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***ONCORHYNCHUS MASOU* VIRUS (OMV) EPIDEMIOLOGY AND ITS CONTROL STRATEGY**

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ABSTRACT

Distribution of salmonid herpesvirus was known in USA and Japan. Herpesviruses isolated in USA were classified to serotype 1 (SaHV-1) and in Japan were serotype 2 (SaHV-2). The reference strain of SaHV-1 is *Herpesvirus salmonis* and SaHV-2 is *Oncorhynchus masou* virus (OMV) strain OO-7812. OMV disease (OMVD) causes oncogenic and skin ulcer conditions. The main susceptible fish species are kokanee salmon (*Oncorhynchus nerka*), masu salmon (*O. masou*), coho salmon (*O. kisutch*) and rainbow trout (*O. mykiss*). Economic losses caused by this virus were recognized among kokanee salmon, coho salmon and rainbow trout. At the beginning of the 1980s, OMV was distributed widely in the northern part of Japan. Since 1988, OMV was isolated from coho salmon and since 1991 this virus was found in rainbow trout. Recently, re-appearing OMVD has become a major problem in pond culture of rainbow trout in the central part of Japan. OMV is sensitive to ultraviolet irradiation and iodophore treatment. Although detection of OMV in carrier fish is difficult using polymerase chain reaction (PCR) and OMV replicates and appears in ovarian fluid at mature stage, it can be controlled by disinfection. OMVD was successfully controlled by disinfection of all facilities and eggs with iodophore just after fertilization and again at early-eyed stage.

Key words: *Oncorhynchus masou* virus, OMVD, epidemiology, salmonid fish, herpesvirus, salmonid herpesvirus, SaHV-2

INTRODUCTION

A herpesvirus infection of salmonid fish in Japan was first described by Sano (1976) isolated from moribund kokanee salmon (*Oncorhynchus nerka*) in Towada Lake, northern part of Honshu, mainland of Japan. Subsequently, in 1978, a herpesvirus was isolated from the ovarian fluid of mature masu salmon (*O. masou*) cultured in Hokkaido. The virus was named *Oncorhynchus masou* virus (OMV) (Kimura *et al.*, 1981a). Following the discovery of OMV, many strains of herpesvirus that can be neutralized with antiserum against OMV have been isolated from cultured and wild salmonid fish in the northern part of Japan (Yoshimizu *et al.*, 1993). Since 1988, OMVD had become a major problem in pen cultures of coho salmon (*O. kisutch*) in the Tohoku district and since 1991 OMV has been found in pond cultures of rainbow trout (*O. mykiss*) in Hokkaido and the central part of Japan (Yoshimizu, 1996).

NERKA VIRUS IN TOWADA LAKE (NeVTA)

High mortality has been observed among the fry of kokanee salmon (landlocked *O. nerka*) from June to September of every year since 1970. Mortality reached over 80% for the 3-mo period. The affected fish demonstrated the following signs: a darkening in body color, sluggish behavior and loss of appetite. From these moribund fish, a syncytium-forming virus was isolated in RTG-2 cells incubated at 10 °C in 1972 and 1974. The virus was classified as a member of Herpesviridae and it was named the nerka virus in Towada Lake, Akita and Aomori Prefectures (NeVTA) (Sano, 1976). NeVTA is pathogenic and lacks oncogenicity.

MASU SALMON HERPESVIRUS; *ONCORHYNCHUS MASOU* VIRUS (OMV)

In 1978, a herpesvirus was isolated from the ovarian

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fluid of an apparently normal mature masu salmon (*O. masou*), cultured in the Otohe Salmon Hatchery in Hokkaido. This virus was named *Oncorhynchus masou* virus, after the scientific name of the host fish and its oncogenicity (Kimura *et al.*, 1981 a,b). The general properties of OMV were similar to those of *Herpesvirus salmonis* and NeVTA, but it differed in virion size and its optimal growth temperature. It was also distinct from *H. salmonis* with respect to its virus-induced polypeptide patterns and serological properties (Kimura and Yoshimizu, 1989). OMV was pathogenic and, more significantly, oncogenic for masu salmon and several other salmonid fish (Kimura *et al.*, 1981b). One-month-old kokanee salmon exhibited the greatest sensitivity. Masu and chum salmon also exhibited high susceptibility. Coho salmon and rainbow trout were shown to be less susceptible to OMV infection (Tanaka *et al.*, 1984). The incidence of tumor-bearing fish approached more than 60%. Epithelial tumors were found on 12-100% of the surviving chum, coho and masu salmon, and rainbow trout beginning at about 4 mo and persisting for at least 1 year post-infection (Yoshimizu *et al.*, 1987). Since its discovery in 1978 at the Otohe Salmon Hatchery, OMV has been isolated from the ovarian fluid and neoplastic tissue of mature masu salmon collected from other places. In 1981, a similar herpesvirus was isolated from the tissues of a basal cell carcinoma that developed on the mouthpart of yamame (another name for masu salmon) cultured at Koide Branch, Niigata Prefectural Inland Fisheries Experimental Station. This virus was named yamame tumor virus (YTV) (Sano *et al.*, 1983). Serologically, NeVTA, OMV and YTV were confirmed as being the same virus by Yoshimizu *et al.* (1995). Six representative OMV strains, which were isolated from ovarian fluid and tumor tissue of cultured as well as wild masu salmon in Hokkaido and Aomori Prefecture, were also confirmed as being the same virus by DNA restriction endonuclease cleavage analysis by Gou *et al.* (1991a). From the results of the comparison of the DNA homologies, OMV and YTV were classified as the same virus and NeVTA was classified as being similar yet distinct from these 2 viruses (Eaton *et al.*, 1991).

