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VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN NORTH AMERICA

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Abstract. The first detections of viral hemorrhagic septicemia virus (VHSV) in North America were in Washington State from adult coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon in 1988. Subsequently, VHSV was isolated from adult coho salmon returning to hatcheries in the Pacific Northwest in 1989, 1991 and 1994. These isolates represented a strain of VHSV that was genetically different from European VHSV as determined by DNA sequence analysis and T1 ribonuclease fingerprinting. The North American strain of VHSV was also isolated from skin lesions of Pacific cod (*Gadus macrocephalus*) taken from Prince William Sound (PWS), Alaska in 1990, 1991 and 1993. In 1993 and 1994, the virus was isolated from Pacific herring (*Clupea harengus pallasi*) in Alaskan waters of PWS, Kodiak Island, Auke Bay and Port Frederick. During 1993 and 1994 the herring fishery in PWS failed from a probable complex of environmental stressors but VHSV isolates were associated with hemorrhages of the skin and fins in fish that returned to spawn. Also in 1993 and 1994, VHSV was isolated from apparently healthy stocks of herring in British Columbia, Canada and Puget Sound, Washington. Thus, the North American strain of VHSV is enzootic in the Northeastern Pacific Ocean among Pacific herring stocks with Pacific cod serving as a secondary reservoir. Although the North American strain of the virus appears to be moderately pathogenic for herring, causing occasional self-limiting epizootics, it was shown to be relatively avirulent for several species of salmonids. Pacific herring are common prey for cod and salmon and were most probably the source of the VHSV isolates from the adult salmon returning to spawn in rivers or at hatcheries in Washington State. Compelling circumstances involving the European isolates of VHSV suggest that this strain of the virus also is enzootic among marine fish in the Atlantic Ocean. The highly pathogenic nature of the European strain of VHSV for salmonid fish may be the result of the exposure of rainbow trout (*O. mykiss*), an introduced species, in a stressful environment of intensive culture and the high rate of mutation inherent in all rhabdoviruses. Consequently, we recommend that efforts be made to eradicate the North American strain of VHSV when detected in live salmonids to reduce the possibility of its evolution into a more virulent salmonid virus.

Keywords. VHSV, Fish disease, North America

INTRODUCTION

Beginning in 1989, viral hemorrhagic speticemia virus (VHSV) has become an increasingly popular and sometimes controversial topic of discussion among fish pathologists in North America following its discovery in several native species of finfish in the Pacific Northwest of the United States. Evidence has shown that the virus was not an exotic import from Europe as first suspected, but, rather, an indigenous strain of the virus that may give some insight into how fish rhabdoviruses evolve in nature. The story of North American VHSV has been interesting and is still unfolding. However, because much of the existing information about the North American strain of VHSV is yet unpublished, a review of the current knowledge may help direct future investigations.

BRIEF REVIEW OF VHSV IN EUROPE

Discovery and pathogenesis

The European strain of VHSV has been isolated from fish in most countries of continental Europe (1) including the western Soviet Union (2) and the British Isles (3). The disease caused by VHSV was first described, possibly as early as 1931, by Schaperclaus (4) who suspected a viral etiology. The disease, originally called "infectious kidney swelling and liver degeneration" was later known as Egtved Disease, denoting the village in Denmark where the disease was first recognized. The viral etiology of the disease was established by Jensen (5, 6) in 1963.

Native European salmonids were believed to be the reservoir of VHSV which demonstrated remarkable virulence for rainbow trout (*Oncorhynchus mykiss*), a naive host imported from North America into Europe, first to France and then to Germany in 1879 and 1882, respectively (7). Causing an acute to chronic disease primarily of rainbow trout in freshwater, the virus can be transmitted in seawater (8) for up to 2 Km (Vestergard Jørgensen, personal communication) causing high fish mortality and a wide variation of clinical signs ranging from pale viscera with mild petechiation to ascites and considerable internal hemorrhaging. Juvenile fish are more severely affected by the virus with mortality approaching 100% but older fish may also develop the disease with mortality of 25–75% depending upon stressors present. Estimated economic losses in Europe due to VHSV may reach $40 mil per year (Vestergard Jørgensen, personal communication). The hemorrhaging became a hallmark feature of Egtved disease which was internationally named viral hemorrhagic septicemia (VHS) in lieu of any other synonyms (9).

Virus transmission is largely by contact with other infected fish or contaminated water, utensils, etc. at optimum water temperatures of 1–12°C with 15°C the uppermost limit (10). The primary portal of entry for the virus is considered to be the gills (11–13). Oral transmission of the virus has been accomplished by feeding infected fish (14) but vertical transmission within the egg has yet to be demonstrated and is considered extremely rare or nonexistent (15). VHSV can be transmitted on the surface of the egg and by regurgitated infected fish from piscivorous birds, but the virus does not appear to survive passage through the avian gastrointestinal (GI) tract (16). Fish surviving the disease may become asymptomatic carriers shedding the virus in urine (13, 17) and female sex products but not feces or seminal fluid (15). Except from fish with active cases of the disease, the virus is best detected at spawning or during low water temperatures (56).

Physical, serological and molecular characteristics

VHSV was first confirmed as a rhabdovirus by Zwillenberg et al. (18) having dimensions of 70 × 180 nm that remained relatively unchanged in later investigations. This virus, like other members of the Rhabdoviridae, has a single-stranded, negative-sense RNA genome (19, 20), a lipid containing envelope and five structural proteins (21–23) that include an RNA polymerase, a glycoprotein, a nucleoprotein and two smaller proteins of which at least one is phosphorylated (24, 25). Earlier serological characterization of European isolates of VHSV by virus neutralization tests using polyclonal rabbit antisera, established three major strains or subtypes of the virus. These included the original F1 isolate (Egtved virus) as strain I (5), isolate 61 and three others as strain II (26) and an isolate from a brown trout, first designated 23/75 by de Kinkelin and Le Berre (27), as strain III. However, more recent studies involving

neutralizing monoclonal and polyclonal antibodies against 90 European VHSV isolates suggested that all Egtved virus isolates share significant antigenic relatedness making them members of a common group with most isolates belonging to a single serotype (28).

Susceptible fish species and cell culture characteristics

Several species of fishes including certain salmonids, grayling, whitefish, pike, seabass and turbot are susceptible to the European strain of VHSV (Table 1). Other salmonids, Eurasian perch and several minnows are refractory to the virus (Table 1). An isolate of VHSV serologically indistinguishable from the F1 strain was recovered from Atlantic cod (*Gadus morhua*) having the ulcus syndrome (29). However, some have suggested this isolate might have resulted from contamination of the fish surface or of the laboratory cultures with virus from a freshwater source (30).

VHSV is best isolated using cell cultures inoculated with kidney/spleen tissue homogenates from fish having an active case of the disease. The virus can also be isolated from the same tissues, brain or ovarian fluid collected from asymptomatic fish (1). Established fish cell lines that have been effective for isolating Egtved virus include: BF-2, CHSE-214, EPC, FHM, PS, RTG-2 and STE-137 (1). Inoculated cell monolayers should be incubated at 15°C and the pH of the culture medium must be maintained between 7.4 and 7.8 because cytopathic effect (CPE) may be inhibited outside this pH range (6, 31). The virus is stable at -20°C for several years but is inactivated by ether, chloroform, glycerol and standard disinfectants including ultraviolet light.

