips were then washed thoroughly in 0.9% NaCl and deionised water (Tween 20, 0.05%) and incubated with goat anti-mouse IgG FITC Conjugate (Sigma Cat No F0257) at 1/50 or 1/100 dilution in PBS for 30 mins. Washing and mounting in Vectamount (Vector Labs) followed and control cultures were also likewise stained. Slides were read with a Nikon Diaphot microscope in UV light with a B2 blue light filter combination.

RESULTS

2/19 fish tested showed positive CPE by day 10 post inoculation in the wells with undiluted inoculum only. The further dilution did not show CPE showing that the level of virus present was borderline for initial growth. These isolates were termed H17/5 and H19/1 for fish haul number and fish number sampled.

Both isolates were identified as VHS virus by IFAT using the cross-reacting monoclonal IP5B11, reactive to the N protein, with fluorescing foci of infected cells observed. Cell controls in the IFAT reaction were uniformly negative and blank.

DISCUSSION

The finding of cod-ulcus lesions in the northern North Sea corroborates the earlier finding of Jensen and Larsen (1979) and extends the distribution map of this apparently common lesion. A significant proportion of cod examined (45/328 =13.7%) showed evidence of the lesions. Also at haul stations where cod were trawled, 13/17 (76%) of stations yielded cod with lesions.

The identification of virus as VHSV by immunofluorescence also corroborates the previous reports of isolations of VHSV from Pacific cod (*Gadus macrocephalus*) (Meyers, 1992, 1994) but more importantly points to a true marine origin of the rhabdovirus isolate of Jensen, Bloch and Larsen (1979) from cod from Danish coastal waters. The latter isolate was also found indistinguishable to VHSV by Vestergard-Jorgensen and Olesen (1987) by an immunofluorescence antibody test. These two isolates from cod were unlikely to be caused by a laboratory contaminant in 1993 as no other VHS isolates were handled at that time.

In broader terms this finding indicates that cod (*Gadus morhua* L.) is a susceptible species to the virus. It is not known to date to what extent this virus can invade the internal tissues of cod and cause pathology but association with the ulcus lesions was shown. Such an association agrees with recent reports that VHS virus can act as an epitheliotropic virus of the fish skin (Estepa, Frias and Coll, 1992) and can cause a cytopathic effect in rainbow trout fin cells in culture.

The epizootiological consequences of these isolations are significant and important for the potential spread of VHS viruses in the marine environment, viz the North Sea especially. It would be interesting to know which other marine wild species are susceptible to VHSV in the coastal waters around Scotland because the outbreak of VHSV in farmed turbot on Gigha Island, off the Kintyre Peninsula, Scotland (Ross *et al.*, 1994) was not traceable to any particular source. From the North American evidence on virus testing on Pacific Ocean VHS carriers (Meyers *et al.*, 1994) another proven carrier is Pacific herring (*Clupea harengus pallasi*). For this reason, Atlantic herring (*Clupea harengus harengus*) is a potential host for future rhabdovirus testing and study in coastal waters around Scotland but other gadoid fish and turbot are also of relevant interest.

It will be of interest in future studies on these isolates from cod and turbot to establish if they fall within the range of isolates from farmed rainbow trout in continental Europe, with respect to virulence, and antigenic and genetic characteristics.

ACKNOWLEDGEMENTS

I thank Mrs E Rough for technical assistance and Dr K MacKenzie for assistance at sea. I also thank the Typing Pool.

REFERENCES

Batts, W.N. and Winton, J.R. 1989. Enhanced detection of infectious haematopoietic necrosis virus and other fish viruses by pretreatment of cell monolayers with polyethylene glycol. *Journal of Aquatic Animal Health*, **1**, 284-290.

Estepa, A., Frias, D. and Coll, J.M. 1992. *In vitro* susceptibility of rainbow trout fin cells to viral haemorrhagic septicaemia virus. *Diseases of Aquatic Organisms*, **15**(1), 35-39.

Fijan, N., Sulimanovic, D., Bearzottm, M., Muzinic, D., Zillenberg, L.O., Chilmonczyk, S., Vautherot, J.F. and de Kinkelin, P. 1980. Some properties of the Epitheliom papulosum cyprini (EPC) cell line from carp *Cyprinus carpio*. *Annals of the Institute Pasteur*, **134**, 207-220.

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

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

McVicar, A.H., Bruno, D.W. and Fraser, C.O. 1991. Fish disease in the North Sea in relation to sewage sludge dumping. *Marine Pollution Bulletin*, **19**, 169-173.

Meyers, T.R., Sullivan, J., Emmenegger, E., Follett, J., Short, S., Batts, W.N. and Winton, J.R. 1992. Identification of viral haemorrhagic septicaemia 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. and Brown, E. 1994. Association of viral haemorrhagic septicaemia virus with epizootic haemorrhages 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.

Ross, K., McCarthy, U., Huntly, P.J., Wood, B.P., Stuart, D., Rough, E.I., Smail, D.A. and 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**(6), 213-4.

Vestergard-Jorgensen, P.E.V. and Olesen, N.J. 1987. Cod ulcus syndrome rhabdovirus is indistinguishable from the Egtved (VHS) virus. *Bulletin of the European Association of Fish Pathologists*, **7**(3), 73-4.

TABLE 1

Examination of cod for ulcus lesions and virological sampling, June 1993, North Sea

Haul No	Haul position		Location	Cod examined	Cod ulcer	Cod virus sample	Virus +ve
	Latitude	Longitude					
5	56°27.36'N	02°14.59'W	Bell Rock	2	0		
8	56°04.82'N	02°02.18'W	St Abbs	54	4	4	0
9	56°04.55'N	02°13.62'W	St Abbs	44	1		
10	56°04.50'N	02°11.48'W	St Abbs	30	0		
11	56°10.26'N	02°03.85'W	St Abbs	8	2		
12	58°10.00'N	02°57.48'W	Beatrice	2	0		
13	59°16.85'N	01°25 00'W	SE F/Isle	22	0		
14	59°12.90'N	01°29.48'W	SE F/Isle	19	2		
15	59°43.45'N	01°17.86'W	S Lumburgh	17	1		
16	60°04.28'N	00°21.12'W	Mousa	12	3	2	0
17	60°18.41'N	00°08.26'E	E Shetland	7	5	5	1
18	60°03.56'N	00°19.95'E	E Shetland	17	8	7	0
19	60°55.77'N	00°34.38'E	Halibut Bk	8	2	1	1
20	61°07.03'N	00°51.50'E	W Cormorant	11	5		
21	61°00.66'W	01°13.76'E	W Cormorant	10	4		
22	60°42.00'W	01°16.20'E	W Cormorant	8	3		
23	60°19.88'N	01°11.54'E	W Cormorant	57	5		
Totals				328	45	19	2

Figure 1 Track of FRS *Scotia*, 5-9 June 1993, from Bell Rock off St Andrews Bay, Fife, to East of Shetland. Crosses mark haul stations and numbers multiple hauls at the same station.