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NOTE

Genetic comparison of viral hemorrhagic septicemia virus isolates from North America and Europe

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ABSTRACT: Viral hemorrhagic septicemia virus (VHSV) is the causative agent of a serious rhabdoviral disease of rainbow trout *Oncorhynchus mykiss* in Europe. The first isolation of the virus in North America occurred in the fall of 1988 when it was recovered from adult chinook *O. tshawytscha* and coho *O. kisutch* salmon returning to 2 hatcheries in the state of Washington, USA. The following year, VHSV was isolated from adult coho salmon at 2 other hatcheries in northwestern Washington. In 1990 and 1991, VHSV was recovered from Pacific cod *Gadus macrocephalus* caught in Prince William Sound, Alaska. Genetic variation among the 4 isolates from salmon and the 1990 isolate from Pacific cod was determined using T1 ribonuclease fingerprinting. In addition, 4 diverse isolates from Europe were included for comparison. The North American isolates of VHSV formed a single fingerprint group in which the 4 isolates from salmonids were highly similar to each other and the isolate from Pacific cod was related but less similar. The 4 European isolates, which included an isolate from Atlantic cod *G. morhua*, formed a second fingerprint group. The genetic diversity among the isolates within each fingerprint group was estimated to be less than 5% while the North American and European strains of the virus were judged to differ by more than 5%. The results indicate that the North American isolates of VHSV are not of European origin and that the virus may be enzootic within the marine environment.

KEY WORDS: Genetic · Rhabdovirus · Salmonids · VHSV

Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus that causes an important disease (VHS) that results in the loss of millions of dollars worth of rainbow trout throughout much of Europe. The virus has been isolated from a number of salmonid as well as non-salmonid species of fish (Wolf 1988). Because of the potential for devastating losses to occur within public and private salmon and trout culture facilities in North America, regulations have been enacted in the United States and Canada to prevent the introduction of VHSV.

The first isolates of VHSV from fish in North America were obtained in the fall of 1988 from adult chinook salmon *Oncorhynchus tshawytscha* returning to the Glenwood Springs Hatchery on Orcas Island in northwest Washington, USA, and from adult coho salmon *O. kisutch* returning to the Makah National Fish Hatchery near Neah Bay, Washington. Both facilities are located near the open ocean. By electron microscopy, the new isolates appeared to be typical rhabdoviruses, and were effectively neutralized by polyclonal antiserum against a European reference strain (F1) of VHSV. Analysis of the 2 isolates by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that the structural proteins had molecular weights indistinguishable from each other or from the F1 reference strain and reacted in a Western blot developed with a polyclonal antiserum made against the F1 reference strain (Winton et al. 1991). In 1989, VHSV was isolated from adult coho salmon at the Lummi Tribal Hatchery near Bellingham, Washington, and from adult coho salmon held at the Soleduck Hatchery near Forks, Washington (Eaton et al. 1991). Viral hemorrhagic septicemia virus was also isolated from Pacific cod *Gadus macrocephalus* caught in the open ocean in Prince William Sound, Alaska, in August 1990 and again in 1991 (Meyers et al. 1992).

The mechanisms by which VHSV is maintained within enzootic regions and the mode of virus transmission are still not completely understood. While

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horizontal transmission from fish to fish by waterborne virus is important for initiating infections and for spread of the disease, vertical transmission of the virus from adult to progeny via the gametes is believed to occur only rarely if at all (Jørgensen 1974). In addition to being maintained by horizontal transmission among susceptible fish in fresh water, the isolation of VHSV from Pacific cod in Alaska and from Atlantic cod *Gadus morhua* in Denmark (Jensen & Larsen 1979, Jørgensen & Olesen 1987) indicates that the virus may also have a marine reservoir.

Improvements in our knowledge of the genetic relationships among diverse isolates of VHSV may lead to a better understanding of the epidemiology of the virus. T1 ribonuclease fingerprinting has been used to compare genomes of closely related RNA viruses (Clewley & Bishop 1982, Kew et al. 1984) by detecting variation in the location of the guanosine residues in the RNA molecule. In the present study, the T1 fingerprinting technique was used to examine genetic variation among VHSV isolates from fish in North America and Europe to determine the possible genetic relatedness of the isolates from the 2 continents.