Table 1. Finfish species that are susceptible or refractory to Egtved virus (adapted from Wolf [1])

Susceptible finfishes
Atlantic salmon (*Salmo salar*)
Brook trout (*Salvelinus fontinalis*)
Brown trout (*Salmo trutta*)
Golden trout (*Oncorhynchus aguabonita*)
Grayling (*Thymallus thymallus*)
Lake Trout (*Salvelinus namaycush*)
Northern pike (*Esox lucius*)
Pollan (*Coregonus lavaretus*)
Rainbow trout (*Oncorhynchus mykiss*)
Sea Bass (*Dicentrarchus labrax*)
Turbot (*Scophthalmus maximus*)
Whitefish (*Coregonus* sp.)
Hybrid (rainbow trout × coho salmon) (*O. mykiss* × *O. kisutch*)
Refractory finfishes
Common carp (*Cyprinus carpio*)
Chinook salmon (*O. tshawytscha*)
Chub (*Leuciscus cephalus*)
Coho salmon (*O. kisutch*)
Goldfish (*Carassius auratus*)
Eurasian perch (*Perca fluviatilus*)
Roach (*Leuciscus rutilus*)
Tench (*Tinca vulgaris*)
Hybrid (triploid rainbow trout × coho salmon) (*O. mykiss* × *O. kisutch*)

Control measures

Beginning in Denmark during 1965, successful control of Egtved virus has been accomplished in several areas of Europe by use of virus-free water supplies, eradication of virus-infected fish stocks, disinfection of rearing facilities, and repopulation of hatcheries with certified virus-free broodstock (31, 32, 58). There is also a strong tendency for rainbow trout to eliminate virus infections at warm water temperatures of 15–20°C provided that there is no source for re-infection (Vestergard Jørgensen, personal communication). Fish farms free of VHSV are registered and inspections for VHSV are conducted yearly at each facility with tight control on fish exports and imports. Control efforts pertain only to hatchery fish while wild or feral fish remain as reservoirs of the virus.

VHSV IN PACIFIC SALMON

Case histories — 1988

Concern that VHSV could be introduced from Europe with imported salmonids produced federal regulations in the United States and Canada known as Title 50 and Fish Health Protection Regulations, respectively. These regulations require all salmonid fish and fresh fish products be inspected for Egtved virus prior to importation into the United States or Canada. However, in 1988, a virus that was serologically indistinguishable from the F1 strain of VHSV was isolated from fish at two Washington State hatcheries; from returning adult chinook salmon (*Oncorhynchus tshawytscha*) at the Glenwood Springs Hatchery on Orcas Island in northern Puget Sound and from returning adult coho salmon (*O. kisutch*) at the Makah National Fish Hatchery approximately 75 miles away near Neah Bay on the Washington coast (Fig. 1).

1. At Glenwood Springs, 68 fall chinook salmon were sampled from a spawning population of 393 adults. VHSV was recovered using CHSE-214 cells inoculated with one of the kidney/spleen pools of the 19 tested (each pool contained tissues from 5 or fewer fish). Only 1 well of 6 replicate wells inoculated with tissue homogenates was positive and ovarian fluids, inoculated on EPC cells, were negative for virus (33).

2. During December of 1988, VHSV was isolated using EPC cells inoculated with 9 five fish pools of ovarian fluids collected from 300 female adult coho salmon returning to the Makah National Fish Hatchery (34). CHSE-214 cells inoculated with the same samples were negative for virus. Kidney/spleen pools from 60 coho salmon were negative for virus on both cell lines as were tissue samples and ovarian fluids from 209 chum salmon (*O. keta*), 258 chinook salmon and 83 steelhead trout (*O. mykiss*). Later in February, VHSV was detected in pooled kidney/spleen tissues from 60 yearling coho salmon and 60 juvenile steelhead trout as well as pooled whole body homogenates from 15 fall chinook salmon fry. These juvenile fish apparently were infected with the virus through exposure to recycled water from the infected coho salmon broodstock, however no mortality was associated with virus infection. Again, virus-positive samples were only observed on inoculated EPC cells where the CPE was similar to that for IHNV with rounding of cells and plaque formation that took up to 14 days in some cases (34). The CHSE-214 cell line was later found to be less sensitive than EPC cells for isolation of this virus (35).

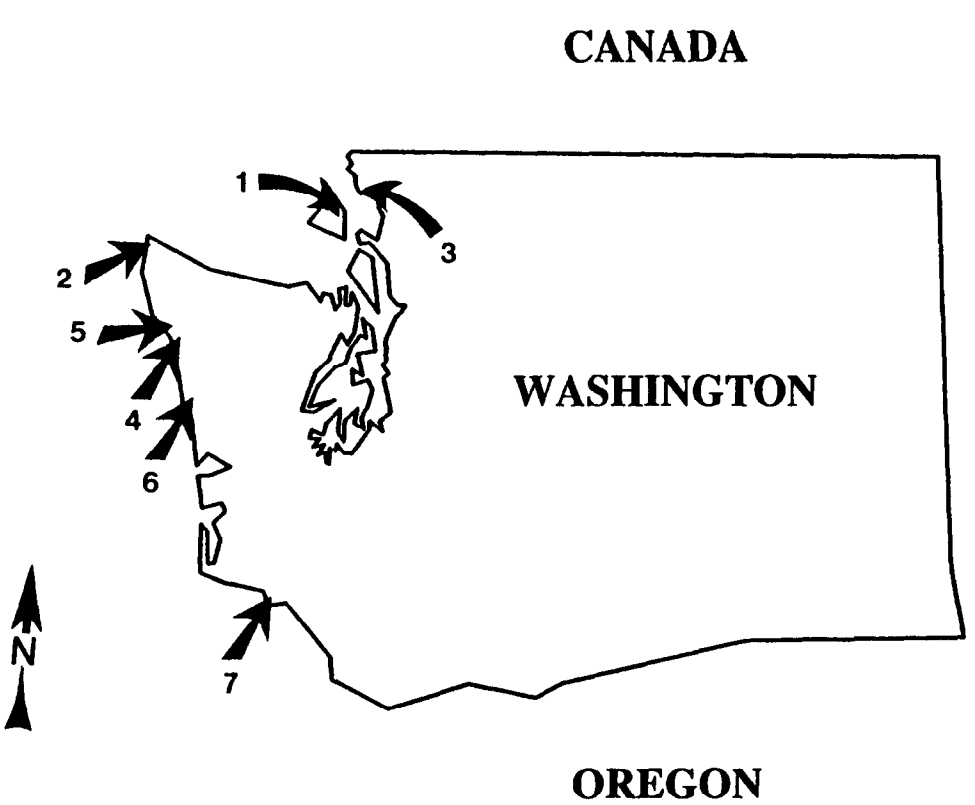

Fig. 1. Locations in Washington State, USA where North American VHSV has been isolated from chinook and coho salmon in 1988, 1989, 1991 and 1994: (1) Glenwood Springs Hatchery; (2) Makah National Fish Hatchery; (3) Lummi Bay Seaponds; (4) Bogachiel River; (5) Soleduck River Hatchery; (6) Queets River/Shale Creek Hatchery; (7) Elokomin River Hatchery.

Case histories — 1989

During the spawning season of 1989, intensive sampling of salmonids (in some cases 100% of returning fish populations) throughout the Pacific Northwest and Canada yielded isolations of VHSV from two more locations.

1. In November and December, adult coho salmon that had returned to seaponds at Lummi Bay Hatchery (36) in Bellingham, Washington (Fig. 1) were positive for VHSV in a pooled sample of 14 ovarian fluids (90 fish tested) inoculated onto EPC cells. Similar samples taken from an additional 1232 fish before and afterwards were negative for virus.

2. Also in 1989, a second isolation of VHSV was made from wild adult coho salmon returning to the Soleduck and Bogachiel Rivers (Fig. 1) in Washington State (37). Fish were captured in the wild and moved into separate holding areas supplied by virus-free water at the Soleduck Hatchery for maturation and virus sampling. Virus was isolated from 1 five-fish pool of ovarian fluid from the Bogachiel stock and from 1 five-fish pool of milt from the Soleduck stock.

