2.2.13 *Oncorhynchus masou* Virus Disease

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A. Name of Disease and Etiological Agent

Oncorhynchus masou virus disease (OMVD) is caused by the *Salmonid herpesvirus 2* (*SalHV-2*), which was first described as an oncogenic virus isolated from *Oncorhynchus masou* (Kimura et al. 1981a, b) (Figs. 1 and 2). This virus is more commonly known as *Oncorhynchus masou* virus (OMV) but other synonyms include the nerka virus in Towada lake, Akita and Aomori Prefecture (NeVTA; Sano 1976), Yamame tumor virus (YTV; Sano et al. 1983), *Oncorhynchus kisutch* virus (OKV; Horiuchi et al. 1989), coho salmon tumor virus (CSTV or COTV; Yoshimizu et al. 1995), coho salmon herpesvirus (CHV; Kumagai et al. 1994), rainbow trout kidney virus (RKV; Suzuki 1993) and rainbow trout herpesvirus (RHV; Yoshimizu et al. 1995). *SalHV-2* is a recognized species in the family *Alloherpesviridae* and the genus *Salmonivirus.*

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Salmonid herpesvirus 2 (*SalHV-2*) is considered to be enzootic only in Japan, but is most likely in the coastal rivers of Kamchatka, Russia that harbor Pacific salmon (Yoshimizu et al. 1993; Yoshimizu and Nomura 2001).

2. Host Species

Epizootics of OMVD have occurred in pond culture of masu salmon, coho salmon (*O. kisutch*) and kokanee salmon (*O. nerka*). Juvenile rainbow trout (*O. mykiss*), yearling rainbow trout and adult rainbow trout have also been shown to be susceptible (Furihata et al. 2003). Chum salmon (*O. keta*) were susceptible to *SalHV-2* in laboratory challenges while Amago salmon (*O. rhodurus*) and iwana salmon (*Salvelinus pluvius*) were not susceptible (Kimura and Yoshimizu 1989; 1991).

C. Epizootiology

In 1988, OMVD was diagnosed in pond cultured coho salmon and marine net pen reared coho salmon in Tohoku district, Japan. OMVD of coho salmon was successfully controlled (Kumagai et al. 1994). In 1991, OMVD was first reported in rainbow trout (Suzuki 1993) and is now a major problem in rainbow trout pond culture in central Japan (Furihata et al. 2003). Horizontal (waterborne) transmission has been demonstrated in the laboratory and field. Clinically infected juveniles and carrier adults are the reservoirs of virus for waterborne transmission. No other reservoirs of virus have been identified.

Observations from naturally occurring disease and experimental infections indicate that fish from one to five months old are most susceptible (Tanaka et al. 1984). In recent years, fewer epizootics have been reported and these epizootics have primarily impacted rainbow trout of 100-500g. Large scale losses in rainbow trout culture have been reported and mortality has reached more than 80% in some cases. Most OMVD occurs at temperatures below 15°C in fresh water (Furihata et al. 2003).