Materials and methods. ***Virus isolates:*** Five North American and 4 European isolates of VHSV were used in this study (Table 1). The isolates were confirmed to be VHSV by serum neutralization using polyclonal antiserum against the F1 strain. The T1 ribonuclease fingerprinting analysis was performed using stocks of virus with a minimum number of passages in tissue culture at a low multiplicity of infection (MOI) except for the F1 isolate which had been passed 254 times at low MOI.

Virus growth and purification: Chinook salmon embryo (CHSE-214, ATCC 1681) cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.3% tryptose phosphate, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, and 100 µg ml^{-1} gentamicin sulfate. The medium was adjusted to pH 7.5 using sodium bicarbonate. Both cells and virus were grown at 15 °C in ridged roller bottles (Corning Glass Works, Corning, NY, USA) containing a surface area of 1700 cm^2. Monolayer cultures of CHSE-214 cells were infected with low passage stocks of VHSV at a MOI of > 0.05 and incubated for 5 to 7 d in 100 ml of culture medium until the cytopathic effect was complete. Virus purification and extraction of viral RNA were done using a modification of the methods described by Hsu et al. (1985) and Kurath & Leong (1985) for IHNV. Briefly, about 200 ml of culture fluid was harvested and the cell debris removed by centrifugation at 10000 × *g* for 20 min at 4 °C. The supernatant was placed on a 0.3 ml pad of 100% glycerol and centrifuged in a Beckman SW 28 rotor for 90 min at 80000 × *g*. Virus pellets were resuspended in TNE buffer (50 mM Tris HCl, pH 7.6, containing 10 mM NaCl and 0.5 mM EDTA). The virus was purified in a discontinuous gradient composed of 20, 35 and 50% sucrose-TNE, layered in 3.5 ml volumes and centrifuged in a Beckman SW 41 Ti rotor for 90 min at 151000 × *g*. The virus band was collected from the interface between the 20 and 35% sucrose-TNE layers and then pelleted by centrifugation in a Beckman SW 50.1 rotor for 60 min at 147000 × *g*. The viral pellet was resuspended in TNE and further purified through a continuous gradient of 10 to 40% sucrose-TNE centrifuged in a SW 50.1 rotor at 84000 × *g*. The virus band was collected and pelleted as before.

Extraction and quantitation of viral RNA: The virus pellet was resuspended in 200 µl of TNE and the virions disrupted with proteinase K (1.0 mg ml^{-1}) and 1% SDS at 37 °C for 30 min. Protein was removed from the mixture using 2 extractions with 100 µl TNE-saturated phenol and 100 µl chloroform-isoamyl alcohol (24:1). The RNA was precipitated and stored in 0.3 M sodium acetate and 5 volumes of absolute ethyl alcohol at −70 °C. The quality and quantity of the genomic RNA

Table 1. Isolates of viral hemorrhagic septicemia analyzed by T1 ribonuclease fingerprinting

Isolate	Location	Year	Host species	
North American isolates				
Orcas Island	Washington	1988	Chinook salmon	*Oncorhynchus tshawytscha*
Makah	Washington	1988	Coho salmon	*O. kisutch*
Bogachiel	Washington	1989	Coho salmon	*O. kisutch*
Lummi	Washington	1989	Coho salmon	*O. kisutch*
Pacific cod	Alaska	1990	Pacific cod	*Gadus macrocephalus*
European isolates				
Egtved (F1)	Denmark	1962	Rainbow trout	*Oncorhynchus mykiss*
07-71	France	1971	Rainbow trout	*O. mykiss*
43-84	France	1984	Rainbow trout	*O. mykiss*
Atlantic cod	Denmark	1979	Atlantic cod	*Gadus morhua*

for each preparation was determined by formaldehyde denaturing agarose gel electrophoresis (Maniatis et al. 1982) using 1/15 of the total volume. A 0.24 to 9.5 kilobase RNA ladder (GIBCO BRL, Gaithersburg, MD, USA) was used in each gel to determine the molecular weight and relative concentration of the viral RNA in the sample. The gels were stained with ethidium bromide (0.5 μg ml^{-1}) for 1 h followed by destaining overnight in deionized water (dH_2O).