Case histories — 1991

During 1990, VHSV was not detected anywhere in the Pacific Northwest or Canada. However, in December of 1991 the virus was again isolated from 1 five-fish pool of ovarian

fluid collected from 31 female Clearwater River coho salmon (B. Stewart, unpublished, Northwest Indian Fisheries Commission, Olympia, WA). These were wild fish spawned at the Shale Creek facility on the Queets River system of the Western Olympic Peninsula in Washington State (Fig. 1). Typical plaques appeared on EPC cells after 4 days with an estimated titer of 5×10^4 plaque forming units (PFU). Pooled seminal fluids from 37 males were negative for virus.

Case histories — 1994

No additional North American isolates of VHSV from salmonids were reported until 1994. Adult coho salmon returning to the Elokomin River Hatchery in Washington State on the north shore of the lower Columbia River (Fig. 1) were positive for VHSV in 1 of 12 pools (5 fish each) of ovarian fluids taken on November 8, 1994 (Pacific Northwest Fish Health Protection Committee newsbrief).

Identification of Pacific salmon VHSV isolates

The Orcas Island and Makah virus isolates were found to be structurally and serologically indistinguishable from the European F1 VHSV (38). Transmission electron microscopy (TEM) of CHSE-214 cells infected with each isolate showed bullet-shaped virus particles with helical symmetry typical of rhabdoviruses. The new virus isolates were completely neutralized by rabbit antisera against the F1 serotype of VHSV but not by antisera against infectious hematopoietic necrosis virus (IHNV) or hirame rhabdovirus (HRV) and infected cell monolayers were stained by fluorescent antibody tests (FAT) using VHSV-specific conjugate. Polyacrylamide gel electrophoresis (PAGE) revealed 5 viral structural proteins having molecular weights typical of viruses in the genus *Lyssavirus*. The protein profiles of the new isolates were unlike those of IHNV and HRV but identical to each other and to those of the VHSV F1 strain. Proteins of the new virus isolates were separated by PAGE and analyzed by western blot assay using antisera against IHNV, HRV and VHSV. Antiserum against VHSV recognized the G, N, M1 and M2 structural proteins of the F1 strain of VHSV and those of both the new isolates. Collectively, this data was evidence that the new isolates were VHSV with a close antigenic relationship to the F1 serotype.

Sequence analyses of the North American isolates of VHSV yielded further information regarding their relatedness with European VHSV and possible origins (39). Utilization of T1 ribonuclease fingerprinting showed that the North American isolates of VHSV formed a single fingerprint group (40). Four isolates from salmonids (Orcas Island, Makah, Bogachiel, Lummi) were highly similar to each other while four European isolates (F1, 07–71, 43–84, Atlantic cod) formed a second fingerprint group. The genetic diversity among isolates within each fingerprint group was less than 5% while the diversity between the two groups was greater than 5% (40). Additional supportive data came from comparison of the nucleoprotein gene sequence of the Makah isolate with that of the European VHSV reference strain 07–71 where a divergence of more than 13% was found (41).

Based upon this data, Batts et al. (42) developed biotinylated DNA probes constructed to hybridize with specific sequences within the messenger RNA (mRNA) of the nucleoprotein (N) gene of VHSV reference strains from Europe (07–71) and North America (Makah). One probe recognized a 29-nucleotide sequence near the center of the N gene common to both North American and European strains of VHSV. A second probe was specific for North American VHSV strains recognizing a nearly unique 28-nucleotide sequence following the

open reading frame of the Makah N gene mRNA. A third probe was specific for the European strain of VHSV recognizing a 22-nucleotide sequence within the 07–71 N gene that had 6 mismatches with the Makah strain. Although 16 VHS isolates from both continents were antigenically related, these probes demonstrated that VHSV isolates from North America and Europe formed 2 genetically distinct strains whereby isolates from different years or species of fish from each continent were more related to each other than to isolates from the other continent.

Both the fingerprinting and DNA probe studies were critical in establishing that the North American VHSV isolates were enzootic and not of European origin due to some recent importation of fish. This information, together with the following case histories and biological characteristics of the North American isolates, suggests the origin is one or more reservoir fish species in the North Pacific Ocean. All the salmonid isolates were from adult fish, freshly returning from the Pacific Ocean (except the isolates from juvenile fish that acquired the infection from maturing adult fish held at the Makah Hatchery). Examination of indigenous freshwater fishes within the same watersheds failed to detect VHSV. The stability of the North American isolates in saltwater was much greater than in freshwater after 1 hr (38). Virus titer reductions were 200-fold in freshwater compared to 10-fold in saltwater. As a comparison, F1 VHSV was slightly more stable in saltwater as well with a titer reduction of 2-fold compared to 50-fold in freshwater. Other biological differences were that the progression of CPE from the North American VHSV isolates was less affected by cell culture pH and they did not grow well in rainbow trout cell lines (35).

Susceptible fish species

Unlike Egtved virus, the North American isolates were unexpectedly avirulent for rainbow trout. Again, this suggested that these viruses were not typical of European isolates but represented an indigenous divergent strain. Replicate groups of 25 rainbow trout (3g) exposed to waterborne virus challenges (10^5 PFU ml^{-1}) for 1 hr of the Orcas Island and Makah isolates sustained 6% (3/50) and 8% (4/50) mortality, respectively, during 15 days compared to no mortality in control fish (38). Similarly exposed coho salmon (2g) sustained mortalities of 4% (2/50) and 2% (1/50), respectively (38).

Control measures

When North American VHSV was first confirmed on February 17, 1989, the information now known about its potential origin was limited and policies requiring destruction of fish and disinfection of facilities were already in place. At Glenwood Springs Hatchery, all fish (approximately 500,000) were buried, the water supply was chlorinated, and the dirt bottom ponds were drained and surfaces covered with unslaked lime. Sediment samples tested prior to pond treatment were negative for virus (33). At Makah Hatchery, a similar depopulation and disinfection of the site resulted in the killing of all fish (approximately 4.5 mil) in late February of 1989 (34).

The Lummi Bay VHSV isolate in the fall of 1989 (36) resulted in the destruction of all stocks on site and complete facility disinfection. However, because the Skookum Creek Hatchery had received nondisinfected green coho salmon eggs from VHSV-positive parents at Lummi Bay, those eggs and others that were potentially exposed in the incubation area were also destroyed followed by disinfection. Yearling coho in outside asphalt ponds at Skookum Creek were not destroyed but monitored for VHSV. No virus was ever detected in these fish.

Between the two facilities, a total of 6 mil. coho salmon eggs and fry, 0.5 mil. pink salmon (*O. gorbuscha*) fry, and 150,000 fall chinook fry were buried in January of 1990.

The Soleduck Hatchery VHSV episode was less fortunate in that some fish and eggs had been moved prior to the discovery of VHSV. Due to an earlier detection of IHNV at the site, certain stocks were in isolation; however, all fish stocks at the hatchery were subsequently quarantined to the Bogachiel and Soleduck systems which would be monitored for VHSV (37). The 1991 isolation of VHSV from Clearwater coho salmon resulted in the destruction of all eggs and milt taken on that particular spawning day. These had been transported to the Quinalt Indian Nation's Lake Quinalt Hatchery for fertilization and incubation where the incubation units holding the fertilized eggs were sanitized and the effluents from the other incubation units were chlorinated for a period after the discovery of the virus. By 1994 enough data had been accumulated that the isolation of VHSV from adult coho salmon returning to the Elokomin River Hatchery resulted in a limited response consisting of the redisinfection of the eggs from VHSV-positive adults, no destruction of any fish or eggs, monitoring of progeny fry, and restrictions on movement of fish outside the Elokomin drainage.

