Incidence of Fish Pathogenic Viruses among Anadromous Salmonids in the Northern Part of Japan, 1976–1987

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ABSTRACT

During the period from September 1976 to December 1987, various species of mature salmonid fish, including masu (*Oncorhynchus masou*), chum (*O. keta*), pink (*O. gorbuscha*), kokanee salmon (*O. nerka*), charr (*Salvelinus leucomaenis*), and rainbow trout (*O. mykiss*), were examined to provide information on the distribution of pathogenic viruses in northern Japan. Virus inspections were conducted on ovarian fluids, mixed kidney and spleen specimens, epithelial tumor tissues, and blood samples. Four viruses were isolated during the course of this investigation. Infectious hematopoietic necrosis virus (IHNV) was found in the ovarian fluid of chum and masu salmon. *Oncorhynchus masou* virus (OMV), discovered in 1978 and specific to masu salmon, has been isolated from ovarian fluids and epithelial tumor tissues at 13 sampling sites. Chum salmon virus (CSV) was isolated from mixed kidney and spleen specimens from healthy chum salmon in 1978 and again in the ovarian fluids of masu salmon in 1987 at two localities on the coast of the Sea of Japan. Infectious pancreatic necrosis virus (IPNV) was isolated from masu salmon at two locations: once from tumor tissue in 1981 and a second time from an ovarian fluid sample in 1987. Viral erythrocytic necrosis (VEN) was found at four locations in the erythrocytes of chum and pink salmon in waters along the Okhotsk coast. Cytoplasmic particles with a hexagonal profile were found in the erythrocytes by electron microscopy.

