Viral Infections of Cultured Fishes in Japan

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Abstract

Since infectious pancreatic necrosis virus and infectious hematopoietic necrosis virus were first isolated in the 1970s, more than 20 fish viruses have been isolated and more than 7 viruses have been observed by electron microscopy, in Japan. Viral diseases are major problems and cause economic losses among cultured fishes in Japan and other countries.

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 (IPNV)(Sano 1971). Subsequently, infectious hematopoietic necrosis virus (IHNV) was isolated from kokanee salmon (*O. nerka*) (Kimura and Awakura 1977). Since then, various viral infections of fish have been reported. At present, more than 20 fish viruses have been isolated and more than 7 viruses have been observed by electron microscopy (Table 1).

Viral Diseases of Salmonid Fishes:

Infectious pancreatic necrosis

Infectious pancreatic necrosis is an acute systemic disease affecting the fry and fingerlings of rainbow trout. Its occurrence is widespread in Japan. Susceptibility of fish to IPNV depends on body weight; smaller fry are more susceptible. The signs of this disease are darkening of body coloring, moderate exophthalmia, and abdominal distention. Internally, the spleen, heart, liver, and kidneys are

pale and the digestive tract is almost always devoid of food. Mainly rainbow trout is the fish most affected by IPNV, but the virus is also isolated from amago (*O. rhodurus*) and masu salmon (*O. masou*). Recently, the fry of rainbow trout were observed to be less susceptible to IPNV and consequently, damage attributed to IPN has decreased (Okamoto et al. 1987).

Infectious hematopoietic necrosis

Infectious hematopoietic necrosis is an acute systemic disease which mainly affects the fry of rainbow trout and masu and kokanee salmon, but has also been isolated from moribund chum salmon (*O. keta*) and ayu (*Plecoglossus altivelis*) (Yoshimizu et al., 1987c). The characteristic sign of IHNV infection is V-shaped hemorrhages located in muscle tissue. Recently, a large rainbow trout, body weight 50-500 g was found to be infected with IHNV and subsequently died. In this case, petechiae were observed in the fatty tissues and on the wall of the body cavity. This virus is widespread and especially prevalent in the central part of Honshu, the Japanese mainland (Sano et al. 1977). In several districts, river waters have been contaminated with IHNV and are now unsuitable for rainbow trout culture. Although the vertical transmission of IHNV is doubtful (Yoshimizu et al. 1988a,b), it can be controlled by disinfecting eggs with iodine during the early eyed stage. Fish at the fry stage are very susceptible to IHNV. They should be reared in either well water or U.V. irradiated river water. When fish have past this sensitive stage, they can be transferred to the usual rearing ponds (Yoshimizu et al. 1990). Recently, IHNV was isolated from brain tissue of moribund rainbow trout. Infected fish showed leaf like swimming, and looked like sleeping (unpublished data).

Herpesvirus infection

A herpesvirus, Nerkavirus in Towada Lake Akita and Aomori Prefecture (NeVTA) was first isolated from diseased kokanee salmon in Towada Lake (Sano 1976). In 1978, another herpesvirus was isolated from the ovarian fluid of apparently normal mature masu salmon (Kimura et al. 1980). This virus was named as *Oncorhynchus masou* virus (OMV) from

the scientific name of the host fish. *Oncorhynchus masou* virus was found to be pathogenic and more significantly oncogenic in young masu salmon and several other salmonid fish (Kimura et al. 1981a,b; Yoshimizu et al. 1987a). In 1983, a similar herpesvirus were isolated from tumor tissue of yamame (landlocked *O. masou*) and was named yamame tumor virus (Sano et al. 1983). Subsequent study showed that OMV is epizootic in the northern part of Japan (Yoshimizu et al. 1988b) and that the characteristics of these herpesviruses were similar except NeVTA lacks oncogenicity (Hedrick et al. 1987, 1991, Sano et al. 1988, Guo et al, 1991). In 1983, we recommended the disinfection of fish eggs with iodine at the early eyed stage in Hokkaido. Now OMV cannot be detected in most of the hatcheries in this area (Yoshimizu et al. 1988b). Although the host species of this virus is primarily masu salmon, OMV has also been isolated from the tumor tissues, kidney, liver, and skin of hatchery reared and pen cultured coho salmon (*O. kisutch*). Herpesviruses isolated from masu salmon and cultured coho salmon were similar (Horiuchi et al. 1989, Kimura and Yoshimizu 1991).