During the years following the first detection of VHSV in North America, control measures for VHSV were modified and integrated on a larger scale encompassing the entire Pacific Northwest as directed by the Pacific Northwest Fish Health Protection Committee (PNFHPC). Established in 1984, the PNFHPC is a consortium of pathologists and administrators from state, federal, tribal and private agencies from Washington, Oregon, Idaho, Montana, California, Alaska and British Columbia, Canada. The PNFHPC meets every 6 months to discuss regional fish disease issues and formulate cooperative disease policies when necessary. This committee was very instrumental in guiding the response and research regarding these first isolations of VHSV in North America.

VHSV IN PACIFIC COD

Case histories

The discoveries of VHSV in Washington State salmonids caused a hightened awareness of VHSV and an increase in surveillance for the virus throughout the Pacific regions of the US and Canada. Fish pathologists within the Alaska Department of Fish and Game (ADF&G) scrutinized their own salmonid stocks for VHSV and found no such virus. Also during this period, ADF&G pathologists were investigating the etiology of skin lesions observed in up to 30% of Pacific cod (*Gadus macrocephalus*) from certain areas including Prince William Sound (PWS) and the Bering Sea. These skin lesions comprised ulcerations and papules similar to those described in Denmark for Atlantic cod having the Ulcus Syndrome (29, 57) from which VHSV F1 and an iridovirus were isolated. Skin lesions from over 100 Pacific cod taken from Alaskan waters were tested for virus on EPC cells. Subsequently, the North American strain of VHSV was isolated from sport-caught cod in PWS in 1990, 1991 and 1993 (44–46). These were the first isolations of VHSV from Pacific cod, a new host species. Kidney and spleen tissues available from the single cod examined in 1990 were negative for virus despite the virus-positive skin lesion. The one cod tested in 1993 yielded virus from both the skin lesion and from a pool of gut/liver tissues. On initial incubation, the cod isolates typically exhibited CPE in EPC cells within 6–9 days in the wells inoculated with the highest dilutions showing titers of at least 10^3 g^{-1} of tissue. Subsequent virus passages in cell culture

produced lower apparent virus titers when plaqued with a semi-solid overlay than when measured by $TCID_{50}$. Viral CPE was slower and titers were lower using CHSE-214 cells than with the EPC and BF-2 cell lines (45).

Histological examination of a skin lesion from the Pacific cod collected in 1990 showed X-type cells and plasmodial forms of a protozoan as the most likely etiology of the lesion. Bacterial organisms were also observed within lesion material, although none were isolated on tryptose soy agar (45).

Identification of Pacific cod VHSV isolates

Ultrastructural examination of EPC cells infected with the 1990 and 1991 cod isolates showed typical rhabdovirus particles with dimensions typical of those reported for VHSV (60–70 nm × 170–180 nm) with occasional longer particles of 400 nm having slightly smaller diameters (Fig. 2). Cod isolates from 1990 and 1991 were neutralized in cell culture and recognized on immunoblot assay using antiserum against the European F1 VHSV (45). All three cod isolates were recognized by the DNA probe (42) specific for the North American strain of VHSV (Fig. 3) (45). Additional T1 ribonuclease fingerprint studies with the 1990 cod isolate showed that it belonged to the North American fingerprint group of VHSV and was similar to isolates from salmonids in Washington State (40).

Susceptible fish species

Like the Pacific salmonid isolates, the Pacific cod isolate of VHSV from Alaska was relatively avirulent for certain salmonid species. Separate groups of 50 juvenile ($\leq$ 2g) coho, chinook, sockeye (*O. nerka*) and pink salmon and rainbow trout were given 1 hr static freshwater challenges of a low (10^3 PFU ml^{-1}) and high (10^5 PFU ml^{-1}) dose of the 1990 Pacific cod VHSV isolate. Afterwards fish were placed in flowing freshwater at 10°C for 30 days of observation. A significant cumulative mortality, 12% above the control group of fish exposed to tissue culture fluid only, was observed among the rainbow trout exposed to the high dose of VHSV. Moribund trout in this group exhibited external petechial hemorrhages and virus was recovered from visceral organs at relatively high titers (10^4–10^5 g^{-1}). No mortality was observed among, nor was virus recovered from, any other groups of exposed fish (ADF&G, unpublished).

VHSV IN PACIFIC HERRING (TABLE 2)

Case histories — Alaska 1993

1. In 1993, Pacific herring (*Clupea harengus pallasi*) from PWS, Alaska were tested for virus. These fish showed varying degrees of external ulceration or hemorrhage beneath the skin, at the bases of fins and around the vent accompanied by lethargic swimming behavior in 15–43% of those fish returning to the spawning grounds. Over 3000 spawning or spawned herring were collected from 8 locations in PWS for field examination between April 17–23 (Fig. 4). Affected herring represented all age classes of recruitment. Particularly disturbing was the very low number of herring returning to spawn (about 37% of the expected total of 134,000 tons). All year classes were represented in this decline which prompted closure of the herring seine fishery. VHSV was isolated from these herring using kidney/spleen tissues from 1 of 2 five-fish pools and from 2 five-fish pools collected at two different locations (Graveyard

Fig. 2. Electron micrograph of Pacific cod VHSV in an infected EPC cell showing viral particles outside the cell (A) and within cell vacuoles (B) and probable unassembled capsid material within the cytoplasm (C) (bar = 1 μm) (taken from Meyers et al. [44] by permission).