T1 ribonuclease digestion and 5'-end labeling of viral RNA: A 5'-end labeling kit (Du Pont NEN Research Products, Boston, MA, USA) was used to label the digested oligonucleotides (Maxam & Gilbert 1977). Approximately 3 μg of viral RNA in 3 μl of dH_2O was added to 52 μl of dH_2O, 3 μl of T1 ribonuclease at 16 units of activity (U) ml^{-1} (GIBCO BRL), 25 μl of phosphate buffer, and 20 μl of alkaline phosphatase solution containing 2.5 U ml^{-1}. This mixture was incubated for 50 min at 37 °C. Fifty to 100 μCi [γ-32P]ATP (Du Pont NEN; 3000 Ci $mmol^{-1}$) and 2.5 U of polynucleotide kinase were added to the T1-digested RNA and incubated at 37 °C for 40 min to label the 5'-end of each fragment.

First- and second-dimensional electrophoresis: The methods used in the fingerprinting protocol were first described by De Wachter & Fiers (1972), modified by Nichol (1987), and further modified for this study. The first-dimensional gel consisted of 10% acrylamide in 6 M urea and 0.025 M citric acid. The sample was suspended in 9 μl digest buffer (8.4 M urea, 12.5 mM sodium citrate (pH 5), 1.25 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), boiled for 1 min, then rapidly cooled on wet ice. The T1-digested RNA was loaded directly onto a 0.8 mm standard gel and electrophoresed at ca 250 V until the bromophenol dye front was 22 cm into the gel (ca 20 h). The gel track was then excised 7 cm from the top and 1.5 cm from the bottom of the gel and soaked in second-dimensional electrophoresis buffer for 15 min. The gel strip was then transferred to the bottom of a 0.75 mm standard TBE second-dimensional gel which was electrophoresed at 425 V under reversed polarity until the xylene cyanol dye was 8 cm from the top of the gel (ca 20 h). Gels were exposed to X-ray film for up to 72 h before developing the autoradiographs. The fingerprinting procedure was repeated at least twice for each isolate to confirm reproducibility.

Fingerprints of different isolates were compared by directly overlapping the autoradiographs. Approximately 100 of the spots generated by the larger oligonucleotides were numbered to create a master pattern. Autoradiographs of each isolate were analyzed for the presence or absence of each of the master spots. The number of spot differences between isolates was used as a measure of the degree of relatedness.

Results. For each isolate, 1 production (2 roller bottles) of virus culture typically yielded 7 to 15 μg of genomic RNA as estimated by denaturing agarose gel electrophoresis. The purified RNA was of the size expected for a full-length genome. In most cases, smaller RNA molecules were not detected indicating that few defective interfering particles were present in the preparations.

T1 ribonuclease fingerprinting analysis was done using genomic RNA from 9 isolates of VHSV from North America and Europe. The autoradiographs containing the spot patterns formed by the labeled RNA fragments were compared by inspection (Fig. 1), a master figure showing the analyzable spots was developed (Fig. 2), and the presence or absence of each of the master spots was cataloged for each isolate (Table 2).

Viruses are commonly placed into separate fingerprint groups when more than 50% of the spots differ between the isolates (Aaronson et al. 1982). The 5 isolates from North America had spot patterns sufficiently alike to be compared as 1 fingerprint group, while the 4 European isolates had patterns that indicated they belonged within a second fingerprint group. Among the North American isolates, 115 different oligonucleotide spots could be analyzed and used to form the master pattern, while 97 large oligonucleotides could be analyzed among the European isolates (Fig. 2). When the number of spot differences among the isolates was determined (Table 3), the variation among the 4 North American salmon isolates was relatively small (5 to 11%); however, when the Pacific cod isolate was included, the spot variation within the North American strain increased to 22 to 27%. Spot variation among the 3 rainbow trout isolates of the European strain was 9 to 17%, and the variation increased to 20 to 27% when the Atlantic cod isolate was included in the comparison.

A computer simulation model by Aaronson et al. (1982) showed that an oligonucleotide spot variation of 50% corresponded to a variation in the nucleotide sequence of about 5%. Based upon this model, the maximum variation of the RNA sequence within either the North American or the European VHSV fingerprint groups was estimated to be about 3%. The variation in the RNA sequence between the 2 fingerprint groups was estimated to be in excess of 5%.