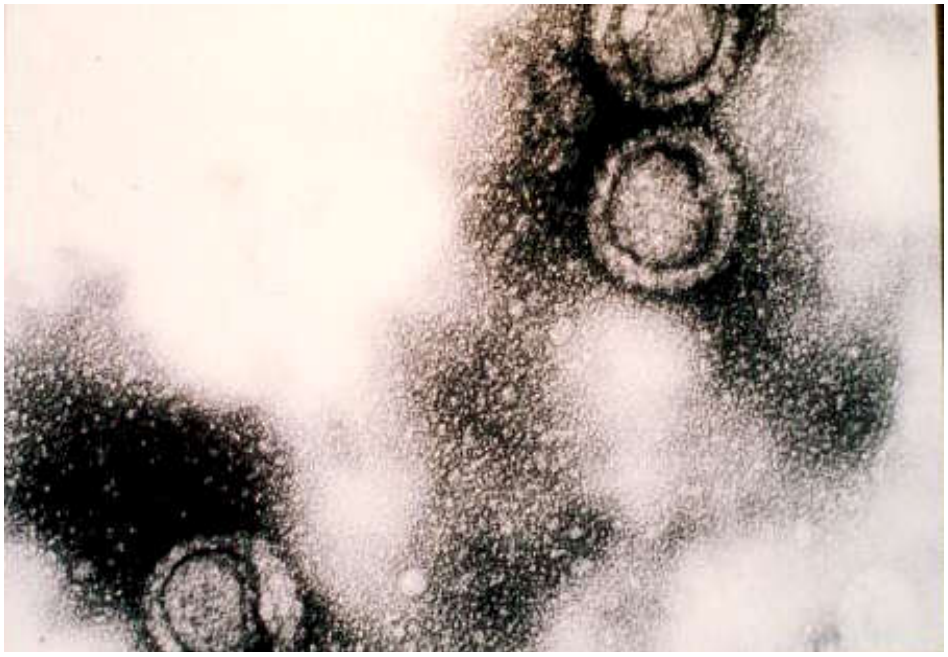

Figure 1. Electron microscopy of negatively stained *Salmonid herpesvirus 2*. Photo by Mamoru Yoshimizu, Hokkaido University.

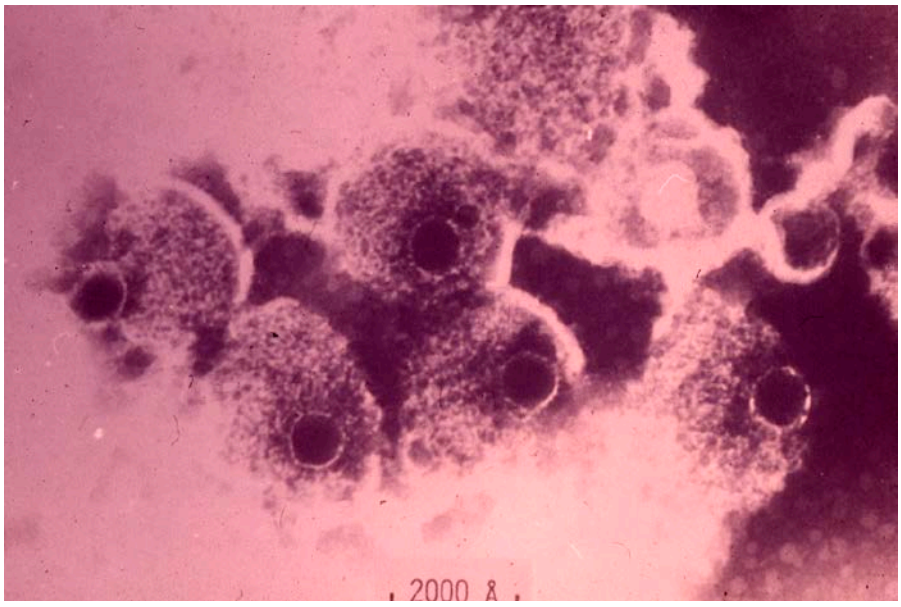

Figure 2. Electron microscopy of cultured *Salmonid herpesvirus 2*. Photo courtesy of Tokuo Sano, Tokyo University of Fisheries.

D. Disease signs

The initial infection by *SalHV-2* appears as a systemic and frequently lethal infection that is associated with edema and hemorrhages. Virus multiplication in endothelial cells of blood capillaries, hematopoietic tissues and hepatocytes underlies the disease signs. Diseased rainbow trout exhibit almost no external signs; although some fish manifest ulcerative lesions on the skin (Fig. 3). Internal signs can include intestinal hemorrhaging, white spots on the liver, skin ulcers and neoplastic tissue around the mouth parts or body surface (Fig. 4, 5 and 6). Four months after the first clinical signs, a varying number of surviving fish exhibit epithelioma occurring mainly around the mouth, upper and lower jaw (Figs 7 and 8) and to a lesser extent, on the caudal fin, operculum and body surface. The neoplasia may persist for up to 1 year post-infection and virus may be isolated from the tumor. A carrier state frequently occurs, which may lead to virus shedding via the sexual products at the time of spawning.