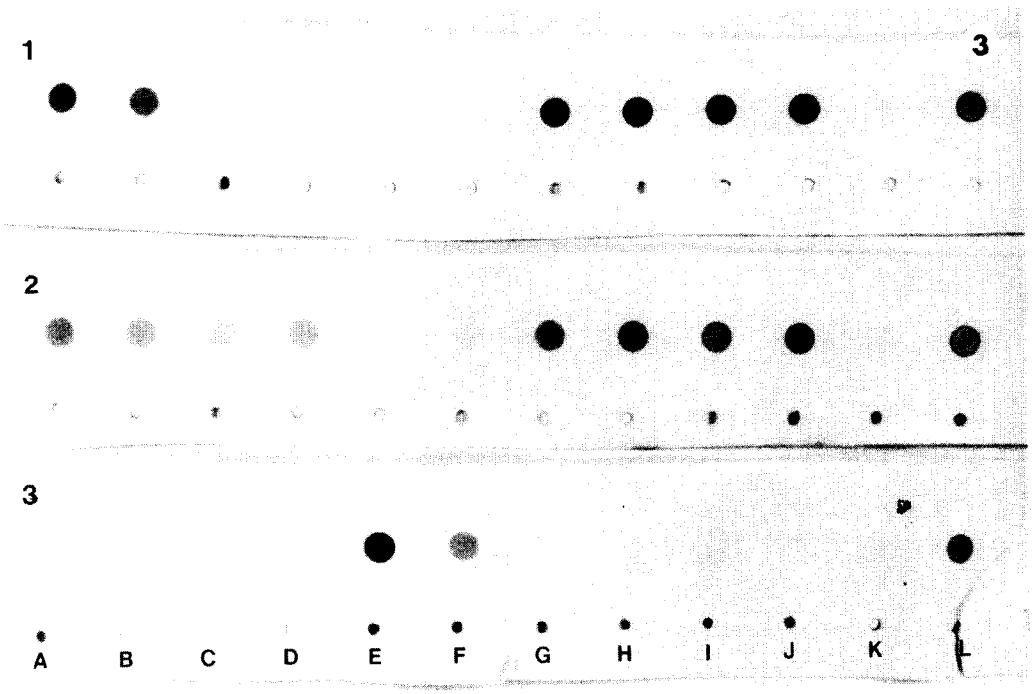

Fig. 3. DNA probe assay: nitrocellulose strips were spotted with 3 isolates of VHSV from Pacific herring *Clupea harengus pallasi*, 1 VHSV isolate from Pacific cod *Gadus macrocephalus*, 4 isolates of reference VHSV and 2 isolates of reference IHNV. They were reacted with the North American VHSV DNA probe (strip 1), European VHSV DNA probe (strip 2) and an IHNV DNA probe (strip 3). All viruses were replicated in either EPC or CHSE-214 cells for 48 hr. Strips 1, 2, 3: (A) 1988 Makah National Fish Hatchery, Washington reference VHSV; (B) 1990 Pacific cod reference isolate of VHSV from Prince William Sound (PWS), Alaska; (C) 1971 European 07–71 reference VHSV from France; (D) 1975 European 23–75 reference VHSV from France; (E) 1980 Cedar River, Washington reference IHNV; (F) 1976 Round Butte, Oregon reference IHNV; (G) 1993 Pacific herring VHSV isolate from Graveyard Point PWS; (H) 1993 Pacific herring VHSV isolate from Red Head, PWS; (I) 1993 Pacific cod VHSV isolate from Mud Bay, PWS; (J) 1993 Pacific herring VHSV isolate from Barling Bay, Kodiak Island, Alaska; (K) MEM-10; (L) homologous PCR positive control (taken from Meyers et al. [46] by permission).

Point; Red Head) and from 1 of 2 four-fish pools from a third group of herring taken from Barling Bay off Kodiak Island (46). Herring from Kodiak Island had similar external hemorrhages but the spawning returns there were normal and a productive fishery ensued. This was the first isolation of VHSV from Pacific herring, a new host species for the virus. It was also during this sample period in 1993 that the VHSV-positive Pacific cod referred to earlier was collected nearby in Mud Bay of PWS (46). Twenty-five normal appearing adult herring collected from PWS in October, 1993 were negative for VHSV.

The PWS herring were examined for other pathogens using bacteriologic media, blood smears for detection of erythrocytic necrosis virus (ENV) and histologic methods. No significant presence of any fish pathogen was found other than VHSV (46). Histological lesions that appeared to be associated with VHSV infection included: varying degrees of passive congestion in large vessels and sinusoids of the liver (67/79 = 85%), spleen (50/74 = 68%), kidney (44/70 = 63%) and subcutis (15/41 = 37%); various sized "active" melanomacrophage foci of reticuloendothelial (RE) type cells in congested livers (23/67 = 34%), kidneys (8/44 = 18%), spleens (3/50 = 6%) and subcutis (26/41 = 63%); hemorrhaging in kidneys (21/41 = 51%),

Table 2. Prevalences of North American VHSV in Pacific herring from Alaska, British Columbia, Canada and Puget Sound, Washington

Location	Date	Prevalence
1. Alaska, USA		
Prince William Sound		
Graveyard Point	4/20/93	1/2 (pools)
Red Head	4/22/93	2/2 (pools)
several locations	10/9/93	0/25
Port Chalmers	4/7–13/94	4/30 (13%)
		5/10 (50%)
		1/10 (10%)
Rocky Bay	4/25–28/94	11/233 (5%)
Stockdale Harbor	4/13/94	2/20 (10%)
Green Island	11/6/94	0/20
Kodiak Island		
Barling Bay	4/22/93	1/2 (pools)
Lynn Canal, Juneau		
Auke Bay	5/16–17/93	20/46 (43%)
Shelter Island	5/10–13/94	22/157 (14%)[a]
Admiralty Island	5/5/93	0/9
Port Frederick, Hoonah Harbor	2/3/94	5/12 (42%)
Ward Cove, Ketchikan	6/16/94	0/17[b]
2. British Columbia, Canada		
Prince Rupert Sound	9/9/93	8/10 (80%)
Campbell River	10/10/93	13/60 (22%)
Sechlet, BC	10/19/93	25/60 (42%)
Departure Bay	9/93	0/70[c]
3. Washington, USA		
Puget Sound	10/93	5/12 (42%)
	11/21/93–4/18/94	7/8 sample dates positive

[a]Captive adult herring used for experimental oil exposure study.
[b]Mass juvenile herring mortality due to ENV.
[c]Herring were held captive for two months during virus testing.

spleens (6/50 = 12%) and subcutis (11/41 = 27%); minor to extensive degeneration and pyknosis of the tubular epithelium in congested kidneys (24/44 = 55%) (46). Other incidental findings included: *Icthyophonus hoferi* in various tissues (4/79 = 5%); mostly low numbers of *Goussia clupearum* in livers with little or no cell damage or host tissue reaction (32/79 = 41%); myxosporidian pansporoblasts in the gall bladder of one fish and in the mesonephric ducts of the kidney in three others; unidentified nematodes encysted in the mesenteries of 2 fish (46). None of these lesions or parasites appeared to be acutely life threatening except for the skin lesions associated with VHSV.

2. To determine susceptibility of herring to the herring VHSV isolates, juvenile herring from Auke Bay in Juneau, Alaska were collected on May 16 and 17 for virus exposure studies. Subsequent holding mortality and isolation of VHS virus from 43% of 46 control fish terminated further herring *in vivo* studies (46). A concommitant infection by ENV was also present in about 70% of these fish which probably contributed to the mortality. Viral CPE in VHSV-positive samples occurred from 3–13 days in EPC cells with titers as high as 10^7 $TCID_{50}$ ml^{-1} while CHSE-214 cells were slower to show virus replication and failed to

