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Abstract. Viruses causing infectious pancreatic necrosis (IPN) and infectious hematopoietic necrosis (IHN) were first isolated in Japan during the 1970s and these two diseases remain among the most serious problems affecting cultured salmonids in Japan. In addition to IHN and IPN, four other viral diseases cause major economic losses among cultured fishes in Japan. These include viral pancreatic hepatic necrosis of yellowtail, rhabdovirus infection of Japanese flounder and black rock fish, Kuchishiro-sho of tiger puffer fish, and epidermal hyperplasia or necrosis of Japanese flounder. Over the years, a number of other viruses have been isolated from, or have been observed by electron microscopy, in moribund fish. Members of 10 of the existing families of animal viruses are represented in this group. Their importance to the aquaculture industry in Japan is reviewed here.

Keywords. Fish virus, Disease, Japan

INTRODUCTION

A virological study of cultured fishes in Japan was initiated when an unknown disease occurred among rainbow trout (Oncorhynchus mykiss) in the 1960s. The causative agent was identified as infectious pancreatic necrosis virus by Sano (1). Subsequently, infectious hematopoietic necrosis virus was isolated from kokanee (landlocked sockeye) and sockeye salmon (O. nerka) by Kimura and Awakura (2). Since then, more than 20 viruses have been isolated, or observed by electron microscopy, in fish. The agents are reviewed below by the hosts that they infect rather than by virus grouping.

VIRAL DISEASES OF SALMONID FISHES

Infectious pancreatic necrosis (IPN)

It is conceivable that IPN occurred in Japan as early as 1955 (3) as there were references at that time to a disease of rainbow trout of unknown etiology. The pathogen was not identified for about 10 years (4) when the disease was diagnosed as IPN by Sano (1). The causative virus is a member of the Birnaviridae family of viruses. IPN is an acute systemic disease of fry and fingerling rainbow trout (5,6) and is widespread in Japan (7) with its distribution shown in Figure 1. The characteristic clinical signs of this disease are body darkening, moderate exophthalmia, and abdominal distention. Internally, the spleen, heart, liver, and kidneys are pale and the digestive tract is almost always devoid of food (8). IPNV is pathogenic to rainbow trout, amago (O. rhodurus), and kokanee salmon fry, but not for yamame (landlocked masu salmon, O. masou) fry (9). Although the major losses caused by IPNV occur among rainbow trout and amago salmon, IPNV has also been isolated from coho (O. kisutch) salmon (10), char (Salvelinus fontinalis), masu salmon and coregonus trout cultured with rainbow trout (Yamazaki, personal communication). Susceptibility of fish to IPNV depends on their body weight with smaller fry being more susceptible. Recently rainbow trout fry in Japan appear to have become less susceptible to IPNV (11) and losses attributed to IPN have decreased (Fig. 2), nevertheless, it is still a major problem for Japanese aquaculture.
Infectious hematopoietic necrosis (IHN)

Severe disease outbreaks occurred in July 1971, and from May through June 1972, among kokanee and sockeye salmon fry in Hokkaido with mortalities reaching a level of 80–100%. A virus was isolated (Fig. 3) from these diseased fish and was subsequently identified as IHNV, a rhabdovirus, by Kimura and Awakura (2). Subsequently, an outbreak of this disease occurred in yamame and rainbow trout cultured on Hokkaido. Since 1974, IHN has been known to infect rainbow trout fry on Honshu Island (12) as well, especially its central part, where in several districts river water was found to be contaminated with IHNV and thus unsuitable for trout culture. Figure 4 shows the current distribution of IHNV in Japan. IHNV has also been isolated from moribund chum (O. keta), masu and amago salmon (12,13), and ayu (Plecoglossus altivelis) (14). In the case of wild masu salmon, the typical clinical sign of V-shaped hemorrhages in the muscle were observed (Fig. 5). Recently, large (≥50–80 g body weight) kokanee and amago salmon, and rainbow trout were found to be infected with IHNV (15,16) with petechiae in the fat and on the body cavity wall regularly seen.