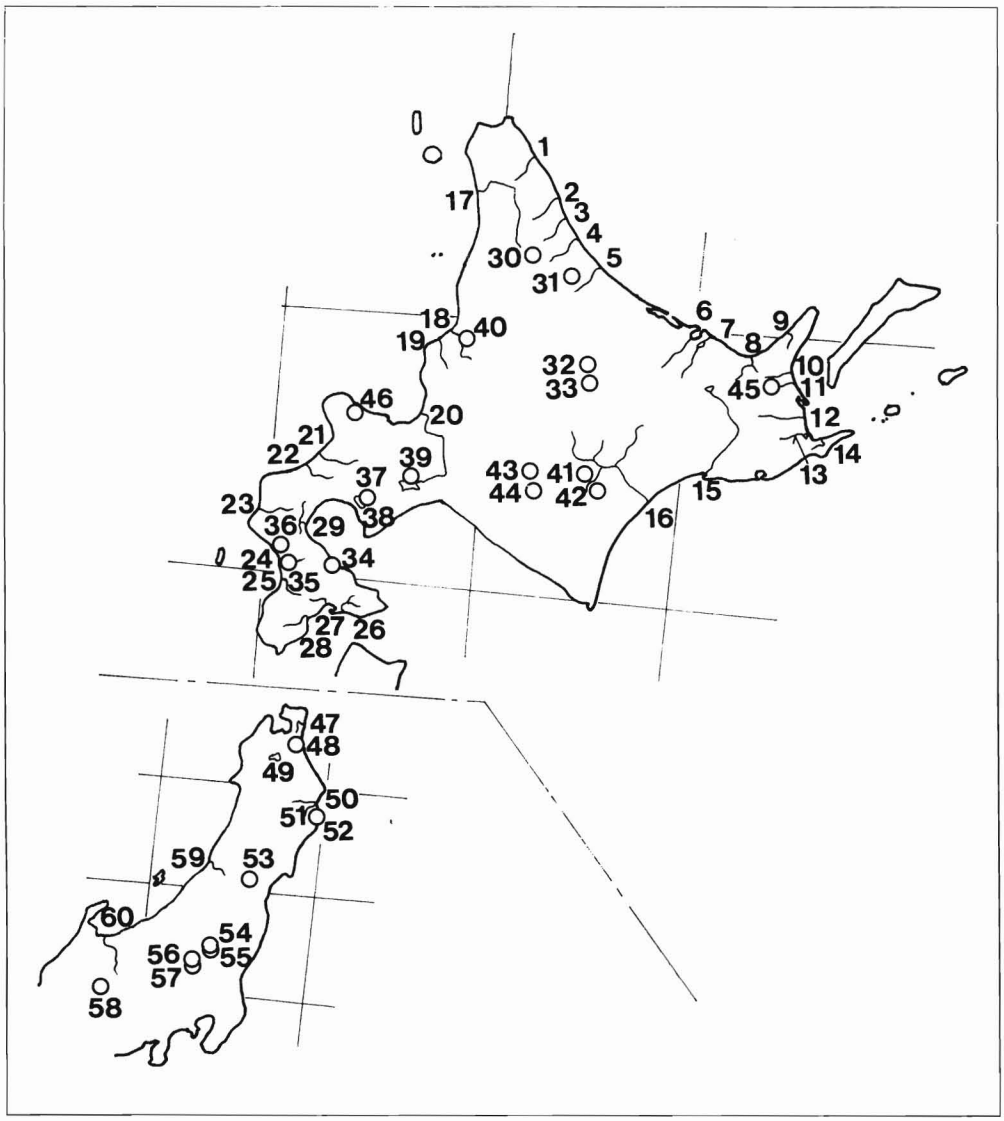
Introduction

Information on the distribution and incidence of fish pathogenic viruses is important for the prevention of transmission to the progeny of mature salmonids. Therefore, we studied the occurrence of pathogenic viruses among mature salmonids in the northern part of Japan. Here, we introduce the results of our investigation from September 1976 to December 1987.

Materials and Methods

Fish Used

From September 1976 to December 1987, we collected 6125 ovarian fluid specimens from 6 species of 11,095 females and 21 seminal specimens from 2 species of 155 males of mature salmonid fishes. Until 1978, 100 fish were sampled at each collection site and were pooled into 10 specimen lots. Subsequently 60 fish were used, and



1: Tonbetsu R.
2: Kitamihorobetsu R.
3: Tokushibetsu R.
4: Horonai R.
5: Okkoppe R.
6: Tokoro R.
7: Abashiri R.
8: Shari R.
9: Iwaobetsu R.
10: Ichani R.
11: Shibetsu R.
12: Nishibetsu R.
13: Fuuren R.
14: Bettoga R.
15: Kushiro R.
16: Tokachi R.
17: Teshio R.
18: Nobusha R.
19: Shokanbetsu R.
20: Chitose R.
21: Shiribetsu R.
22: Shubuto R.
23: Toshibetsu R.
24: Toppu R.
25: Assabu R.
26: Shiodomari R.
27: Hekirichi R.
28: Shiriuchi R.
29: Yuurappu R.
30: Bifuka
31: Nishiokkoppe
32: Kamikawa-A
33: Kamikawa-B
34: Mori H.
35: Otobe H.
36: Kumaishi H.
37: Toya L.H.
38: Toya L.
39: Shikotsu L.H.
40: Nobusha H.
41: Memuro
42: Sarabutsu
43: Hidaka-A.
44: Hidaka-B.
45: Nakashibetsu
46: Shakotan
47: Oippe R.
48: Aomori P.F.E.S.
49: Towada L.H.
50: Hei R.
51: Tsugaruishi R.
52: Tsugaruishi H.
53: Yamagata P.F.E.S.
54: Chuuzenji L.H.
55: Nikko H.
56: Gunma-K P.F.E.S.
57: Gunma-H P.F.E.S.
58: Gifu P.F.E.S.
59: Miomote R.
60: Jintsu R.

