

2.2.3 Herpesvirus Diseases of Salmonids

W. D. Eaton and R. P. Hedrick*

Science and Technology
Malaspina College
900 5th St.
Nanaimo, BC, Canada V9R 5S5
604/753-3245

*School of Veterinary Medicine
Department of Medicine
University of California, Davis
Davis, CA 95616
916/752-3411
rphedrick@ucdavis.edu

I. *Herpesvirus salmonis* Type 1

A. Name of Disease and Etiological Agent

Herpesvirus disease of salmonids is caused by *Herpesvirus salmonis* Type 1 previously *Herpesvirus salmonis* (HPV) and steelhead herpesvirus (SHV).

Herpesvirus salmonis and steelhead herpesvirus from salmonids in western USA have been shown to be closely related strains of the same virus as demonstrated by serological and DNA homology comparisons (Hedrick et al. 1987; Eaton et al. 1991). The North American viruses have properties in common with herpesviruses found among salmonids in Japan but differ sufficiently to separate them into two respective groups, *Herpesvirus salmonis* Type 1 and Type 2 (HPV-1 and HPV-2) based on both serology and DNA homology (Hedrick et al. 1987; Eaton et al. 1991).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Herpesvirus salmonis Type 1 has been found only in the states of Washington and California.

2. Host Range

HPV-1 was first recognized in 1971 in rainbow trout adults at the Winthrop National Fish Hatchery. However, both rainbow trout *Oncorhynchus mykiss* and chinook salmon *Oncorhynchus tshawytscha* juveniles were found to be susceptible to experimental infection by bath exposure. Atlantic salmon *Salmo salar*, brown trout *Salmo trutta*, and brook trout *Salvelinus fontinalis* were refractory to the virus following intraperitoneal (IP) injection. A second strain of HPV-1 was found in 1985 in California among hatchery-reared steelhead trout

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and rainbow trout adults and later among steelhead alevins suffering mortality. The HPV from California was experimentally transmitted to rainbow trout and chinook salmon juveniles by the IP and waterborne routes but not to brown trout *Salmo trutta* or coho salmon *Oncorhynchus kisutch*.

C. Epizootiology

HPV-1 was isolated from 1971 to 1975 at the Winthrop Hatchery from the ovarian fluid of moribund adult rainbow trout that suffered up to 50% post spawning losses. HPV-1 was never isolated from alevins or other juvenile rainbow trout at the hatchery. The virus was transmitted experimentally to juvenile rainbow trout and chinook salmon but severity and prevalence of infection seemingly decreased with fish age. HPV-1 has also been isolated from ovarian fluids of several healthy rainbow and steelhead trout brood stocks in California since 1985 (W. H. Wingfield, California Department of Fish and Game, personal communication). The sole isolation of HPV-1 from juvenile steelhead trout occurred in 1991. These fish were dying from an infestation of ectoparasites and the role of the virus in the mortality remains unknown (Hedrick et al. 1992). The presence of HPV-1 in ovarian and seminal fluids indicates a potential for vertical transmission. Horizontal transmission is also suspected since experimental waterborne exposures to HPV have resulted in infections.

D. Disease Signs

The role of HPV in post-spawning losses is unknown but these fish exhibit overall darkening of the body, a slightly distended abdomen, and occasional exophthalmia. A mild ascitic fluid, pink discoloration of liver and adipose tissues accompanied by a flaccid condition of visceral organs and skeletal muscle resulting from edema occurs.

Experimentally-infected fish show darkened bodies, abdominal distention, and exophthalmos with hemorrhages in the orbital regions and the base of the fins. Mucoid fecal casts and pale gills are also evident. Ascites is evident as is a mottled liver, pale kidney, and empty anterior digestive tract. The liver, spleen, kidney, and heart area are flaccid.