Figure 3. Rainbow trout infected with *Salmonid herpesvirus 2* exhibiting ulcerative skin lesions. Photo by Mamoru Yoshimizu.

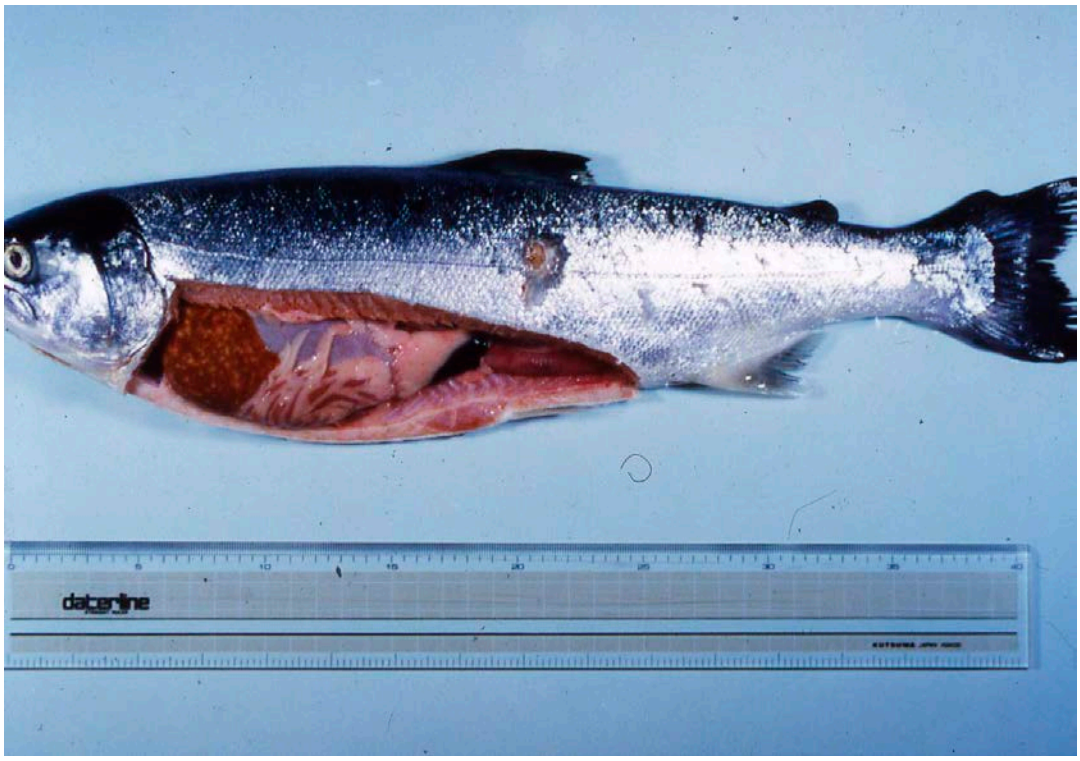

Figure 4. Coho salmon infected with *Salmonid herpesvirus 2* exhibiting skin ulcers and white spots on the liver. Photo courtesy of Akira Kumagai, Miyagi Prefectural Fisheries Experimental Station.

Figure 5. Chum salmon infected with *Salmonid herpesvirus 2* exhibiting hemorrhaging and white spots on the liver. Photo by Mamoru Yoshimizu.