Although Mulcahy and Pascho (17) reported that IHNV absorbs to fish sperm and may play a role in the vertical transmission of the virus, we have found that IHNV entering the fertilized eggs is probably inactivated by yolk components and vertical transmission of IHNV is considered doubtful (18). In Nagano Prefecture, IHN is controlled by the following method (19): the eggs are disinfected with iodine in the early eyed stage, since fry are the most susceptible to IHNV; they are then reared in well water or ultraviolet-irradiated river water (20). After the fish pass through this sensi-

Fig. 2. Percent composition of disease outbreaks in Japan observed in cultured rainbow trout, 1980 to 1984.

Fig. 3. Electron micrograph of an ultrathin section of RTG 2 cells infected with IHN virus. Bullet shaped virus particles were observed (bar = 200 nm).
Viral diseases of fish in Japan

Fig. 4. Geographic distribution of IHN virus in Japan, 1975 to 1988.

In the active stage they can be transferred to normal ponds for culture. Other control measures being investigated include establishment of an IHN-resistant strain of rainbow trout and development of a vaccine using a low virulence clone of the virus (21,22).

Herpesvirus infection

From June to September of every year since 1970, high mortalities (greater than 80%) have occurred among kokanee salmon fry in Towada Lake. A herpesvirus, Nerka virus in Towada Lake Akita and Aomori Prefecture (NeVTA), was first isolated from diseased kokanee salmon in 1972 and 1974 (23). The affected fish showed darkening in body color, sluggish behavior, loss of appetite, and a tendency to assemble at the water supply. Some diseased fry were also infected by fungi. Artificially, NeVTA virus shows pathogenicity for landlocked salmonids, such as kokanee and yamame, but a lower susceptibility has been reported in sea-going salmonids such as chum and pink salmon, and in rainbow trout (24).

In 1978, another herpesvirus was isolated (Fig. 6) from the ovarian fluid of apparently normal, mature masu salmon at Otobe hatchery. This virus was named *Oncorhynchus masou* virus (OMV), after the scientific name of the host fish (25). Subsequently, OMV has been isolated from ovarian fluids and epithelial tumor tissues of mature masu salmon at all but one of 60 sites (Fig. 7) that were monitored (26). OMV proved to be pathogenic and, more significantly, oncogenic for young masu salmon (Fig. 8) and several other salmonid fish (27,28). Affected fish lose their appetites and become exophthalmic. They show petechiae on the body surface (Fig. 9) and cumulative mortality can reach 80 to 100% in masu, chum, and kokanee

Fig. 5. Clinical signs of IHN in a wild masu salmon. V-shaped hemorrhages (arrow) were observed.
salmon and 30–40% in coho and rainbow trout (29). Epithelial tumors appeared around the mouth area of surviving fish with an incidence of more than 60% in some populations. Beginning at four months and persisting for at least one year postinfection, 12–100% of surviving chum, coho and masu salmon, and rainbow trout developed differentiated epithelioma type tumors (30).

In 1983, a herpesvirus similar to OMV was isolated from tumor tissue of yamame cultured in Niigata Prefecture and was named yamame tumor virus (YTV) (31). The characteristics of these three herpesviruses were similar, except NeVTA lacks oncogenicity (32,33). In 1983, we recommended the disinfection of fish eggs in Hokkaido with iodine at the early eyed stage as a control measure and now, OMV cannot be detected in most of the hatcheries in this area (26). The host species of this virus was believed to be masu salmon, but OMV was also isolated from the liver, kidney (34), and tumor tissues (Fig. 10) of pen cultured coho salmon (35). OMV infections cause significant economic losses to the coho salmon industry in Japan.

**Chum salmon virus (CSV) infection**

In 1978, a reovirus was isolated from normal-appearing, adult chum salmon returning to their hatchery in Hokkaido (36). After initial isolation and characterization of CSV, the virus was not recovered again until 1986. A large mortality among masu salmon fry (Fig. 11) resulted in the isolation of a virus (Fig. 12), which was identical to CSV (37). The titer of infectious viral particles in diseased fish was as high as 10⁵/g of internal organ, however, no severe histopathological changes were
Fig. 8. OMV infected chum salmon. Exophtalmia and petechiae are evident.