As seen in the autoradiograms (Fig. 1), there was an apparent lack of uniformity among the molarities of some of the larger oligonucleotides. We assumed this was due to formation of secondary structures among some of the T1-digested oligonucleotides, which affected the relative efficiency of the kinase reaction. Similar observations have been noted for other RNA viruses (Frisby 1977, Stephenson & Ter Meulen 1982, Kamahora et al. 1985, Kusters et al. 1987). The pres-

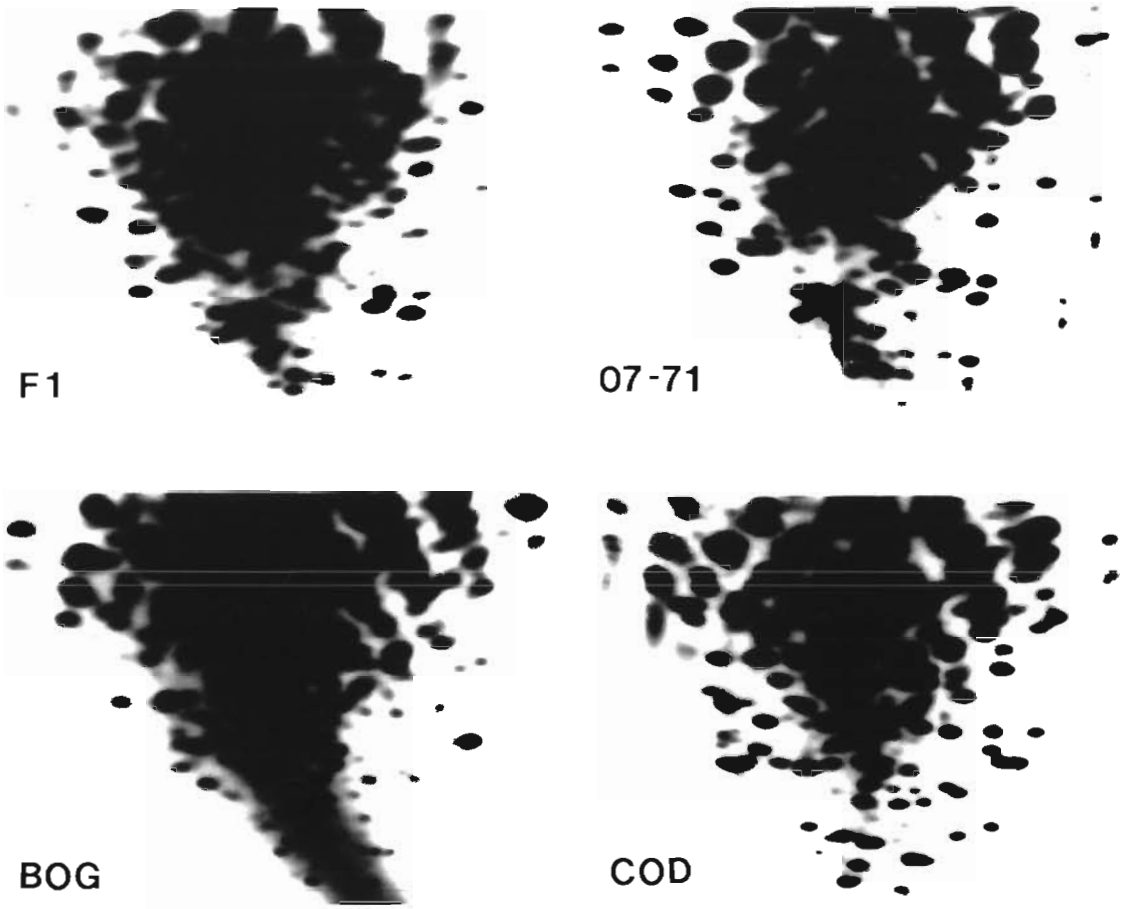

Fig. 1. Representative T1 ribonuclease fingerprints of 2 European (F1 and 07-71) and 2 North American (BOG and COD) isolates of viral hemorrhagic septicemia virus. The isolates from Europe (top) formed 1 fingerprint pattern while the North American isolates (bottom) formed a second fingerprint group

ence of several small genetic subpopulations (a result of the high mutation rate of RNA viruses) in addition to the dominant population (quasispecies) may also be a factor (Steinhauer et al. 1989b). These variations, however, were judged not to have affected the validity of the conclusions because the fingerprint patterns were reproducible for repeated analyses of the same RNA preparations and for different RNA preparations made from the same virus stock.