Microscopic pathology of natural infections include a generalized edema of visceral organs and hyperemia of the liver and adipose tissue. In experimentally infected fish, edema of cardiac and skeletal muscle, congestion and necrosis of the hematopoietic tissues of the kidney are evident. Congestion and necrosis are also observed in the gill, heart, digestive tract, liver and pancreas. Syncytium formation and small multifocal regions of inflammatory cells in the acinar pancreas and among hepatocytes is considered pathognomonic for HPV-1 infections. Syncytium formation and small multifocal regions of inflammatory cells in the liver are the only signs in rainbow trout experimentally infected with HPV-1 from California.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Presumptive diagnosis results from the observation of typical herpesvirus CPE in inoculated salmonid tissue culture cells. Final dilution of ovarian fluid should not exceed 1:10 and final

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dilution of tissue samples should not exceed 1:50 prior to inoculation onto either CHSE-214 or RTG-2 cells. Cells should be inoculated before a completed monolayer is formed (50% monolayer) because these viruses often take several weeks to induce CPE. Inoculated cells should be incubated at 10°C because HPV and SHV do not grow well at higher temperatures. Cells should be observed for typical herpesvirus CPE, which consists of cell fusion resulting in plaques and multinucleated giant cells with intranuclear Cowdry type A inclusion bodies. Samples containing very low levels of SHV or HPV will appear negative after 2 to 3 weeks on tissue culture cells, yet after one or two blind passages, the viral-induced CPE can occur. Thus, routine sampling procedures should include a three week initial incubation period followed by a blind passage and another three week incubation before a sample is considered negative.

2. Confirmatory Diagnosis

Confirmatory diagnosis requires serum neutralization assays using anti-HPV or anti-SHV antisera. This will allow differentiation between the strains of Type 1 *Herpesvirus salmonis* (HPV and SHV) and Type 2 (OMV, YTV, CSTV, NeVTA, etc.).

F. Procedures For Detecting Subclinical Infections

No procedures have been reported.

G. Procedures For Determining Prior Exposure to the Etiological Agent

No procedures have been reported.

H. Procedures For Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

See Section 1, 2.1 General Procedures for Virology.

II. *Herpesvirus salmonis* Type 2

A. Name of Disease and Etiological Agent

Herpesvirus disease of salmonids is caused by *Herpesvirus salmonis* Type 2. Previously the oncogenic viruses include *Oncorhynchus masou* virus (OMV) and H-83, yamame tumor virus (YTV), coho salmon tumor viruses (CSTV, OKV, COTV, CSLV), and the nononcogenic Nerka virus from Towada Lake, Akita and Amori Prefecture (NeVTA).

The salmonid herpesviruses from Japan appear to be related strains of *Herpesvirus salmonis* Type 2 (HPV-2). NeVTA appears to be a unique isolate among HPV-2 strains because it does not show the oncogenic properties shared by other strains. A serological and DNA homology comparison of OMV, YTV, and NeVTA, with two HPV-1 strains supports their separation into a related but distinct group designated HPV-2.

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Found in Japan.

2. Host Range

OMV has been isolated from masou salmon *Oncorhynchus masou*, but juvenile kokanee *Oncorhynchus nerka*, coho *Oncorhynchus kisutch* and chum salmon *Oncorhynchus keta*, and rainbow trout *Oncorhynchus mykiss* are susceptible to experimental infections via the waterborne route. YTV has been isolated from a mandibular tumor in yamame salmon *Oncorhynchus masou* and it has been shown to be pathogenic for juvenile yamame salmon and chum salmon. CSTV and additional coho salmon tumor isolates have been obtained from fin and mandibular tumors of pen-reared coho salmon. NeVTA has been isolated from kokanee salmon fry suffering mortality as high as 80%. The virus has only been recovered from fry reared at the hatchery between 1970 to 1974 at Towasa Lake, Akita and Amori Prefecture.