Fig. 9. Tumor developing around the mouth of a cultured masu salmon fry.
observed (Yasutake, personal communication). Since then, the virus has been recovered from stocks of adult masu salmon at new locations in Hokkaido in 1987 and 1988 (26). Artificial infection studies with this virus showed no significant mortality in the several species of salmonid fishes tested (38).

Viral Erythrocytic necrosis (VEN)

Inclusion bodies were observed in Giemsa stained erythrocytes of chum, pink (O. gorbuscha) and coho salmon collected in Okhotsuku and along the north Pacific coast of Hokkaido and Tohoku. The causative agent of VEN, an iridovirus, was observed by electron microscopy (39). Recently, Hayakawa et al. (40) reported that mortalities of pen-cultured coho salmon in Miyagi Prefecture were due to severe anemia. Blood smears showed few inclusion body-like structures of a size from 1–2 μm with virus particles being observed in the cytoplasm. The viruses isolated from salmonid fish in Japan are summarized in Table 1.

VIRAL INFECTIONS OF EEL

A number of viruses have been isolated from cultured eel (Anguilla anguilla, A. japonicus, and A. rostrata), and these agents have been reviewed by Sano (23) and Sano and Fukuda (41) (Table 2). These include an eel virus from the European eel (EVE), which is a member of the birnavirus group; eel virus of America (EVA) and eel virus of Europe.

Fig. 10. Tumor developing on the mouth of a pen-cultured coho salmon.

Fig. 11. Mortality patterns of cultured masu salmon infected with CSV in Hokkaido. Cumulative mortality reached 100%.
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Fig. 12. Electron micrograph of negatively stained virions of CSV isolated from masu salmon (bar = 100 nm).

X (EVEX) which are both rhabdoviruses; a papovavirus; a herpesvirus; a picornavirus; and a reovirus. EVE was most frequently isolated from both Japanese and European eels and was found to be widely distributed over the main eel culturing district in Japan (42). Pathogenicity of these viruses (EVE, EVA, EVEX) for eel was demonstrated only for EVE (43).

Table 1. Viruses isolated from salmonid fish in Japan

| Virus*   | Host (Natural) | Reference                  |
|----------|----------------|----------------------------|
| RNA viruses |                |                            |
| IPNV     | Rainbow trout  | Sano (1)                   |
|          | Amago salmon   | Sano (9)                   |
| IHNV     | Kokanee & Sockeye salmon | Kimura & Awakura (2) |
|          | Rainbow trout  | Sano et al. (12)           |
|          | Chum salmon    | Winton et al. (36)         |
| Chum salmon virus (CSV) | Masu salmon | Yoshimizu (37)             |
| DNA viruses |                |                            |
| NeVTA    | Kokanee salmon | Sano (23)                  |
| OMV      | Masu salmon    | Kimura et al. (27)         |
|          | Coho salmon    | Horiuchi et al. (34)       |
| YTV      | Yamame         | Sano et al. (31)           |
| Observed by E.M. |       |                            |
| VEN      | Chum & Pink salmon | Yoshimizu et al. (39) |
|          | Coho salmon    | Hayakawa et al. (40)       |

*Nerka virus Towanda Lake, Aomori and Akita Prefecture (NeVTA); Oncorhynchus masou virus (OMV); yamame tumor virus (YTV); viral erythrocytic necrosis (VEN).*
Table 2. Viruses isolated from cultured eel in Japan

| Virus               | Host              | Reference                      |
|---------------------|-------------------|--------------------------------|
| **DNA viruses**     |                   |                                |
| ICDV                | Japanese eel      | Sorimachi and Egusa (42)       |
| Herpesvirus         | Japanese eel      | Sano and Hukuda (41)           |
| **RNA viruses**     |                   |                                |
| EVE                 | European eel      | Sano (23)                      |
| EVA                 | American eel      | Sano (23)                      |
| EVEX                | European eel      | Sano et al. (2)                |
| Papovavirus         | Japanese eel      | Sano and Fukuda (41)           |
| Picornavirus        | Japanese eel      | Sano and Fukuda (41)           |
| Reovirus            | Japanese eel      | Sano and Fukuda (41)           |