Figure 1

Waters in Hokkaido and northern part of Honshu where salmonid populations were sampled and examined for presence of IHNV, OMV, IPNV, and CSV, and coastal waters of Okhotsk where certain species were examined for VEN, 1976–1978. O = Hatchery or fish farm; R = River; H = Hatchery; L = Lake; P.F.E.S. = Prefectural Fisheries Experimental Station.

specimens were collected individually. Species sampled included masu (*Oncorhynchus masou*), chum (*O. keta*), pink (*O. gorbuscha*), and kokanee salmon (*O. nerka*), charr (*Salvelinus leucomaenis*), and rainbow trout (*O. mykiss*) from the following 60 collecting stations: catching stations (29 in Hokkaido, 5 in Honshu), hatcheries (6 in Hokkaido, 9 in Honshu), 10 fish farms and 1 lake all in Hokkaido. From 1981 to 1987, 140 tumor tissues observed among 4115 fish were used for *Oncorhynchus masou* virus (OMV) inspection. Furthermore, 190 mixed kidney and spleen specimens were taken from 858 of these fish and blood smears prepared from 660 fish were employed for virus inspection and for microscopical examination for evidence of viral erythrocytic necrosis VEN, respectively. Thin sections of blood of these fish were observed by electron microscopy (EM). Collection sites of the specimens are noted on the map of Figure 1.

Collection of Ovarian Fluid Specimens

Ovarian fluid specimens were collected according to the method of Yoshimizu et al. (1985). A sterilized automated pipette tip was inserted into the urogenital opening of the mature fish. One mL of ovarian fluid was taken from the fish and sterilized by one of two methods. Until 1981, a filtration method with a millipore filter HA (0.45 μm) was employed and subsequently the antibiotic treatment method of Amos (1985). Both filtrate and antibiotic treated specimens were transported to the laboratory in ice.

Virus Inspection and Identification

RTG-2 (Wolf and Quimby 1962) and CHSE-214 (Fryer et al. 1965) cell lines cultured in roller tubes or 24-well tissue culture plates were employed for virus inspection. We inoculated 0.1 mL of specimen into 2 tubes or wells, and observed them for 10 days at 15°C. Isolated viruses were identified using the rabbit antisera against infectious hematopoietic necrosis virus (IHNV), OMV, and infectious pancreatic necrosis virus (IPNV), and chum salmon virus (CSV). Some ovarian fluid specimens that showed positive results of virus inspection were measured for virus titer using the RTG-2 cell line with the microtiter plate. For the inspection of VEN, smears of erythrocytes were fixed by methanol, stained by 10% Giemsa solution, and viewed by light microscopy (× 400). Thin sections of the erythrocytes were prepared from specimens in which we found inclusion bodies, and the virus particles were observed by E.M.

Isolation of the Virus

From 1981, epithelial tumor tissues observed around the mouth were used for the OMV inspection according to the method of Yoshimizu et al. (1987). Tumor tissue was cut off from the fish and disinfected with iodophore (50 ppm, 15 min), washed with Hanks' balanced salt solution (HBSS) containing antibiotic, and brought to the laboratory with ice.

Light and Electron Microscopy

Prior to egg collection in female fish, blood was collected from the veins under the backbone of the tail for electron microscopy; 1 or 2 drops of blood was fixed in 1 mL of 1.25% glutaraldehyde with 0.05M phosphate buffer's saline (PBS, pH 7.2) and 4% sucrose. After 1-h fixation, the blood was washed with PBS, centrifuged at 3000 rpm for 10 min, and postfixed in 2% osmic acid. After collection of the eggs, blood was collected in a capillary tube from a small hole opened in the kidney and spread on a glass slide. After air-drying, it was fixed with methanol for 20 min and stained with 10% Giemsa solution.

Results and Discussion

IHNV

Results of the virus inspections are shown in Figure 2. Also included are the results of the examination of blood smears for VEN. IHNV was isolated from the ovarian fluid (each of 10 pooled specimen lots) of 100 chum salmon at the Abashiri River in 1976 and at the Yuurappu River in 1977. In the following year, IHNV was discovered at the Mori Hatchery in masu salmon; the incidence of infection was 60 percent. For three consecutive years, from 1979 to 1981, the entire physical facilities at Mori received an annual disinfection with chlorine, while the eggs were treated with iodophore. As a result of this cleaning project, IHNV has not been isolated again from mature masu salmon at the Mori Hatchery until now (Awakura, unpubl. data).