C. Epizootiology

In 1978, OMV was isolated from the ovarian fluid of healthy appearing adult female masou salmon whose progeny had a history of poor fry survival rates. In the laboratory, fry were exposed to water containing OMV to determine the pathogenicity of the virus. Kokanee salmon were found to be the most susceptible to OMV, followed by the masou, chum and coho salmon, and rainbow trout. Both vertical and horizontal transmission are likely. Susceptibility decreased with increasing age; by eight months of age salmon were completely refractory to the virus; however, squamous cell epitheliomas developed in the opercula, head, mouth, eyes, caudal, fin, and kidney of chum and coho salmon surviving experimental OMV infections. OMV was isolated from some of the tumors and from primary cell cultures derived from tumor tissue.

YTV was originally isolated from mandibular tumors on juvenile yamame salmon in Japan. Five-month-old yamame salmon were more susceptible to infections of YTV than chum salmon. Some surviving yamame and chum salmon developed tumors. The tumors were classified as basal cell epitheliomas.

Only limited information is available on the pathogenesis and epizootiology of CSTV. Virus isolated from tumors of coho salmon was infectious for other coho salmon injected with the virus. The experimental challenge resulted in less than 28% mortality, and tumor formation occurred in 8% of the coho salmon.

NeVTA epizootics in sockeye salmon (kokanee) fry in Towada Lake (Honshu) between 1970 and 1974 caused mortality as high as 80%. The virus has been isolated from adult kokanee on Honshu.

D. Disease Signs

1. OMV

Fish experimentally infected with OMV became lethargic, anorexic, and congregate at the water intake. Gross signs include exophthalmia and petechiation of the body surface. Naturally infected fish only showed a darkened body color. Experimentally challenged fish have mottled

and swollen livers, swollen spleens, and empty digestive tracts. Surviving fish may develop tumors on the external surfaces of the opercula, heads, mouths, eyes, and caudal fins. Microscopic examination of tissues from fish infected with OMV show necrosis and syncytia formation in the liver and hematopoietic tissue of the kidney and edema, and less severe necrosis of the spleen, pancreas, cardiac muscle, and brain. One fish developed an epithelioma in the kidney.

Fish experimentally infected with YTV developed exophthalmia, abdominal distention, and hyperemia of the anus. Mandibular tumors have been observed in fish naturally infected with YTV. Fish infected with YTV develop basal cell epitheliomas but showed no other histopathological changes.

2. CSTV

Fish naturally infected with CSTV develop fin and mandibular tumors. No other gross external signs are observed.

3. NeVTA

Infected fish are lethargic clustering around water intake and are anorexic, and some swim erratically. External gross signs include darkened bodies and abdominal distention. No internal gross signs are reported. Microscopic findings include granular degeneration of the skeletal muscle, proteinaceous fluid in Bowman's spaces, kidney tubule necrosis, hypertrophy and desquamation of the gill epithelium, vacuolation of the pancreatic acinar cells and syncytia, and cytoplasmic inclusions in the kidney interstitium.

E. Disease Diagnostic Procedure

1. Presumptive Diagnosis

Ovarian fluid and tissue samples should be diluted as described previously for HPV and SHV; however, CHSE-214 or RTG-2 cells should be incubated at 15°C rather than 10°C. OMV, YTV, and NeVTA replicate at 10 to 20°C and virus replication at 15°C suggests that neither HPV nor SHV is involved. Infected tissue culture cells should exhibit the typical herpesvirus CPE.

2. Confirmatory Diagnosis

Serum neutralization assays using antiserum against OMV, YTV, or CSTV can be performed. However, this will only differentiate OMV, YTV, CSTV, and NeVTA from SHV and HPV. There is serological cross-reaction among OMV, YTV, CSTV, and NeVTA.

F. Procedures for Detecting Subclinical Infections

No procedures have been reported. Subclinical infections may be detected by cell culture assays.

G. Procedures for Determining Prior Exposure to the Etiological Agent

No procedures have been reported. Anti-OMV neutralizing activity has been detected in serum of experimentally infected chum salmon. Titers up to 1:320 were detected.

H. Procedures For Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

See Section 1, 2.1 General Procedures for Virology.

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