Discussion. Oligonucleotide fingerprinting has been used extensively in molecular studies of several RNA viruses (Clewley & Bishop 1982, Kew et al. 1984). For VHSV, the technique provided an independent approach to identifying variation in VHSV isolates based on genetic rather than antigenic relationships. The high degree of sensitivity inherent in the T1 fingerprinting method has made it very useful for detecting small variations in nucleic acid sequences. It is estimated that only about 10% of the RNA genome is analyzed by T1 fingerprinting because the shorter oligonucleotides are not well resolved and may occur more than once within the genome. Thus, these are not used for analysis, but the larger fragments have a high statistical probability of containing unique sequences that occur only once in a viral genome. Because these unique sequences are thought to occur randomly throughout the genome, the method provides a good approximation of the overall genetic diversity among RNA virus isolates. Quantitative estimates of relatedness are most reliable when RNA molecules share >95% sequence homology because a sequence variation greater than 5% generally results in greater than 50% spot variation, requiring the separation of virus isolates into different fingerprint groups (Aaronson et al. 1982).

The placement of the European and the North American isolates of VHSV into separate fingerprint groups was in good agreement with results obtained by Bernard et al. (1990, 1991, 1992), who compared the nucleoprotein (N) gene sequences of a European (07-71) and a North American (Makah) isolate of VHSV. They determined that these 2 N gene sequences

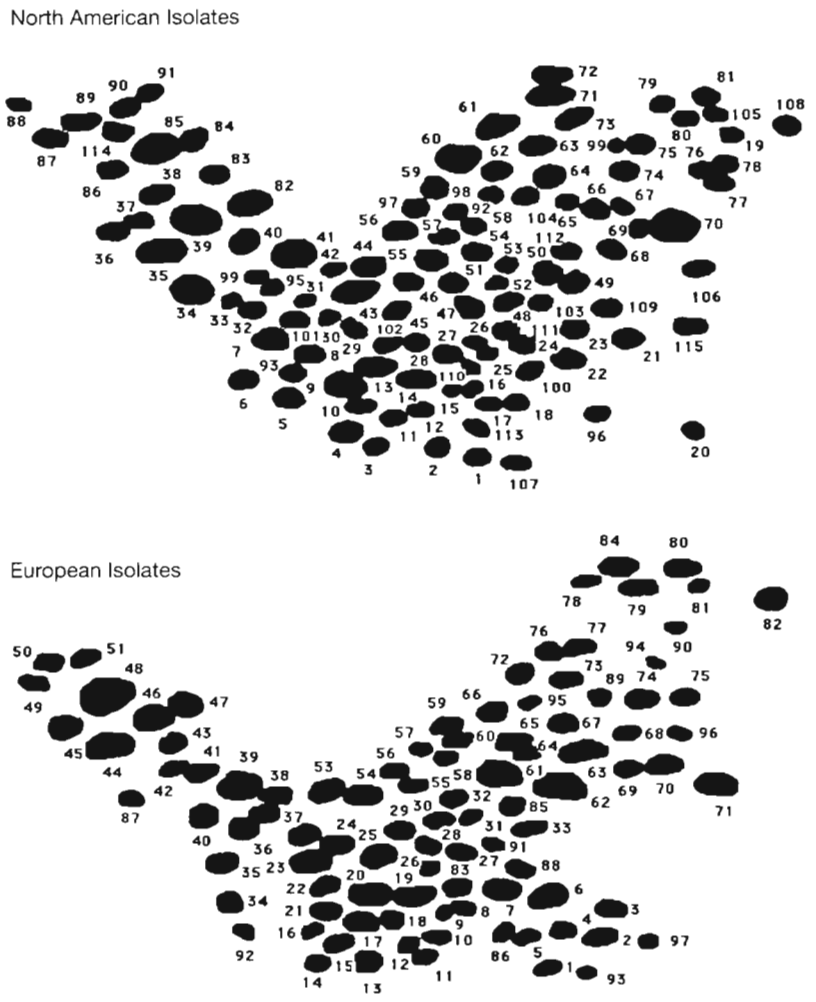

Fig. 2. Master spot patterns used to analyze the North American (top) and European (bottom) T1 ribonuclease fingerprint groups of viral hemorrhagic septicemia virus