*aIcosahedral cytoplasmic deoxyribovirus (ICDV); eel virus Europe (EVA); eel virus American (EVE); eel virus Europe X (EVEX).*

Sorimachi and Egusa (42) and Sorimachi (44) reported the isolation of an icosahedral, cytoplasmic deoxyribovirus (ICDV) from diseased eel. Infected eels showed clinical signs of discoloration, congestion of the anal, pectoral and dorsal fins, and an increase of mucus on the body surface. This virus showed pathogenicity for the Japanese eel following artificial infection. Mortality was 40-75% at water temperatures of 14.5-18.5 °C, 15% at 22.8 °C, and 0% at 24.1 °C. The artificially infected fish showed the same pathological signs as those observed in naturally infected fish (45).

**VIRAL INFECTIONS OF CARP**

**Herpesvirus cyprini** (CHV) was initially isolated from papillomas of cultured fancy asagi carp (Cyprinus carpio) in October 1981 to November 1983 and was shown to be virulent for carp fry. Cumulative mortality for two-week-old common carp (C. carpio) was 85.7% but it was only 20% for four-week-old fancy carp. CHV also proved to be oncogenic in common carp and fancy carp fry. The neoplasms appeared at a high frequency (83%) five to six months after the carp had been exposed to the virus by immersion. CHV also induced papillomas in 13.3% of adult mirror carp (C. carpio) and 9.8% of adult fancy carp approximately five months after the intraperitoneal inoculation of virus. Neoplasms were located on the fins, skin, mandible, and at the inoculation sites. CHV was also isolated from naturally occurring papillomas in Japanese fancy carp and was reisolated from 50% of the experimentally induced tumors in carp fingerlings (46,47).

A coronavirus (Coronavirus cyprini) was isolated from a disease outbreak in common carp raised in the laboratory (48). Fish began to succumb without showing any external clinical signs except erythematous skin on the abdomen. Experimentally, the carp coronavirus (CACV) was virulent for carp fry at 20 °C with the cumulative mortality for three-week-old fry being 72.5%. The affected fish manifested swollen and hemorrhagic abdomens filled with ascites prior to their death. Being a recent isolate, the importance of this virus to carp culture in Japan is not yet clear.

**VIRAL INFECTIONS OF MARINE FISH**

**Pancreatic-hepatic necrosis of yellowtail**

In early summer 1983, an acute disease characterized by ascites occurred among yellowtail fingerlings, Seriola quinquergadiata, cultured on a farm in Seto Inland Sea, Japan. A birnavirus, yellowtail ascites virus (YAV), was isolated from yellowtail fry by Sorimachi and Hara (49). This acute viral infection of naturally grown or hatchery-raised fry (50,51) has spread to the south west of Japan, where yellowtail are cultured (Fig. 13) and has become a major problem there. The epizootic period generally occurs during May to June at water temperatures of 18-22 °C. The moribund fry typically show anemic gills, hemorrhaging in the liver, ascites, and petechiae in the pyloric caeca (Fig. 14). The name, viral pancreatic-hepatic necrosis, was proposed by Egusa and Sorimachi (52).

**Rhabdovirus infection of Japanese flounder**

A disease of unknown etiology occurred during March 1984 in cultured hirame or Japanese flounder (Paralichthys olivaceus), held in shore pens and among ayu fry held in sea water tanks at Hyogo Prefecture, Japan (53,54). A rhabdovirus was isolated from both the diseased hirame and ayu and was designated hirame rhabdovirus (HRV) (Fig. 15). Characterization of HRV has shown it to be sufficiently distinct from other known fish rhabdoviruses; therefore, this virus has been given a separate species name Rhabdovirus olivaceus, after the scientific name of its host species (54). HRV has a broad host range, being pathogenic for hirame, black sea bream (Milio macrocephalus), red sea bream (Crysophrys major), black rock fish (Sebastes inermis), redspotted grouper (Epinephelus aukaara), spottybelly greenling (Hexagrammos agrammus), yellowfin goby (Acanthogobius flavimanus), and sunrise sculpin (Pseudoblennius cottoides) among oceanic fishes (41,55,56,57). Salmonid species, especially rainbow trout are also susceptible (58). The
Viral diseases of fish in Japan