Chum salmon virus infection

In 1978, a reovirus was isolated from an apparently normal adult chum salmon (O. keta) returning to its hatchery in Hokkaido (Winton et al. 1981). After the initial isolation and characterization, it was named chum salmon virus (CSV), the virus was not observed again until 1986. CSV was isolated during an epizootic of mass mortalities of masu salmon fry at Nakagawa Hatchery, Hokkaido in 1977. Since then, the virus has been detected in stocks of adult masu salmon at several locations in Hokkaido (Yoshimizu et al. 1992). Artificial infection studies of this virus showed no significant mortality in several species of salmonid fishes (Winton et al. 1989).

Viral erythrocytic necrosis

Inclusion bodies stained with Giemsa were observed in the erythrocytes of chum and pink salmon (*O. gorbuscha*) collected in Okhotsuku and along the North Pacific coast of Hokkaido, and the causative agent of viral erythrocytic necrosis (VEN), an iridovirus, was

observed by electron microscopy (Yoshimizu et al. 1988b).

Erythrocytic inclusion body syndrome

Erythrocytic inclusion body syndrome (EIBS) is a serious disease among hatchery reared and pen cultured coho salmon in the Tohoku district. The death due to this disease in sea cultured coho salmon has been recorded from February to May, 1985 (Hayakawa et al. 1989, Takahashi et al, 1992). Disease signs of infected fish were pale gills, yellowish liver, blood retain in the atrium and watery blood. The diseased fish exhibited lower hematocrits, erythrocyte counts, hemoglobin concentrations, and greater numbers of immature erythrocytes. Icosahedral virions are observed in the cytoplasm and the size was 70 - 80 nm in diameter. Recently, EIBS is distributed widely in the hatchery reared coho salmon and is a major contributor to mortality with fungus infection. Some hatchery recorded the severe cumulative mortality (more than 70 %).

Viral Infections of Eels:

From cultured eels (*Anguilla anguilla*, *A. japonicus* and *A. rostrata*), many viruses were isolated by Sano (1976) and Sano and Fukuda (1987). They include a birnavirus, eel virus from the European eel (EVE); the rhabdoviruses, eel virus of America (EVA) and eel virus of Europe X (EVEX); papovavirus; herpesvirus; picornavirus; and a reovirus. These viruses are not recognized as pathogenic against eel except for EVE (Nishimura et al. 1981). Sorimachi (1982, 1984), reported isolating icosahedral cytoplasmic deoxyribovirus (ICDV) from a diseased eel. This virus showed a pathogenicity against Japanese eel by artificial infection. Mortality was 40 - 75 % at the water temperature of 14.5 - 18.5 C, 15 % at 22.8 C, and 0 % at 24.1 C. Infected fish showed signs of decoloration, congestion of the anal, pectoral and dorsal fins and increase of mucus on the body surface.

Viral Infection of Carp:

Herpesvirus cyprini was isolated from papilloma tissue of cultured fancy carp (*Cyprinus carpio*, also called common or asagi carp) and confirmed as the agent of infection by induction of epithelial tumors by

artificial infection (Sano et al. 1985). *Coronavirus cyprini*, carp coronavirus (CACV), was isolated from diseased common carp raised in the laboratory. Fish infected with CACV showed acute mortality showing no external signs except erythematous skin on the abdomen. Experimentally, CACV was virulent for carp fry at 20 C. Cumulative mortality for 3-week-old fry was 72.5 %. The affected fish manifested swollen and hemorrhagic abdomens filled with ascites and eventually died. Reovirus was also isolated from common carp (Sano and Fukuda, 1987).