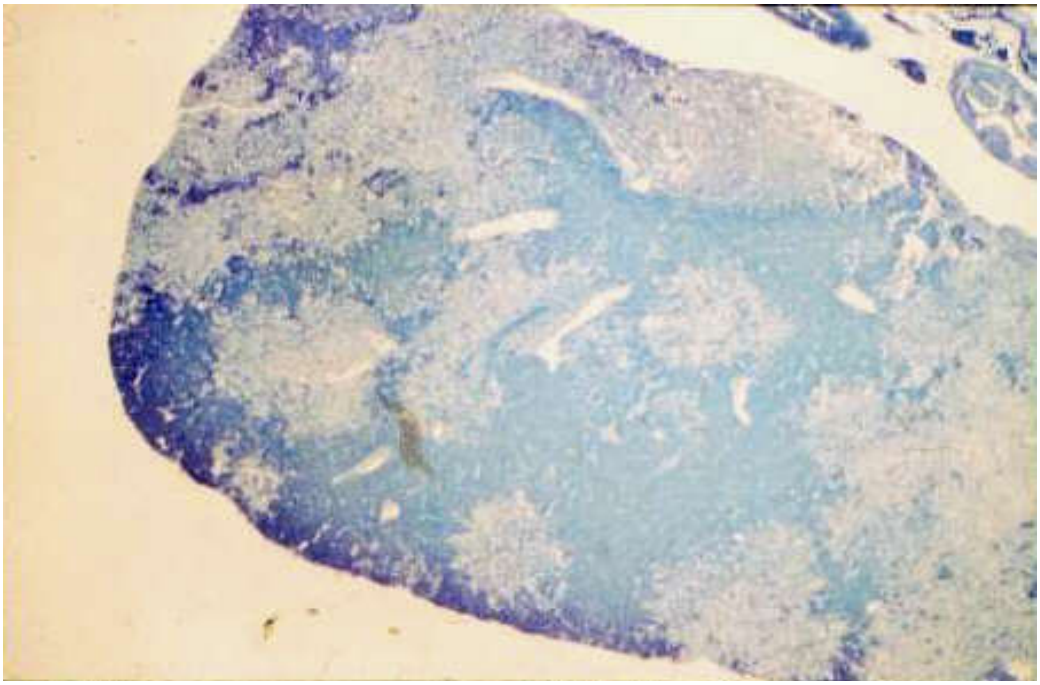

Figure 6. Liver section of *Salmonid herpesvirus 2* infected chum salmon. Photo by Mamoru Yoshimizu.

Figure 7. Tumor on the jaw of chum salmon induced by *Salmonid herpesvirus 2*. Photo by Mamoru Yoshimizu.