Fig. 13. Geographic distribution of viral pancreatic-hepatic necrosis (VPHN) in Japan, 1986–1987.

The clinical signs of infection are congestion of the gonads, focal hemorrhaging in skeletal muscle and fins, and the accumulation of ascitic fluid (Fig. 16). Histopathologically, the kidney and spleen tissues showed necrotic changes and hemorrhaging. Skeletal muscle, testis, and ovary revealed hyperemia and hemorrhaging of capillary vessels (59). HRV is distributed widely in Japan (Fig. 17) ranging from Hokkaido to Honshu (55). Keeping the water temperature at 15 °C seems to be an effective measure for blocking HRV infection (60) as natural outbreaks did not occur when this temperature was maintained.

Kuchishiro-shou of tiger puffer

A small unidentified virus was isolated (61) from cultured tiger puffer (Takifugu rubripes) fish. The epizootic period is from May to June when water temperatures range from 18–22 °C. Moribund fish showed necrosis around the mouth (Fig. 18) and were often observed to be fighting with each other. From the clinical signs of this infection, the disease was named Kuchishiro-shou, from the Japanese words kuchi, meaning mouth, shiro, meaning white, and shou, meaning disease. Viral particles were observed in the brains of affected fish by electron microscopy. Kuchishiroshou is found in the southwest of Japan where the tiger puffer is cultured (Fig. 19).

Epidermal hyperplasia (or necrosis) of Japanese flounder

From 1985 to 1987, outbreaks of a disease, resulting in mass mortalities in larval and juvenile Japanese flounder, were reported by Iida et al. (62). The disease occurred in 10 to 30-day-old fish...
Fig. 15. Electron micrograph of an ultrathin section of RTG-2 cells infected with HRV. Bullet shaped virus particles were observed (bar = 200 nm).

Fig. 16. Clinical signs of HRV infected Hirame. Congestion of the gonad, focal hemorrhaging of skeletal muscle, and accumulation of ascitic fluid were observed.
that were reared at 18–20 °C. Once the disease appeared in a pond, the fish population decreased significantly within one month. Affected fish were characterized by opaqueness of the fins. Histopathologically, hyperplasia was observed in the epidermal layer of the fins and skin. Hexagonal virus particles were observed in the nucleus (100–140 nm in diameter without an envelope) and cytoplasm (190–230 nm with an envelope) of infected epidermal cells by electron microscopy. Experimentally, exposure of fish to the filtrate of an infected tissue homogenate produced 18–50% mortalities in flounder larvae, with more than 90% of the survivors exhibiting epidermal hyperplasia. The morphological features of the virus along with its sensitivity to treatment with ether, pH 3, or a 30-min exposure to 50 °C indicated it was a member of the herpesvirus group. Miyazaki et al. (63) reported a similar disease of larval and juvenile Japanese flounder. Diseased fish had white cloudy fins and body surfaces, as well as occasional ascites. Skin lesions contained necrotic epidermal cells that had intranuclear inclusion granules and displayed vascular degeneration. These necrotic cells contained both intranuclear and intracytoplasmic viral particles that displayed features of a herpesvirus.