Viral Infection of Marine Fishes:

Viral pancreatic-hepatic necrosis of yellowtail

A yellowtail ascites virus (a birnavirus) was isolated from the fry of yellowtail (*Seriola quinqueradiata*) (Sorimachi and Hara, 1985). This epizootic is an acute viral infection of naturally grown and net pen-raised fry. The epizootic period occurs from May to June at water temperatures of 18 to 22 C. The moribund fry typically showed anemic gills, hemorrhaging in the liver, ascites and petechiae in the pyloric caea. The disease name, viral pancreatic-hepatic necrosis, was proposed by Egusa and Sorimachi (1986).

Rhabdovirus infection of Japanese flounder

The rhabdovirus, *Rhabdovirus olivaceus* - also referred to as hirame rhabdovirus (HRV) - was isolated from diseased hirame (Japanese flounder), *Paralichthys olivaceus*, and black sea bream, *Milio macrocephalus* (Gorie et al. 1985; Kimura et al. 1986). This virus is pathogenic for marine fish such as hirame, black sea bream, red sea bream *Pagrus major* and black rockfish *Sebastes inermis*, and also for salmonid species, especially rainbow trout and masu salmon (Yoshimizu et al. 1987b). Signs of HRV infection are gonadal congestion, focal hemorrhage of skeletal muscle and fins, and accumulation of ascitic fluid. Although HRV was distributed widely from Hokkaido to Honshu in Japan, HRV infection is controlled by keeping the water temperature more than 15 to 18 °C (Oseko et al, 1988).

Kuchishiro-shou of tiger puffer

From cultured tiger puffer, *Takifugu rubripes*, an unidentified small virus was isolated (Inoue et al. 1986, 1992). The epizootic period is from May to June at water temperatures of 18-22 °C. Moribund fish had necrosis around the mouth and have been observed to be fighting with each other. From the signs of this infection, the disease was named "Kuchishiroshou", from the Japanese words "kuchi", meaning mouth, "shiro", meaning white, and "shou", meaning disease. Viral particles were observed in the brain by electron microscopy. Kuchichiroshou occurs in southwest Japan where tiger puffer are cultured.

Epidermal hyperplasia of Japanese flounder

Outbreaks of a disease resulting in mass mortalities of larval and juvenile Japanese flounder was reported by Iida et al. (1989). Once the disease occurs in a pond, the resident fish populations usually become extinct within one month. Affected fish are characterized by opaque fins. Histopathologically, hyperplasia is observed in the epidermal layer of the fins and skin. In the epidermal tissues of infected fish, hexagonal virus particles were observed by electron microscopy. Japanese flounder larvae experimentally exposured to the filtrate of infected tissue homogenate suffered 18 - 50 % mortalities with 93 - 100 % of the survivors exhibiting epidermal hyperplasia. This virus has not been isolated in any of the 33 fish cell lines in which culture has been attempted, including cell lines from the host species.

Lymphocystis disease

In several species of marine fishes, suzuki (*Lateolabrax japonicus*), yellowtail, red sea bream, Japanese flounder, and others, lymphocystis disease was reported and iridovirus was observed by electron microscopy (Matsusato 1975; Miyazaki and Egusa 1972; Tanaka et al. 1984). Seasonal variation in the prevalence of lymphocystis was noted with increased prevalence in summer. Lymphocystis cells were observed mainly on the fins or body surface. The virus particles were polyhedral, presenting hexagonal or pentagonal profiles in tissue

sections. They may be seen in a crystalline array and are always located in the cytoplasm.