In 1980, IHNV was isolated from rainbow trout at the Aomori Prefectural Fisheries Experimental Station and from kokanee salmon at the Towada Lake Hatchery. The incidence of infection was 8 and 3 percent, respectively, increasing the next year to 42 and 70 percent. In 1982, infection rates at the Towada Lake Hatchery had increased 98% owing to a failure to disinfect the facilities (Yoshimizu et al. 1988a). The difference between Mori and Towada lake Hatcheries suggests that to prevent IHNV outbreak, early measures to disinfect the eggs and facilities are very important. IHNV was also isolated from rainbow trout at the Chuzenji Lake Hatchery in 1983 with a frequency of 8 percent.

Recently, in 1985, IHNV was isolated from the ovarian fluid of masu salmon taken from the Shari River; the incidence of infection was 60 percent. In this case, infectivity of IHNV in the ovarian fluid was measured at 10^2 $TCID_{50}$/mL with the exception of 2 fish whose infectivity was 10^4 $TCID_{50}$/mL. All eggs and facilities had been disinfected by iodophore before the early eyed stage, thus avoiding an outbreak of IHNV.

In Hokkaido, most of the hatcheries culturing masu salmon also culture chum salmon. when we compared the susceptibility of chum and masu salmon to IHNV, chum salmon showed low mortality (less than 25%), compared with masu salmon (Yoshimizu et al. 1989). Recently, an epizootics of IHNV among chum salmon at Kitoi, Russell Creek, and Eklutna in Alaska was reported (Follett 1987) and again at Iwate Prefecture in Japan (Yoshimizu et al. 1988b). Thus IHNV is not a virus to be neglected when raising chum salmon.

OMV

OMV was first isolated from ovarian fluid specimens of masu salmon at the Otobe Hatchery in 1978 with an infection rate of 7.5 percent. In 1978 the rate increased to 61 and in 1983 to 75 percent (Yoshimizu et al. 1988a).

