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Author(s)	Yoshimizu, Mamoru; Kimura, Takahisa
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VIRAL INFECTIONS OF CULTURED FISHES IN JAPAN

Mamoru Yoshimizu and Takahisa Kimura

Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University, Minato, Hakodate, Hokkaido, 041 Japan

Abstract

Infectious pancreatic necrosis virus and infectious hematopoietic necrosis virus were isolated in the 1970's. These two viral diseases are still serious problems among the cultured salmonids in Japan. Since then, various viral infections of fish have been reported, more than 20 fish viruses have been isolated and more than five viruses have been observed by electron microscopy. Six viral diseases are major problems and cause economic losses among cultured fishes in Japan. These are IPN and IHN of salmonids, viral pancreatic-hepatic necrosis of yellowtails, rhabdovirus infection of Japanese flounder and black rock fish, "Kuchishiro-sho" of tiger puffer and epidermal necrosis of Japanese flounder. Besides these, there are several viral infections: herpesvirus infections of salmonids, especially epithelial tumor (induced by OMV or YTV); epithelial tumor of carp (*Herpesvirus cyprini*); and lymphocystis disease of several marine fishes. Eel virus from the European eel, eel virus of America and eel virus of Europe X were also isolated from cultured eel. Recently however, many other viruses were isolated from diseased and healthy fishes, including icosahedral cytoplasmic deoxyribovirus, papovavirus, herpesvirus, picornavirus and reovirus from cultured eel. Chum salmon virus was isolated from masu salmon. Reovirus and *Coronavirus cyprini* were isolated from common carp and birnavirus was isolated from Japanese flounder and red sea bream. In the blood of salmonids, the causative agent of viral erythrocytic necrosis was observed and in moribund fry of ishida and epithelial necrosis of black sea bream and red sea bream, picornavirus and paramyxovirus were observed by E.M.

A virological study of cultured fishes in Japan was initiated when an unknown disease occurred among rainbow trout (*Oncorhynchus mykiss*) in the 1960's. The causative agent was identified as infectious pancreatic necrosis virus (Sano, 1971). Subsequently, infectious hematopoietic necrosis virus was isolated from kokanee salmon (*Oncorhynchus 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

five viruses have been observed by electron microscopic studies (Table 1).

Viral diseases of salmonid fishes:

Infectious pancreatic necrosis (IPN). IPN is an acute systemic disease of fry and fingerlings of rainbow trout. This disease is spread widely in Japan. Susceptibility of fish to IPNV depends on body weight; smaller fry are more susceptible. The signs of this disease are 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. The fish mainly affected by IPNV is rainbow trout, but IPNV is also isolated from amago (*Oncorhynchus rhodurus*) and masu salmon (*O. masou*). Recently, the fry of rainbow trout were observed less susceptible to IPNV and damage attributed to IPN decreased (Okamoto et al., 1987).

Infectious hematopoietic necrosis (IHN). IHN is an acute systemic disease of fry of rainbow trout, masu and kokanee salmon. IHNV was also isolated from moribund ayu (*Plecoglossus altivelis*) (Yoshimizu et al., 1987a). Signs of V shaped hemorrhages were observed in the muscle. Recently, a large-size rainbow trout, body weight 50 to 80 g or more, was found to be infected with IHN and died (Mori et al., 1987). In this case, petechiae were observed on the fat and on the wall of the body cavity. This virus is wide spread especially in the central part of Honshu, the mainland of Japan (Sano et al., 1977). In several districts, river waters were contaminated with IHNV and became unsuitable for rainbow trout culture. Vertical transmission of IHNV is doubtful (Yoshimizu et al., 1988a,b) and can be controlled by the following method: Eggs are disinfected with iodine in the early eyed stage. Fish at fry stage has great susceptibility to IHNV. They may be reared in well water or U.V. irradiated river water. When the fish pass through this sensitive stage to IHNV, they will be transferred to the usual pond.

Herpesvirus infection. A herpesvirus, Nerkavirus in Towada Lake Akita and Aomori Prefecture (NeVTA) was isolated from diseased kokanee salmon in Towada Lake (Sano, 1976). In 1978, another herpesvirus was isolated from ovarian fluid of normal appearing mature masu salmon (Kimura et al., 1980). This virus was named as *Oncorhynchus masou* virus from the scientific name of the host fish. OMV was pathogenic and more significantly oncogenic to the young masu salmon and several other salmonid fish (Kimura et al., 1981a,b; Yoshimizu et al., 1987b). In

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
<u>Oncorhynchus masou</u> virus (OMV)	Masu salmon
Yamame tumor virus (YTV)	Yamame*
ICDV	Japanese eel
<u>Herpesvirus cyprini</u>	Fancy carp
Herpesvirus	Japanese eel
Unidentified samll virus	Tiger puffer
RNA virus	
Infectious pancreatic necrosis virus (IPNV)	Rainbow trout (Salmonids)
Infectious hematopoietic necrosis virus (IHNV)	Rainbow trout (Salmonids)
Chum salmon virus (CSV)	Masu salmon
Yellowtail ascitic virus (YAV)	Yellowtail
<u>Rhabdovirus olivaceus</u> (HRV)	Hirame**
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	Yellowtail
"	Hirame**
"	Red sea bream
<u>Coronavirus cyprini</u> (CACV)	Common carp
Picornavirus	Japanese eel
Reovirus	Fancy carp
"	Japanese eel
Observed by E.M.	
Viral erythrocytic necrosis virus	Marine fish
Lymphocystis virus	Marine fish
Paramyxovirus	Black rockfish
Herpesvirus	Hirame**
Picornavirus	Ishidai***

*: Masu salmon, **: Japanese flounder

***: Oplegnathus fasciatus

1983, similar herpesvirus were isolated from tumor tissue of yamame (O. masou) and was named yamame tumor virus (YTV) (Sano et al., 1983). Subsequent study shows that OMV is enzootic in the northern part of Japan (Yoshimizu et al., 1988b) and that the characteristics of these three herpesviruses were similar except NeVTA lacks oncogenicity (Hedrick et al., 1987, Sano et al., 1988). In 1983, we recommended the disinfection of eggs of fish 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). The host species of this virus was masu salmon, but OMV was also isolated from the tumor tissue observed in pen cultured coho salmon (O. kisutch).