differed by about 13%. Similarly, Batts et al. (1993) showed that a DNA probe designed to hybridize with a unique sequence in the N gene of the Makah isolate reacted with all North American and none of the European isolates tested, while a probe designed to recognize a sequence in the 07-71 isolate reacted with only the European isolates.

Our results indicate that the isolations of VHSV from fish in North America were not the result of a direct introduction of infected fish or eggs from Europe. If a direct introduction had occurred, greater genetic similarity between the North American and European isolates would have been expected, and the isolates would have originated within a more geographically restricted area. On the contrary, the North American isolates of VHSV were obtained from fish in the open ocean or at hatcheries on separate river systems with no known contact between these facilities. Most of the salmon hatcheries were not located near marine net-pen facilities, and none had a record of ever receiving eggs from Europe.

The isolation of VHSV from Pacific cod in Alaska on 2 occasions indicates that fish in the marine environment may serve as a reservoir of the virus. This would account for the isolation of VHSV from adult salmonids returning from the open ocean to hatcheries at 4 unrelated locations in Washington. Virus transmission to salmonids (and to Pacific cod) may have occurred from feeding on marine fish infected with the virus. In this regard, stocks of Pacific cod are common at various times of the year in Puget Sound, Washington, near the hatcheries where VHSV was first isolated.

The inclusion of the VHSV isolate from Pacific cod within the North American fingerprint group indicates that this strain of VHSV may be more widely disseminated in the northeast Pacific Ocean than is known because this isolate was obtained a great distance from Washington State. Similarly, the relatedness of the 1979 isolate from Atlantic cod to the isolates obtained from rainbow trout in Denmark and France over more than a 20 yr period indicates that the European strain of VHSV may be enzootic in some species of marine fish as well. Further surveillance is needed to determine the extent of VHSV in these species, and studies to determine the virulence of both strains of the virus for marine fishes seem warranted.

Many RNA viruses, including rhabdoviruses, are capable of accumulating high numbers of mutations in the RNA genome (Holland et al. 1982). The error frequency of the RNA polymerase used by these viruses to replicate may be as high as 1×10^{-4} to 4×10^{-4} substitutions per base (Steinhauer & Holland 1986). Under certain conditions (e.g. high MOI passage in cell culture), RNA viruses have been shown to exhibit rapid genome evolution, while other conditions (e.g. low MOI passage in the same cell line) produce a relatively more stable 'quasispecies' (Holland et al. 1979, 1982, 1991, Spindler et al. 1982, O'Hara et al. 1984, Steinhauer et al. 1989a, b); therefore, it is difficult to estimate how long the isolates forming the European fingerprint group of VHSV have been sepa-

Table 2. Catalog of spots present (•) in the autoradiograph of each isolate of viral hemorrhagic septicemia virus. The 5 North American and 4 European isolates formed 2 fingerprint groups that could not be directly compared

North American isolates	Spot no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•
Makah	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lummi	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•
Bogachiel	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Pacific cod	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Makah	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•
Lummi	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Bogachiel	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Pacific cod	•	•	•	•		•	•	•	•	•	•			•	•	•	•	•	•	•
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Makah	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lummi	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Bogachiel	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Pacific cod	•		•		•	•	•	•	•	•	•	•	•	•	•	•	•		•	•
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Makah	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•		•	•	•
Lummi	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Bogachiel	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Pacific cod	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•			•	
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•	•
Makah	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lummi	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		
Bogachiel	•	•	•	•	•	•	•	•		•	•	•	•	•	•		•	•	•	•
Pacific cod	•	•	•	•	•	•	•	•				•		•	•			•	•	
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115					
Orcas Island	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•					
Makah	•	•					•		•	•	•		•		•					
Lummi	•	•	•	•	•	•	•	•		•	•	•	•		•					
Bogachiel	•	•	•	•	•		•	•		•	•	•	•	•	•					
Pacific cod	•	•					•	•	•	•		•	•	•	•					