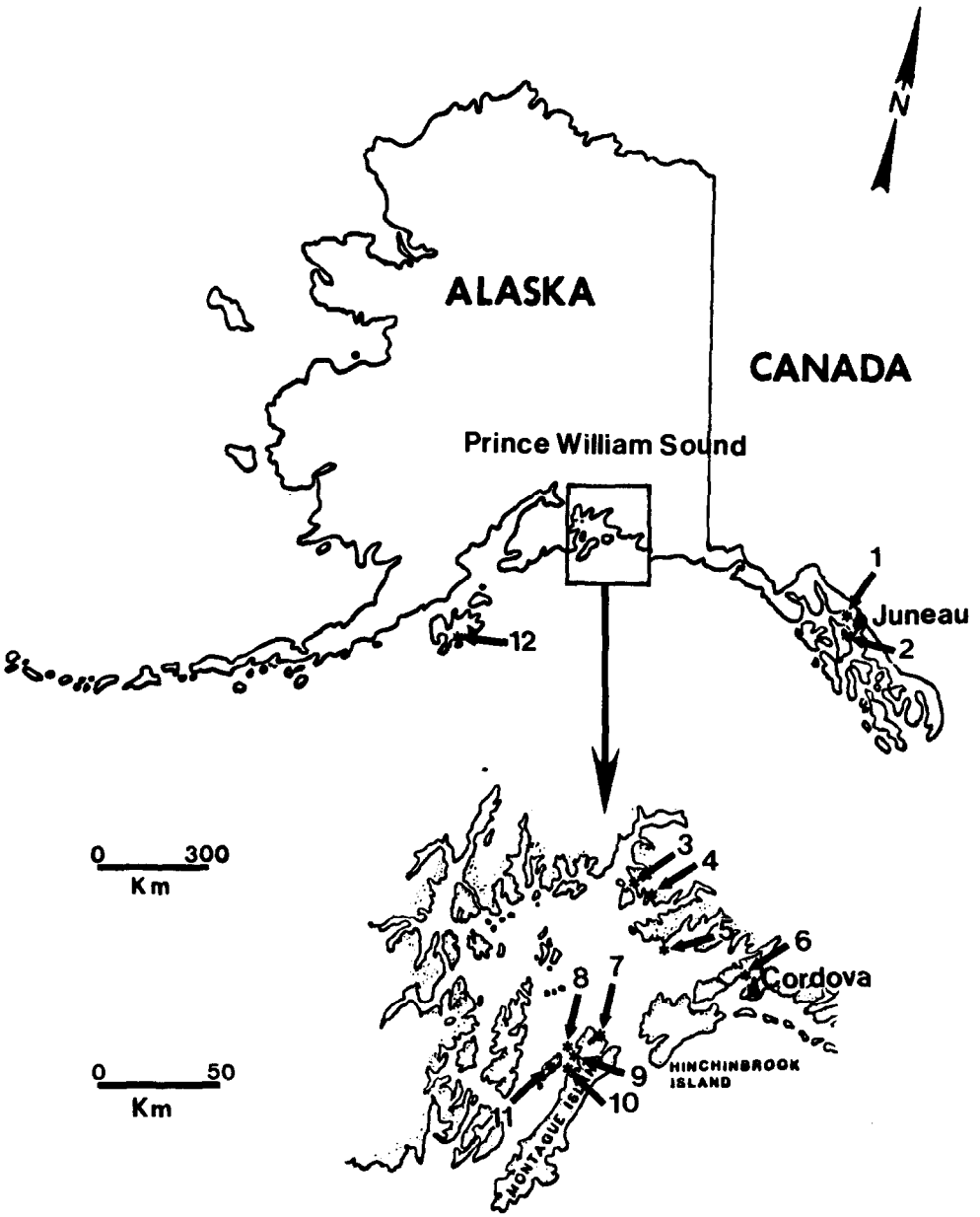

Fig. 4. Locations in Alaska where Pacific herring *Clupea harengus pallasi* and a Pacific cod *Gadus macrocephalus* were sampled for skin lesions during April and May, 1993: (1) Auke Bay; (2) Oliver Inlet; (3) Virgin Bay; (4) Landlocked Bay; (5) Red Head; (6) Mud Bay; (7) Rocky Bay; (8) Graveyard Point; (9) Stockdale Harbor; (10) Port Chalmers; (11) Green Island; (12) Barling Bay (taken from Meyers et al. [46] by permission).

show CPE when incoculated with samples containing low virus titers. Higher titered samples inoculated onto EPC cells often failed to produce CPE at the lowest dilution (10^{-2}) suggesting

defective virus particle interference. Earlier TEM studies of Pacific cod VHSV replicating in EPC cells showed considerable amounts of apparent unassembled capsid material (44) in the cell cytoplasm that may contribute to this observed interference at high virus concentrations (Fig. 2).

Case histories — British Columbia 1993

Isolates of the North American strain of VHSV were obtained from visceral organ pools of Pacific herring collected in the fall of 1993 at these locations in British Columbia, Canada (47): Prince Rupert Sound on September 9 from 8/10 fish after reports 11 days earlier of a diesel fuel spill and dead herring with external lesions and hemorrhages; Campbell River, Vancouver Island on October 10 from 13/60 fish (15–20 cm) from a bait pond — no external lesions or mortality present; and the Sechlet area on October 19 from 25/60 herring obtained from a bait supplier — no external lesions or mortality. Herring (4.2g) collected in September from a fourth site, Departure Bay, were negative for VHSV after testing 70 captive fish during a two month period.

Case histories — Puget Sound, Washington 1993–1994

1. Five of 12 samples of viscera from Pacific herring collected in late October from a bait shop in Puget Sound, Washington were positive for VHSV with titers ranging from 2×10^3 to 9×10^4 PFU ml^{-1} (J.R. Winton and W.N. Batts, unpublished, National Biological Service, Seattle, Washington, USA). Six of the 12 fish had degrees of external hemorrhages which correlated well with the occurrence of light to moderate levels of ENV infections. The bait dealer apparently obtained the herring in the commercial net fishery operating in the vicinity of Tacoma, Washington. Pooled herring sera had no detectable neutralizing antibody against VHSV but the endogenous virus replicated over 1000-fold in fin tissues excised and incubated for 72 hr in tissue culture medium while skin explants produced only 100-fold increases of virus after 7 days of incubation (Winton and Batts, unpublished). These results indicated that North American VHSV and, as determined subsequently, European VHSV could both replicate in herring fin tissues *in vitro*.

2. Several additional VHSV isolates were made from herring collected from another bait dealer in south Puget Sound near the Tacoma Narrows. Herring collected from this dealer were positive for VHSV in 7 out of 8 sample dates between November 21, 1993 and April 18, 1994 (R. Brunson, unpublished, Fish and Wildlife Service, Olympia Fish Health Ctr., WA, USA).

Case histories — Alaska 1994

1. A mortality among juvenile 2 year old herring (4.4–6.9 g; total length 98–110 mm) was reported in Hoonah Harbor of Port Frederick on Chichagof Island about 40 miles southwest of Juneau on February 3 (Meyers, unpublished lab. report, Acc. No. 94–0571). Large numbers of dead herring were visible at low tide and moribund fish reportedly swam in a spiral motion at the seawater surface. Large schools of apparently normal herring in the tens of thousands were present as well. Four of the 12 herring examined had low to high levels of ENV while at least 5 (2 fish were pooled) fish had moderate to high titers of VHSV ranging from (3.16×10^4 to 5.62×10^7 $TCID_{50}$ ml^{-1}) which correlated well with those fish showing external hemorrhages. Natural epizootics caused by ENV have been associated with mass mortality of juvenile herring in Alaska but external hemorrhaging has not been a clinical sign of infection (48). Unlike the Auke Bay juvenile herring, there was almost no correlation between fish infected with ENV with those infected with VHSV except for one fish where both viruses

were detected. The herring mortality in Hoonah Harbor, involving hundreds rather than thousands of juvenile herring, subsided within 2–3 weeks.

2. Additional adult herring were sampled from PWS during April, May and November of 1994 due to the lower than expected returns to spawning areas, resorbing gonads prior to spawning, and external skin hemorrhages among 21% or less of the fish that were present in PWS. Virus titrations of more than 300 individual herring collected from Stockdale Harbor, Rocky Bay and Port Chalmers (Fig. 4) showed prevalences of VHSV that correlated with the occurrence of congestion or hemorrhages of the skin among the fish examined. However, herring collected in early November near Green Island in PWS were negative for VHSV (0/20) despite apparent skin lesions on 10 of the fish.

In Stockdale Harbor, herring skin lesions ranged from barely visible pinpoint reddening to moderate subsurface hemorrhaging (field prevalence not provided). Among 10 fish examined with skin lesions, only two had substantial skin hemorrhaging. One of these fish was positive for VHSV with a titer of 3.16×10^4 $TCID_{50}$ ml^{-1} in the skin lesion while a kidney/spleen pool from the same fish was positive for virus but only after a blind passage. Among 10 fish examined without lesions, only one kidney/spleen pool was positive for VHSV after a blind passage indicating a low level of endogenous virus. No virus was detected using CHSE-214 cells inoculated in parallel and blood smears from these fish were negative for ENV (0/20).

Herring from Rocky Bay had a relatively low prevalence of skin lesions (less than 5% as reported by field biologists) with a prevalence of VHSV in randomly sampled herring of 4.7% (11/233). Virus titers in these fish ranged from less than 10^1 (detected by blind passage) to 3.16×10^6 $TCID_{50}$ ml^{-1} which came from a skin lesion. Four fish had skin lesions that were positive for VHSV but three of these fish had kidney/spleen pools that were negative for virus. The severity of the virus-positive skin lesions varied from mild to severe but virus titers of the mild lesions were often much higher than the titers in the severe lesions. The other positive fish produced kidney/spleen pools having initial cell culture titers ranging from 3.14×10^3 to 3.14×10^5 $TCID_{50}$ ml^{-1}. Blood and kidney impression smears from all fish indicated no prevalence of ENV (0/232).