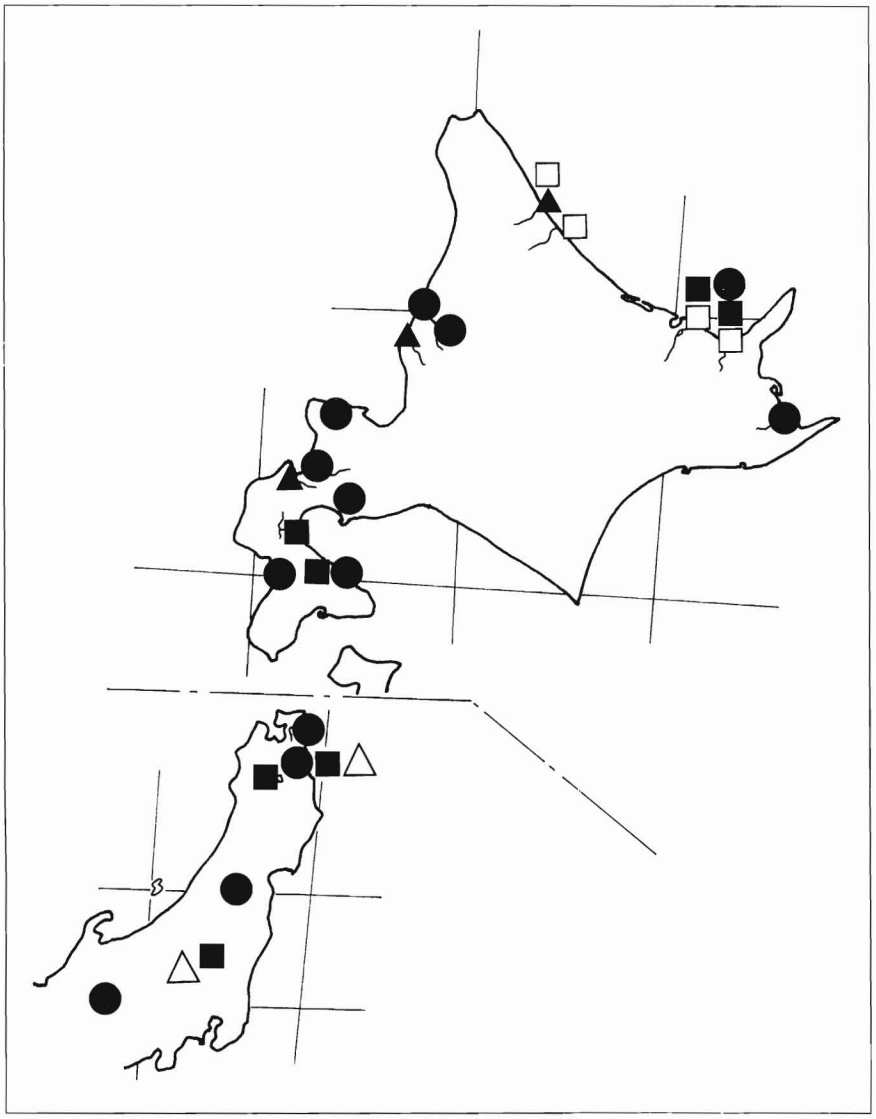


Figure 2
Location of rivers, lakes, hatcheries and fish farms where IHNV, OMV, IPNV, and CSV were isolated; and coastal waters where VEN infected fish was found, 1976–1987. ■: IHNV; ●: OMV; △: IPNV; ▲: CSV; □: VEN.

Over an 8-year period the virus would be detected in fish at a total of 13 locations in northern Japan. This virus was specific to masu salmon and could not be isolated from other species of salmonid fish. Recently, a herpes virus neutralized with anti-OMV rabbit serum was isolated from juvenile coho salmon (*O. kisutch*) cultured in fresh water (Horiuchi et al. 1989). *Renibacterium salmoninarum* and *Flexibacter columnaris* were also isolated at the same time.

It appears that OMV belongs to the herpes viruses and has a pathogenicity to salmonid fish, as well as an oncogenicity (Kimura et al. 1980, a and b; 1981, a, b, and c; 1983; Yoshimizu et al. 1987). Although the size of this virus is 200 to 240 nm (Kimura et al. 1981a), a 0.45 μm membrane filter reduced the infectivity by 99.4 percent. In 1982 we decided to compare the filter sterilization and antibiotic treatment methods using ovarian fluid sampled at the Otobe Hatchery. Although OMV was not isolated from the filter sterilized specimens, it was found in 21 percent of the samples treated with antibiotic only. Because of these results, we changed the method of sterilization of ovarian fluid specimens to the antibiotic method (Yoshimizu et al. 1988a). Following that change, we isolated OMV from six locations that year: the Otobe, Mori, and Shakotan Hatcheries, the Aomori P.F.E.S., and the Shiribetsu and Oippe Rivers (Yoshimizu et al. 1988a). The following year (1983), OMV was discovered at the Fuuren River to an even greater degree. From the autumn of 1983, when we suggested iodophore treatment at the early eyed state, the number of places where OMV has been isolated has decreased and in 1986, OMV was not isolated from the localities where we collected specimens (Yoshimizu et al. 1988a).

From 1981, we observed the body surface, especially around the mouth, and found epithelial tumors at 12

localities. The same basal epithelial tumors have been previously reported at several localities in Japan (Kimura 1976). Sano et al. (1983) also isolated the herpes virus, yamame tumor virus (YAV), from tumor tissues of masu salmon at Niigata Prefecture. OMV was isolated from all the epithelial tumor tissues used for the virus inspection by means of either the primary culture of co-culturing method (Yoshimizu et al. 1987). Tumors induced by OMV were histopathologically similar to those of the tumors observed on the parent fish (Yoshimizu et al. 1987).