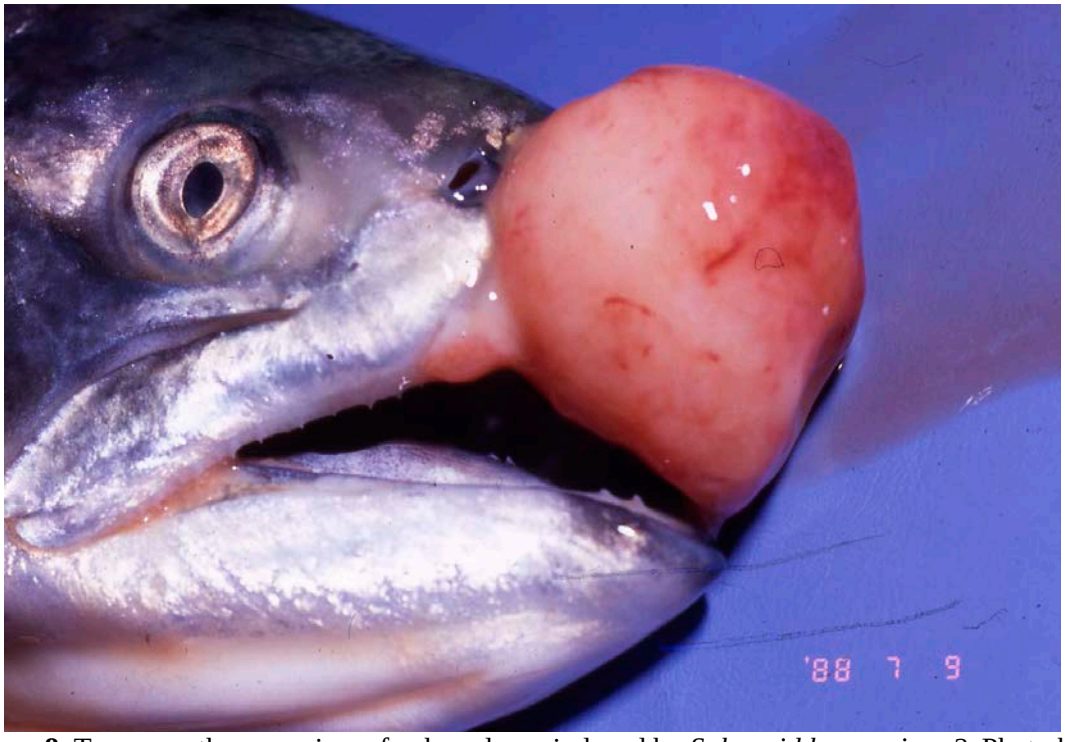

Figure 8. Tumor on the upper jaw of coho salmon induced by *Salmonid herpesvirus 2*. Photo by Mamoru Yoshimizu.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Certain relative key features such as life stage and species of fish, water temperature, disease signs, and disease history of the farm and stock of fish aid the diagnosis of OMVD. To isolate *SalHV-2*, affected fish tissues such as the liver, kidney and spleen are examined by standard cell culture techniques (See Section 2, Chapter 4 Virology).In the case of the tumor tissue, tissue is cut and disinfected with iodophore (50 ppm, 15 min), then washed with Hanks's BSS. Tumor tissue must be prepared for the primary culture or co-culture with RTG-2 cells. 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 equivalent volume of antibiotic solution and overnight incubation at 5°C.

Processed specimens must be inoculated onto the rainbow trout gonad cells (RTG-2) or Chinook salmon embryo cells (CHSE-214). Cytopathic effect includes cell rounding and giant syncytium formation (Fig. 9). Plaque assay procedures of Kamei et al. (1987) which use a methyl cellulose overlay are also used for isolation and enumeration of *SalHV-2*. After one subculture of primary culture cells, the culture medium should be subjected to confirmatory tests (described below).

2. Confirmatory Diagnosis

Confirmation of *SalHV-2* in cell culture is accomplished by serum neutralization tests with polyclonal rabbit antisera or monoclonal antibodies (Hayashi et al. 1993). An ELISA and indirect fluorescent antibody test (I-FAT) for *SalHV-2* have also been reported (Kumagai et al. 1995). The FAT was specific and reacted with all isolates of *SalHV-2* tested and required less time than the

ELISA to obtain a definitive diagnosis (Yoshimizu 1996). A polymerase chain reaction (PCR) method (Aso et al. 2001) has been accepted for confirmation of OMV (see Section Polymerase Chain Reaction (PCR) Method for Confirmation and Worksheet B.5 – *Oncorhynchus masou* virus (OMV)). PCR using the F10 primer, (5'-GTA CCG AAA CTC CCG AGT C) and the R05 primer (5'-AAC TTG AAC TAC TCC GGG G) amplifies a 439 base-pair segment of DNA from OMV strains isolated from masu salmon, coho salmon and rainbow trout, liver, kidney, brain and nervous tissues. Sensitivity of this PCR was $10^{0.8}$ $TCID_{50}$/ml. The PCR test can distinguish *SalHV-2* from *Salmonid herpesvirus 1* (*SalHV-1*), with the later species producing an 800 bp product. For further information on identification of viruses see Section 2, Chapter 4.6, which includes information on confirming '*Oncorhynchus masou* virus'.