Herring from Port Chalmers were collected in two groups. Fish in group I were randomly collected and had a VHSV prevalence of 13% (4/30) with 2 fish having virus titers of 3.16×10^4 $TCID_{50}$ ml^{-1} and 2 others requiring blind passage for virus detection. Fish in group II consisted of 10 fish with and 10 fish without skin lesions. There were five VHSV-positive fish in the lesion subsample and only one positive fish in the group without lesions. In the lesion subsample, two of the virus-positive fish had skin lesions with viral titers of 1.10×10^2 and 3.16×10^7 $TCID_{50}$ ml^{-1} while kidney/spleen pools from the same two fish were negative. Three other fish were positive for VHSV with kidney/spleen pools of two of these having titers of 3.16×10^4 and 10^7 $TCID_{50}$ ml^{-1} while the third fish required a blind passage for virus isolation. Once again, CHSE-214 cells were much less sensitive for initial virus isolation and required a blind passage to detect positive fish. Port Chalmers had the highest field prevalence of skin lesions in 1994 (reported as 21%) which coincided with the highest prevalence of virus detection in those fish having skin lesions. Blood smears from the group II fish were negative for ENV (0/20).

Overall, 27% (7/26) of the skin lesions from PWS herring tested for virus were positive for VHSV at titers of 10^2 to 10^7 $TCID_{50}$ ml^{-1}. Five of these fish had virus-negative kidney/spleen pools, 1 fish had a positive kidney/spleen sample after blind passage and 1 fish had a concurrently positive kidney/spleen pool which was 100-fold lower in virus titer. Among the remaining 19 fish with virus-negative skin lesions, only one had a kidney-spleen pool that was virus-positive.

3. Also in Port Chalmers, about 6 mil chum salmon fry were being maintained in two netpens in very close proximity to a school of herring having a biomass of about 200 tons. No significant losses of chum salmon fry were evident, but due to the occurrence of VHSV in these herring, 10 healthy and 10 moribund chum salmon were tested for virus. These fish were negative for virus (Meyers, unpublished lab report, Acc. No. 94–0581) suggesting that the VHSV being shed by herring was relatively avirulent for chum salmon via seawater exposure.

4. National Marine Fisheries Service (NMFS) personnel of the Auke Bay Laboratory conducted oil exposure studies on 4–5 year old adult Pacific herring collected from waters off Shelter Island in Lynn Canal near the Juneau area (Fig. 4) (M. Carls, personal communication, NMFS, Auke Bay Laboratory, Juneau, AK). Tissues from these fish were examined for a number of parameters including VHSV and ENV to determine if stress from oil exposure would affect the prevalence of either virus. Although detailed reporting of this study will be published elsewhere, the virus results are briefly described. Five groups of herring consisted of controls (unexposed) and fish exposed to trace, low, medium and high (300 ppb hydrocarbon) concentrations of oil continuously for 16 days. Fish were sampled at 16, 17 and 18 days after exposures began. Prior to the study, minor losses were observed, probably due to a flexibacterial infection. However, neither ENV (0/5) nor VHSV (0/4) were detected. Following the oil exposures, no VHSV was detected in control fish (0/31) but an increasing prevalence of VHSV was detected among groups exposed to trace (2/31 = 6.4%), low (5/35 = 14.3%), medium (5/30 = 16.7%) and high (10/30 = 33.3%) concentrations of oil (Meyers, unpublished lab report, Acc. No. 94–0599). The VHSV titers of these oil-exposed herring, expressed as $TCID_{50}\,ml^{-1}$ were: $<10^1$ in 7 fish (requiring a blind passage for virus detection); 3.14 to 5.62×10^2 in 2 fish; 5.62 to 3.16×10^4 in 12 fish; and 5.62×10^6 in 1 fish. Only one of these fish had external hemorrhages about the head and on the anal/pelvic fins. The increasing prevalence of virus suggested a dose response, probably due to increasing stress on the herring caused by increasing oil exposure concentrations. Stress of any kind has been suggested to bring on epizootics of VHSV in herring (46). This may explain the mortality and high prevalences of VHSV in herring exposed 11 days earlier to hydrocarbons from the reported diesel spill in Prince Rupert Sound, British Columbia (47).

ENV was present in these herring at a very low level (1/140) which did not appear to be influenced by oil exposure.

Identification of Pacific herring VHSV isolates

The 1993 virus isolates from herring in PWS (Graveyard Point, Red Head), Kodiak Island (Barling Bay) and Auke Bay were confirmed by DNA probe (Fig. 3) (42) as the North American strain of VHSV (46). The 1993 VHSV isolates from British Columbia and Puget Sound were also recognized by the DNA probe (Batts, unpublished), as were the 1994 herring isolates from Alaska (Meyers, unpublished).

Susceptible fish species

Studies regarding the susceptibilities of Pacific herring, shiner perch (*Cymatogaster aggregata*) and Atlantic salmon (*Salmo salar*) to Canadian herring isolates of North American VHSV have been conducted by researchers within the Department of Fisheries and Oceans of Canada at the Nanaimo, B.C. Pacific Biological Station (G. Traxler and D. Keiser, unpublished). All three fish species were challenged with virus via waterborne exposures ($1 \times 10^4\ ml^{-1}$) for 1 hr

or via intraperitoneal injections (9×10^2 per fish). Waterborne virus exposures resulted in significant mortality in both herring (8/30 = 26.7%) and shiner perch (3/8 = 37.5%) but not Atlantic salmon (0/12). Injection of VHSV caused significant mortality in all three species: herring = 4/25 (16%); shiner perch = 8/8 (100%); 200 g Atlantic salmon = 9/12 (75%); smaller Atlantic salmon (wt not given) = 3/15 (20%). No virus was isolated from uninjected control fish. Over 100 unexposed herring had been tested for virus and were negative for VHSV. Although water exposed or injected herring that died did not have skin hemorrhages, their kidneys and spleens were necrotic and samples of these tissues produced VHSV titers of 10^6–10^7 gm^{-1} of tissue (G. Traxler, personal communication). Clearly, North American VHSV could be pathogenic depending on the fish species and the route of virus exposure.

DISCUSSION

Host origin and transmission of VHSV in North America

Molecular studies proved conclusively that the VHSV strains isolated from fish in Washington, British Columbia and Alaska were not of European origin. Other biological features of the North American isolates also differed from the European isolates of VHSV suggesting their origin was one or more marine fish species in the North Pacific Ocean. The salmonid case history information was consistent with this assumption which was later confirmed by isolations of North American VHSV from Pacific cod and herring. The widespread occurrence of the virus in Pacific herring indicates that this strain of VHSV is enzootic from Kodiak Island, Alaska to Puget Sound Washington. While other marine fish species may be found to carry the virus, Pacific herring are a primary natural host and reservoir for the North American strain of VHSV with Pacific cod and occasionally adult chinook and coho salmon as incidental hosts and minor reservoirs. Because herring are a major forage base for predator fish species, including Pacific salmon, it is not surprising that VHSV may occasionally be transmitted to adult salmonids in the marine environment. This likely occurred with coho and chinook salmon in Washington State where the fish became infected with the virus through the GI tract after ingestion of infected herring. Virus contact with epithelial cells of the integument during exposure to large schools of VHSV-infected herring is another plausible route of infection. However, studies with one North American isolate (Makah) indicated only moderate replication of virus in fin tissues excised from rainbow trout and cultured *in vitro*. No replication was detected in excised gill or skin tissues nor in fin, gill or skin tissues from chinook salmon (49). Like salmonids, Pacific cod forage on herring and have been found in close proximity to large schools of the virus-infected baitfish where contact with virions is likely. Although few internal organs from cod have been available for testing, it appears that VHSV infections in cod are self-limiting to the skin and secondary to other pathogens that may be the primary causes of the skin lesions. The one positive liver/gut cod sample may have been due to virus in the gut following ingestion of infected herring rather than systemic infection of the liver.