COHO SALMON HERPESVIRUS

Since 1988, herpesvirus has been isolated from the liver, kidney, and developing neoplasms in pond and pen-cultured coho salmon (Kimura and Yoshimizu, 1989; Kumagai *et al.*, 1994). Affected fish showed the following disease signs: skin ulcers, white spots on liver, and neoplastic tissues around mouthpart or body surface. Coho salmon culture was economically damaged by this disease. The herpesviruses isolated from coho salmon were tentatively named as coho salmon tumor virus (CSTV), *O. kisutch* virus (OKV) by Horiuchi *et al.* (1989), coho salmon

tumor virus (COTV) by Kimura and Yoshimizu (1991) and coho salmon herpesvirus (CHV) by Kumagai *et al.* (1994). All of these viruses were neutralized by anti-OMV or NeVTA rabbit serum (Yoshimizu *et al.*, 1995), and the oncogenicity of CSTV, OKV and COTV was confirmed by artificial infection. In addition, restriction endonuclease profiles of CSTV were the same as those of NeVTA and YTV (Igari *et al.*, 1991). CHV showed strong pathogenicity to coho salmon (Kumagai *et al.*, 1994).

RAINBOW TROUT HERPESVIRUS

Since 1992, massive mortality has occurred among 1-year-old rainbow trout in pond cultures. The diseased fish exhibited almost no external signs. Some fish did manifest ulcerative skin lesions. Internally, intestinal hemorrhage and white spots on the liver were observed. No bacterial, fungal or parasitic agents were found and the herpesvirus was isolated from the kidney, liver, and ulcerative skin tissues. The rainbow trout culture industry experienced serious economic losses due to this disease, since rainbow trout of marketable size were affected and died. This herpesvirus was tentatively named rainbow trout kidney herpesvirus (RKV) by Suzuki (1993). The virus was neutralized with anti-OMV rabbit serum (Sung *et al.*, 1996) and its main characteristics were the same as OMV. RKV showed strong pathogenicity to marketable-size rainbow trout and masu salmon (Suzuki, 1993).

ROOTS OF OMV

Since 1978 to 2000, 25,753 females of 6 species of mature salmonid fish were collected to survey the incidence of this virus in Hokkaido and the northern part of Honshu. Herpesvirus was isolated from masu salmon at all the investigated sites with the exception of one hatchery. All of the isolates were neutralized with anti-OMV rabbit serum (Yoshimizu *et al.*, 1993). Based on our epizootiological and epidemiological study, the roots of OMV in Japan was assumed to be along the Japan Sea coast of Hokkaido and the presumed original host species was masu salmon. In the 1960s, eggs of masu salmon were collected from the rivers of the Japan Sea coast of Hokkaido, and transported to Honshu Island, mainland of Japan. With the unrestricted fish movement, the virus spread to several places in Honshu where the first cancer disease of masu salmon was observed (Kimura, 1976), and also in Hokkaido where OMV was already detected. Subsequently, coho salmon and rainbow trout were cultured in the same water systems where masu salmon was cultured. Coho salmon might be infected with OMV at fry stage in freshwater because when we found the tumor tissue around the mouth of pen cultured coho salmon, the

hatchery from where coho salmon was transplanted to pen had a history of OMVD (Kimura and Yoshimizu, 1991).

DIAGNOSIS

Detection of OMV in carrier fish is difficult but the virus replicates and appears in ovarian fluid at mature stage. For the purpose of virological survey of mature salmonid fish, ovarian fluid is collected by the method described by Yoshimizu *et al.* (1985), with the addition of an equivalent volume of antibiotic solution and reacted at 5°C, overnight. In the case of the tumor tissue, tissue is cut and disinfected with iodophore (50 ppm, 15 min), then washed with Hanks' balanced salt solution (BSS). Tumor tissue must be prepared for the primary culture or co-culture with RTG-2 cells. After one transplantation of primary culture cells, virus inspection of the culture medium should be carried out. In the laboratory, rabbit serum or monoclonal antibody against OMV was used for fluorescent antibody test (Hayashi *et al.*, 1993), and also polymerase chain reaction (PCR) and DNA probe were used for detection of unsymptomatic or diseased fish (Gou *et al.*, 1991b). PCR using a F10 primer, GTACCGAAACTCCGAGTC and R05 primer AACTTGAAGTACTCC GGGG amplified a 439 base-pair segment of DNA from OMV strains isolated from masu salmon, coho salmon and rainbow trout, and liver, kidney, brain and nervous tissues. Agarose gel profile of amplified DNA was able to distinguish a SaHV-1 and SaHV-2. Sensitivity of this PCR was $10^{0.8}$ TCID₅₀/ml (Aso *et al.*, 2001).

CONTROL OF OMVD

OMV is sensitive to ultraviolet irradiation, ozone or iodophore treatment (Kimura and Yoshimizu, 1989). Since 1983, we have strongly recommended the inspection of the ovarian fluid from matured fish and the disinfection of collected eggs in almost all hatcheries in Hokkaido with iodine at the early-eyed stage as a control strategy. Currently OMV is no longer detected in most of the hatcheries in this area. Nowadays, all eggs and facilities have been disinfected by iodophore just after fertilization and again at the early-eyed stage. Formalin-killed OMV vaccine produced from OMV isolated from rainbow trout have been able to reduce the number of OMV replicating in ovarian fluid. As a result, OMV cannot be isolated in most of the hatcheries in this area, and could avoid the outbreak of OMVD (Yoshimizu *et al.*, 1993, 1995).

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