Nervous necrosis of marine fish

New viral diseases associated with mass mortalities have been recorded among hatchery reared larvae and juveniles of marine fish such as Japanese parrotfish, *Oplegnathus fasciatus* (Yoshikoshi and Inouye 1990), redspotted grouper, *Epinephelus akaara* (Mori et al. 1991), and striped jack, *Pseudocaranx dentex* (Mori et al. 1992). Similar viral etiologies were also reported. Virus particles morphologically similar to picornavirus were observed in the neural cells and some other cells of the affected central nervous system and retina of the eye (Yoshikoshi and Inouye 1990). The virus purified from larval striped jack consists of non-enveloped particles, about 25 nm in diameter, and contains two single strand, positive-sense RNA, and two structural proteins. This virus was classified to family of Nodaviridae and was designated as striped jack nervous necrosis virus (SJNNV) (Mori et al. 1992). To date, these viruses have not been isolated.

Iridovirus infection of marine fish

Occurrence of a new viral epizootic in red sea bream in Shikoku Island in 1990 was reported (Inouye et al. 1992). Diseased fish swam inactively and showed severe anemia with 20 - 60 % mortality. Typically enlarged cells characterized by batsophilic stain were observed in the spleen, heart, kidney, liver, and gill. From the morphology and localization of these cells, they are considered leukocytes. Hexagonal virions were found in the cytoplasm of these cells. Each virion consisted of a central electron-dense core (120 nm) and an electron translucent zone and measured 200 - 240 nm in diameter. Feulgen staining of the enlarged cells suggested DNA in the virus and the virus belongs to the family Iridoviridae. The virus slowly replicated and produced CPE of enlarged and rounded cells in RTG-2, CHSE-214, FHM, BF-2, and KRE-3 cells at 20-25°C. Intraperitoneal inoculation of the spleen homogenate filtrate to red sea bream fingerling induced similar pathological changes (Inouye et al. 1992). Same diseased signs were observed in several marine fish

cultured in west part of Japan in 1991 (FIDIC 1992).

Other diseases.

Miyazaki et al.(1989) reported herpesvirus in the epidermal necrosis of Japanese flounder and paramyxovirus in the epithelial necrosis of black sea bream. These viruses have not been isolated. Birnaviruses were isolated from Japanese flounder and red sea bream (Kimura and Yoshimizu 1991). These viruses were neutralized with antibody against IPNV; the pathogenicity of these birnaviruses have not been clarified.

Conclusion

Viral infections of marine and freshwater fishes may become major problems in the aquaculture industry in Japan. Now, more than 20 fish viruses have been isolated and more than 7 viruses have been observed by electron microscopy.

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Table 1. Viral infection of cultured fishes in Japan

Isolated virus	Host
DNA virus	
Nerka virus Towada Lake, Aomori and Akita Prefecture (NeVTA)	Kokanee salmon
Oncorhynchus masou virus (OMV)	Masu salmon
Yamame tumor virus (YTV)	Yamame (Masu salmon)
Icosahedral cytoplasmic deoxyribovirus (ICDV)	Japanese eel
Herpesvirus cyprini	Fancy carp
Herpesvirus	Japanese eel
Iridovirus	Red sea bream
Unidentified small virus	Tiger puffer
RNA virus	
Infectious pancreatic necrosis virus (IPNV)	Salmonid fish
Infectious hematopoietic necrosis virus (IHNV)	Salmonid fish
Chum salmon virus (CSV)	Masu salmon
Yellowtail ascitic virus (YAV)	Yellowtail
Rhabdovirus olivaceus (HRV)	Various marine fish
Eel virus from European eel (EVE)	European eel
Eel virus of America (EVA)	American eel
Eel virus of Europe X (EVEX)	European eel
Papovavirus	Japanese eel
Birnavirus	Yellow tail
"	Japanese flounder
"	Red sea bream
Coronavirus cyprini (CACV)	Common carp
Picornavirus	Japanese eel
Reovirus	Common carp
"	Japanese eel
Observed by electron microscopy	
Viral erythrocytic necrosis virus	Various marine fish
EIBS virus	Coho salmon
Lymphocystis virus	Various marine fish
Paramyxovirus	Black rockfish
Herpesvirus	Japanese flounder
Picornavirus	Japanese parrotfish
Nodavirus	Striped jack