Chum salmon virus (CSV) infection. In 1978, a reovirus was isolated from normal appearing adult chum salmon (O. keta) returning to its hatchery in Hokkaido (Winton et al., 1981). After the initial isolation and characterization of CSV, the virus was not recovered again until 1986, during mass mortalities of masu salmon fry caused by infection of this virus. Since then, the virus has been recovered from stocks of adult masu salmon at new locations in Hokkaido (Yoshimizu 1988). Artificial infection studies of this virus

showed no significant mortality in several species of salmonid fishes (Winton et al., 1989).

Viral erythrocytic necrosis (VEN). Inclusion bodies stained with Giemsa were observed in erythrocytes of chum and pink salmon (O. gorbuscha) collected in Okhotsku and along the north Pacific coast of Hokkaido, and the causative agent of VEN, irridovirus, was observed by E.M. (Yoshimizu et al., 1988b)

Viral infection of eel:

From cultured eel (Anguilla anguilla, A. japonicus and A. rostrata), many viruses were isolated by Sano (1976) and Sano and Fukuda (1987). They are birnavirus, eel virus from the European eel (EVE); rhabdovirus, eel virus of America (EVA) and eel virus of Europe X (EVEX); papovavirus; herpesvirus; picornavirus; and reovirus. Pathogenicity of these viruses against eel were not recognized except for EVE (Nishimura et al., 1981). Sorimachi (1982, 1984), reported an icosahedral cytoplasmic deoxyribovirus (ICDV) from 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:

From papilloma tissue of cultured fancy carp (asagi carp) Herpesvirus cyprini was isolated and induction of the epithelial tumor by artificial infection was confirmed (Sano et al., 1985). From diseased common carp raised in the laboratory, Coronavirus cyprini, carp coronavirus (CACV) was isolated (Sano et al. 1989). Fish began to acutely succumb, not showing any external sign 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 up with ascites and eventually died. Reovirus was also isolated from common carp (Sano and Fukuda, 1987).

Viral infection of other marine fishes:

Viral pancreatic-hepatic necrosis of yellowtail. A yellowtail ascites virus (YAV), 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 or hatchery-raised fry. The epizootic period is observed as May to June at water temperatures of 18 to 22 °C. The moribund fry typically show anemic

gills, hemorrhaging in the liver, ascites and petechiae in the pyloric caecum. The disease name, viral pancreatic-hepatic necrosis, was proposed by Egusa and Sorimachi (1986).

Rhabdovirus infection of Japanese flounder. From diseased hirame, Japanese flounder (*Paralichthys olivaceus*), and black sea bream (*Milio macrocephalus*), a rhabdovirus, *Rhabdovirus olivaceus*; hirame rhabdovirus (HRV) was isolated (Gorie et al., 1985; Kimura et al., 1986). This virus is pathogenic for marine fish such as hirame, black sea bream, red sea bream (*Chrysophrys major*) and black rockfish (*Sebastes inermis*), and also salmonid species, especially rainbow trout and masu salmon (Yoshimizu et al. 1987c). Signs of HRV infection are congestion of the gonad, focal hemorrhage of skeletal muscle and fins, and accumulation of ascitic fluid. HRV is distributed widely from Hokkaido to Honshu in Japan.

Kuchishiro-shou of tiger puffer. From cultured tiger puffer (*Tahifugu rubripes*), an unidentified small virus was isolated (Inoue et al., 1986). The epizootic period is May to June at water temperatures of 18 to 22 °C. Moribund fish show necrosis around the mouth and were 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 E.M. "Kuchichiroshou" is found in the south west of Japan where tiger puffer is cultured.

Epidermal hyperplasia of Japanese flounder. Outbreaks of a disease resulting in mass mortalities occurring in larval and juvenile Japanese flounder was reported by Iida et al. (1989). Once the disease occurs in a pond, the fish populations of the pond usually become extinct within one month. Affected fish are characterized by opaqueness of the 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 E.M. Experimentally, exposure of fish to the filtrate of the tissue homogenate produced 18 - 50 % mortalities in flounder larvae, and 93 - 100 % of the survivors exhibited epidermal hyperplasia. This virus have not been isolated using the 33 cell lines derived from fish including the host species. From Japanese flounder and red sea bream, birnaviruses were also isolated. These viruses were neutralized with antibody against IPNV and the pathogenicity of this is not clarified.

Lymphocystis disease. In several species of marine fishes, suzuki (*Lateolabrax japonicus*), yellowtail, red sea bream, Japanese flounder and others, lymphocystis disease were reported and iridovirus was observed by E.M. (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 crystalline array and they are always located in the cytoplasm.

Yoshikoshi and Inoue (1988) reported picornavirus in moribund fry of ishikida (*Oplegnathus fasciatus*), and Miyazaki et al. (1989) reported herpesvirus and paramyxovirus in epidermal necrosis of Japanese flounder and epithelial necrosis of black sea bream. But until now, these viruses have not been isolated.

Viral infections of marine and freshwater fishes may become major problems in the aquaculture industry in Japan.

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