This virus has not been isolated although attempts were made in 33 cell lines derived from fish, including one from the host species (Yoshimizu et al., unpublished data). Recently, outbreaks of this disease (Fig. 20) have occurred along the coast of the Japan Sea and the northern part of Japan (Fig. 21).
Lymphocystis disease

Lymphocystis disease has been reported in several species of marine fishes, including Suzuki (Lateolabrax japonicus), yellowtail, red sea bream, Japanese flounder, and others, and the causative iridovirus was observed by electron microscopy (64, 66). Seasonal variation in the prevalence of lymphocystis was noted with the highest incidence occurring in summer. As occurs in many other species, lymphocystis infection does not result in large numbers of mortalities but infected fish are not marketable because the unsightly appearance of their surface lesions makes them aesthetically undesirable. Lymphocystis cells were observed mainly on the fins or body surface. The virus particles were polyhedral, presenting hexagonal or pentagonal profiles in tissue sections (Fig. 22). They may be seen in crystalline array and they are always located in the cytoplasm. The viruses isolated from marine fish in Japan are summarized in Table 3.

**OTHER VIRAL INFECTIONS OF MARINE FISH**

**Viral nervous necrosis**

From 1985 to 1987, mass mortalities of hatchery reared Japanese parrotfish (Oplegnathus fasciatus) larvae and juveniles occurred in Nagasaki Prefecture (67). By light microscopy, necrosis of nerve tissue in the spinal cord, spinal ganglia, and brain was observed. Numerous noneveloped icosahedral virus particles, about 34 nm in diameter, were seen in the cytoplasm of affected neurons and glial cells by electron microscopy. The role of these particles in the disease etiology has not been established.

**Epithelial necrosis**

In 1987, epithelial necrosis occurred among hatchery-reared black sea bream larvae (63). The separation of rounded, necrotic epithelial cells in the epidermis of the fins and body surface, oral mucosa, gills, intestine, and rectum was observed. In the necrotic cells, intracytoplasmic, enveloped virions that were similar to paramyxoviruses were observed by electron microscopy. Until now, this virus has not been isolated.

**Epidermal necrosis**

In 1990, epidermal necrosis of cultured fox jacopever (Sebastes vulpes) larva was recognized. Herpesvirus-like particles were observed by electron microscopy (Yoshimizu et al., unpublished data).

**Table 3. Viruses isolated from marine fish cultured in Japan**

| Virus                  | Host (Natural)                  | Reference               |
|------------------------|---------------------------------|-------------------------|
| DNA virus              |                                 |                         |
| Unidentified small virus | Tiger puffer fish             | Inoue et al. (61)       |
| RNA viruses            |                                 |                         |
| Yellowtail ascitic virus | Yellowtail                     | Sorimachi & Hara (49)  |
| Rhadovirus olivaceus   | Hirame, Black sea bream         | Kimura et al. (54)      |
| Birnavirus             | Red sea bream, Japanese flounder | (This paper)            |
| Observed by E.M.       |                                 |                         |
| Lymphocystis virus     | Marine fish                     | Miyazaki & Egusa (65)   |
| Paramyxovirus          | Black sea bream                 | Miyazaki et al. (63)    |
| Herpesvirus            | Japanese flounder               | lida et al. (62)        |
| Picornavirus-like      | Japanese parrotfish             | Yoshikoshi & Inoue (67) |
Fig. 20. Epidermal hyperplasia of the fins of Hirame larve cultured in Iwate Prefecture. White cloudy fins were observed.

Fig. 21. Electron micrograph of virus particles in epidermal cells of a diseased Hirame (bar = 100 nm).
Birnaviruses

Birnaviruses have been isolated from the larvae of Japanese flounder and red sea bream. These viruses are neutralized by antibody against IPNV; however, their pathogenicity has not been established.

SUMMARY

From the above descriptions it can be seen that viral infections are widespread in both marine and freshwater fishes in Japan and represent major problems to the aquaculture industry of that country.

Since effective vaccines are only now being developed, the best approach currently available for controlling these viral diseases is to prevent their introduction into culture facilities. This is best done by the screening of brood stocks for the presence of virus, so that contaminated eggs can be culled from the rest, and eggs and fish shipped to other hatcheries do not serve as vehicles for spreading the virus. Therefore, the development of nonlethal testing procedures for rapid virus identification is a high-priority research area, not only in Japan, but worldwide as the aquaculture industry continues its global expansion.

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