At the Otobe Hatchery, OMV was isolated annually from 1978, and the incidence of tumor bearing fish was increasing. In 1983, we examined the infectivity of various organs of masu salmon at the hatchery. Although OMV could be isolated from the ovarian fluid specimens of these fish, OMV could not be isolated from the kidney, spleen, liver, intestine, and heart tissues. The infectivity of the OMV in the ovarian fluid was also low, ranging from $10^{1.8}$ to $10^{2.1}$ $TCID_{50}$/mL. In the autumn of 1984, all fish cultured in this facility were killed and the facility disinfected with chlorine. Then fish were transplanted from Kumaishi Hatchery where no virus had been isolated. Because Otobe Hatchery did not keep brood stock, we could not check the mature fish; however, tumors induced by OMV were not recognized among the fingerlings (Yoshimizu et al. 1988a).

In the case of the Aomori P.F.E.S. in 1981, three kinds of viruses, OMV, IHNV, and IPNV were isolated from the same tumor tissue. Additionally, in the case of the Shiribetsu River (1983) all the fish bearing the tumor were tagged, indicating they had been cultured and released from the Shiribetsu Hatchery (Yoshimizu et al. 1988a). OMV was isolated from either ovarian fluid or tumor tissue at all 13 locations where we examined more than 60 individual specimens (except 4 hatcheries). This suggests that OMV is distributed widely in the northern part of Japan.

CSV

CSV (chum salmon virus) (Winton et al. 1981) was isolated from kidney and spleen mixed specimens collected at Tokushibetsu River in 1978 (Winton et al. 1981). This virus was recognized as an orphan virus and did not show severe pathogenicity for salmonid fish (Winton et al. 1981). In 1986 an unknown disease broke out among masu salmon near Tokushibetsu, and CSV was isolated from the diseased fish (Yoshimizu 1988). The next year, in 1987, CSV was isolated from ovarian fluid of mature masu salmon at Shokanbetsu River and Shubuto River, both located on the Sea of Japan coast. We need to study the pathogenicity of CSV in masu salmon.

VEN

An agent of viral erythrocytic necrosis (VEN) could not be isolated with the tissue culture method. We therefore used Giemsa stain for erythrocytes and observed inclusion bodies. In 1980, 1 out of 60 chum salmon collected from the Abashiri River showed a positive result and, in 1981, the same inclusion bodies were found in chum and pink salmon collected from the Tokushibetsu River, Horonai River, and Shari River, and again in the Abashiri River. We found the iridovirus in thin sections of erythrocytes of chum salmon collected from the Abashiri River (Yoshimizu et al. 1988a).

IPNV

IPNV was isolated from tumor tissue of masu salmon with OMV and IHNV at Aomori P.F.E.S. in 1981 and also from the ovarian fluids of masu salmon cultured at the Gunma P.F.E.S. in 1987 (Yoshimizu et al. 1988a). According to the annual reports of the Hokkaido Fish Hatchery (1976, 1981, 1982), IPNV has been isolated from the ovarian fluid of masu salmon, rainbow trout, and coho salmon cultured in fresh water fish farms, but the prevalences were not high.

Conclusion

From the results of this investigation, IHNV, OMV, IPNV, CSV, and the agent of VEN were distributed widely in the northern part of Japan. In most cases, fish infected with these viruses were masu salmon. An effective method for reducing the incidence of these pathogenic viruses is presently needed.

Four viruses and an agent of viral erythrocytic necrosis were isolated during the course of this investigation.

1. IHNV was isolated at 7 collection sites from either masu or chum salmon.
2. OMV was first discovered in masu salmon in 1978. OMV was isolated from ovarian fluid or epithelial tumor tissues. The incidence of OMV was decreased when we suggested iodophore treatment at the early eyed stage.
3. CSV was discovered in healthy chum salmon at the Tokushibetsu Hatchery in 1978 and again from the ovarian fluids of masu salmon at two places on the Sea of Japan coast in 1987.
4. IPNV was isolated from tumor tissue of masu salmon at Somori P.F.E.S. in 1981 and also from ovarian fluid of masu salmon at Gunma Prefecture in 1987.
5. VEN was found in the erythrocytes of both chum and pink salmon taken in the waters along the Okhotsk coast.

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