The epitheliotropic nature of European VHSV has been described for salmonids in which the gills and epidermal cells of skin and fins were important foci of infection enhancing horizontal transmission (1, 49). Studies with North American VHSV show a similar tropism with significant VHSV replication in excised herring fin and skin tissues *in vitro* (Winton and Batts, unpublished) and presence of high virus titers in herring skin lesions but not in visceral organs from the same fish (Meyers, unpublished). Virus titrations of individual kidney/spleen

tissues and skin lesions from the same herring suggested the following: 1) infection of herring by VHSV can result in skin congestion and/or hemorrhaging from which virus can be isolated; 2) VHSV can be transmitted horizontally to Pacific herring in which skin and fin tissues are the initial sites of replication leading to a viremia and infection of kidney and spleen tissues; 3) the severity of the skin lesions does not necessarily coincide with virus titer as VHSV-positive herring can be asymptomatic having no skin lesions suggesting either skin lesions did not initially occur or the lesions were minor and resolved spontaneously. Finally, all skin lesions observed in herring were apparently not caused by VHSV. Other causes for hemorrhaging and even ulceration may include post-mortem changes, handling or other environmental stressors. In adult herring, ENV was either absent or present at such low levels that it could not have been a cause of the observed skin lesions. In juvenile fish ENV was present but hemorrhagic or ulcerative skin lesions in Alaskan herring have not been clinical signs of infection by this virus (48). This observation is logical since ENV infects peripheral erythrocytes and their precursor cells in the kidney causing anemia rather than hemorrhaging.

Epizootiology of North American VHSV

The epizootiology of VHSV in herring appears to be that of an opportunistic pathogen triggered by stress (46). This relationship is analogous to the association of ENV and herring in which ENV is enzootic but only causes occasional epizootic mortality in weaker juvenile fish when they are stressed (48). Mass mortality of juvenile herring due to ENV has occurred repeatedly in one location near Ketchikan, Alaska and in Auke Bay in 1989 (48, 50). In these epizootics, predisposing factors were strong juvenile year classes producing high fish densities in confined areas with bird predation during low tides. The prevalence of ENV infections among 3–5 year old Alaskan herring has ranged from 17–80% with no associated mortality or external lesions (48).

Epizootics of VHSV in herring have involved similar stressors including exceptionally strong year classes of fish, infection by other diseases such as ENV, the rigors of spawning in shallow water, harassment by predators, colder than normal winter seawater temperatures, pursuit and capture in a pound net fishery, acute exposure to pollutants such as hydrocarbons, and nutritional deprivation from reduced levels of forage. All but two of these factors were present in PWS just prior to and during the herring decline in 1993 (46). Variables not present in PWS at that time were significant levels of ENV and hydrocarbon exposure. ENV was nearly absent from the blood smears examined. The oil from the Exxon Valdez spill in 1989 had largely dissipated and had no demonstrated longterm effects on exposed herring that might make them more susceptible to pathogens such as suppression of their immune response. Microbiological and histopathological examinations indicated few other pathogens present which would not be the case if fish were immunosuppressed. Furthermore, normal cellular immune responses were evident against those incidental pathogens that were present in the herring tissues examined by histological methods.

As herring populations become stressed, VHSV may become more prevalent with higher titers per fish accompanied by some level of mortality. Mortality among juvenile herring is likely due to acute necrosis of liver and kidney caused by systemic virus infection while skin lesions predominate in older herring, suggesting a chronic condition and greater host resistance to the virus. The percentage of skin lesions in adult fish that progress to ulceration is not known and may be variable in each case. However, once ulcerated, these skin lesions become life-threatening for herring resulting in osmoregulatory shock, secondary microbial infections

and/or predation due to a weakened condition and altered behavioral pattern. It seems very unlikely that VHSV alone was responsible for the herring decline in PWS, but rather the epizootic was a result of a much larger and more complex ecological interaction composed of many variables that include normal cyclical fluctuations of the herring population itself.

North American VHSV and potential evolution of Egtved virus

Rhabdoviruses have an error-prone RNA polymerase that results in a high rate of mutation. This leads to a population of virions containing a large number of different genotypes (quasi-species) which are acted upon by selective pressures from the host or the environment to favor the most fit virus genotype which dominates over time. Thus, when the conditions of the environment change a different genotype may be rapidly selected that is better adapted to exist under the new conditions leading to a novel strain or even serotype of virus (51). This is an important mechanism by which viruses adapt to new conditions and evolve in nature. Among fish rhabdoviruses, the potential is great for evolution of new strains of virus resulting from the selective pressures of intensive fish culture conditions. These conditions include high rearing densities, novel host species, continuous addition of susceptible fish, different temperatures and the presence of fish immunized by exposure to wild type virus or vaccines (52).

Using existing protein electrophoretic data, fish rhabdoviruses have been placed into one of two established genera, *Lyssavirus* and *Vesiculovirus*, within which further subdivisions can be made based upon biological and serological relationships reported in the literature (52). Members of each group within a genus generally form a single serotype, but the groups are not unrelated as indicated by comparisons using fluorescent antibody tests (53) and western blot analysis (54) in which significant serological cross reactions exist between some of the groups. The North American isolates of VHSV are antigenically related to, but genetically different from, Egtved virus, therefore they represent a different strain of the same serotype.

Is it possible that Egtved virus evolved from the marine environment where it existed much like the North American strain of VHSV does today? Several arguments add strength to this hypothesis. Firstly, F1 VHSV is more stable in seawater than in freshwater suggesting a marine origin (38). Although saltwater stability was less pronounced than for the North American strains, the heritability of this trait could have become diluted by many years of selective pressure exerted by intensive freshwater culture of virus-susceptible fishes. Secondly, there is evidence that marine fish species are susceptible to European VHSV. The earlier isolation of F1 VHSV from Atlantic cod (29) was discounted as a laboratory contaminant (30); however, the isolate was less virulent for trout than typical European strains (P. Jørgensen, personal communication in [38]). Other studies have determined that turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*) are experimentally susceptible to the European isolates of VHSV (58). A natural outbreak of Egtved virus in cultured turbot was later reported in Germany (55). More recently, Egtved virus has been isolated from previously virus-free farmed turbot in Scotland (3) that were fed raw marine fish (B.J. Hill, personal communication, MAFF, Weymouth, UK). These natural outbreaks in turbot are most convincing that the marine environment is still a reservoir for the European strain of VHSV. The feeding of unpasteurized raw marine fish to intensively cultured hatchery fish is also a very plausible vehicle to explain how European VHSV may have been initially introduced to a freshwater host such as the rainbow trout prior to the use of high quality processed fish feeds.

Although the North American strain of VHSV is apparently enzootic in the North Pacific Ocean and appears to be relatively avirulent for most salmonids, its future importance or

threat to intensive salmonid culture should not be minimized. The rapid mutation rate of rhabdoviruses in response to novel conditions provides the potential for an isolate of the North American strain of VHSV to become as virulent as Egtved virus if introduced and allowed to perpetuate in an intensive salmonid culture environment. This is not unprecedented with regard to a similar rhabdovirus, IHNV, which has evolved into a strain capable of killing large trout as well as juveniles in the commercial hatcheries of the Hagerman Valley of Idaho, USA (38). Hence, very conservative methods should be employed to eradicate even the North American strain of VHSV if, and when, it is detected in stocks of live salmonids.

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