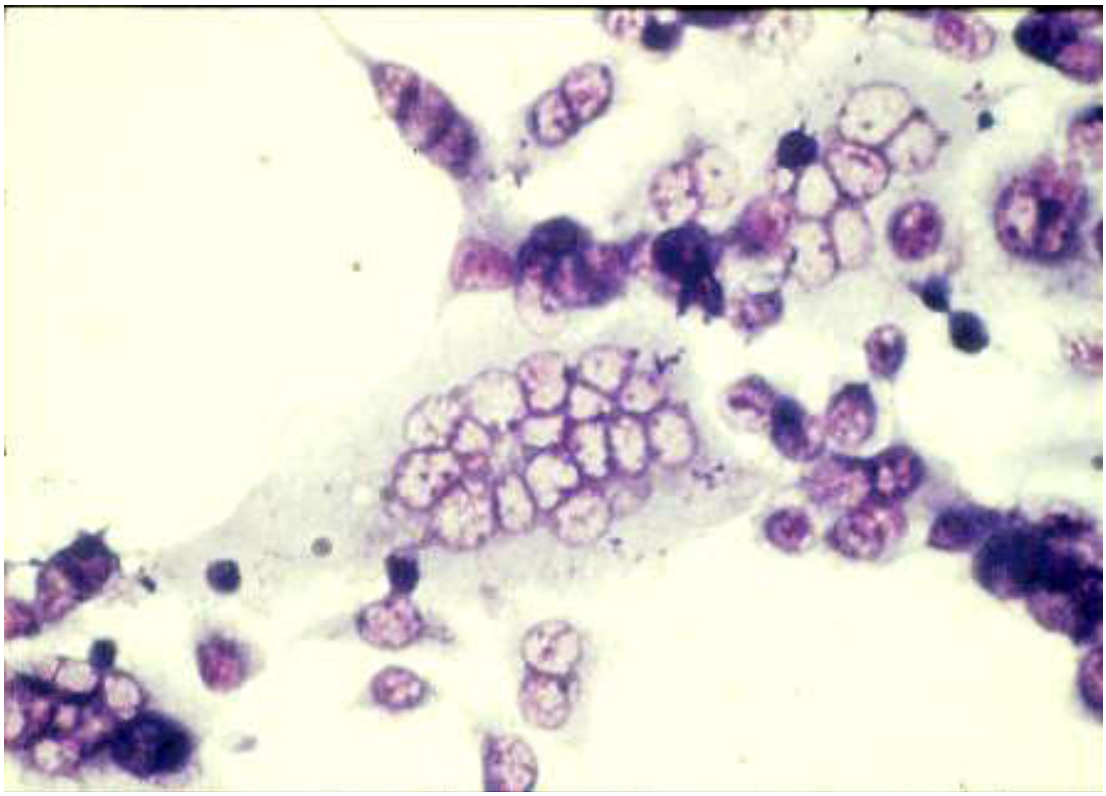

Figure 9. Cytopathic effect of *Salmonid herpesvirus 2* infected CHSE-214 cells. Photo by Mamoru Yoshimizu.

F. Procedures for Detecting Subclinical Infection

The virus can be readily isolated by cell culture from specimens obtained during active epizootics and can be detected in yearling and adult trout and salmon exhibiting no clinical signs. In adult salmonids infected with OMV, the highest prevalence of infection may be found in the latest spawning fish and in post-spawn fish. Tissues from adult fish from late in the spawning run should be examined to optimize chances for *SalHV-2* detection. It is difficult to detect the subclinical infections with PCR, ELISA or I-FAT.

G. Procedures for Determining Prior Exposure to the Etiological Agent

Techniques that have been used to detect antibodies to OMV in fish serum include: plaque

neutralization test and/or ELISA (Yoshimizu 1996). The antibody ELISA could be a useful and convenient technique for sero-epizootiology.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiology Agent

See Section 1, 2.1, General Procedures for Virology.

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