European isolates	Spot no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Egtved (F1)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
43-84	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•
07-71	•	•	•	•	•	•	•	•	•	•			•				•	•	•	•
Atlantic cod		•	•	•		•	•	•	•	•			•	•	•		•	•	•	•
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Egtved (F1)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
43-84	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•
07-71	•	•	•	•	•		•	•	•	•		•	•	•		•	•	•	•	•
Atlantic cod	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Egtved (F1)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
43-84	•	•	•	•		•	•	•		•	•	•	•	•	•	•	•	•	•	•
07-71	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•
Atlantic cod	•	•		•	•	•	•	•		•	•	•	•	•	•	•		•	•	•
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Egtved (F1)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
43-84	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
07-71	•	•	•	•	•	•	•	•	•		•	•			•	•	•	•	•	•
Atlantic cod	•	•	•	•	•	•	•			•	•	•	•	•	•		•	•	•	•
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97			
Egtved (F1)	•	•	•	•	•	•		•			•	•	•							
43-84	•	•	•	•	•	•		•	•	•	•	•	•	•						
07-71	•	•	•	•	•	•	•	•	•	•	•	•	•	•						
Atlantic cod	•	•	•	•								•	•	•	•	•	•			

Table 3. Number of spot differences among isolates of viral hemorrhagic septicemia virus placed within the North American and the European fingerprint groups

North American fingerprint group	Orcas	Makah	Lummi	Bogachiel	Pacific cod
Orcas Island	–				
Makah	12	–			
Lummi	5	13	–		
Bogachiel	7	13	8	–	
Pacific cod	31	24	31	29	–

European fingerprint group	Egtved (F1)	43-84	07-71	Atlantic cod
Egtved (F1)	–			
43-84	7	–		
07-71	16	11	–	
Atlantic cod	19	22	25	–

rated from the isolates comprising the North American group. However, because the overall level of genetic variation found by T1 fingerprinting was relatively low among the isolates from each continent and because sequence data revealed a 13% nucleotide diversity between the N genes of North American and European reference strains of the virus (Bernard et al. 1991), it seems likely that the strains have been separated for a considerable time.

The genetic variation between the North American and European strains of VHSV was greater than the variation observed among diverse isolates of infectious hematopoietic necrosis virus (IHNV), a closely related salmonid rhabdovirus. Oshima et al. (unpubl. data) used T1 ribonuclease fingerprinting to analyze 26 isolates of IHNV obtained from fish throughout western North America and found that all isolates could be placed within a single fingerprint group where the genetic variation was estimated to be less than 5% at the sequence level.

To some extent, the genetic differences observed in our study are also reflected in biological differences between the North American and European isolates of VHSV obtained from salmonid fish. In Europe, coho salmon and chinook salmon are largely refractory to VHSV infection (de Kinkelin et al. 1974, Ord et al. 1976), whereas in North America VHSV was first isolated from these species. Batts et al. (1991) compared the efficiency of plating of 3 isolates of VHSV from trout in Europe and 2 isolates from salmon in North America using 5 different fish cell lines. They reported that the European strains had relatively high efficiency of plating on the rainbow trout cell line, RTG-2, in which the North American salmon isolates grew poorly. Following intraperitoneal or waterborne challenges, the North American VHSV isolates produced only low levels of mortality among any of the salmonid species tested, including rainbow trout (Winton et al. 1991). This is in contrast with the high virulence typical of European isolates of VHSV recovered from trout.

Neutralization assays have detected antigenic variation among European isolates of the virus (Wolf 1988). In a recent study, 127 isolates of VHSV were examined by plaque neutralization tests with a panel of 5 antisera (4 monoclonal and 1 polyclonal). Results indicated that these isolates could be divided into 3 reaction patterns (groups); however, there seemed to be only 1 major serotype of VHSV because 120 of the isolates were neutralized by the polyclonal antiserum and reacted strongly in Western blots (Olesen et al. 1993). Further genetic and serological studies would increase our understanding of the relationships between the European and North American VHSV isolates. Although we included some relatively diverse European strains of VHSV in this study, more strains will need to be examined to accurately estimate the overall level of genetic diversity of the virus.

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