CHAPTER 2.1.12.

ONCORHYNCHUS MASOU VIRUS DISEASE

1. Scope

Oncorhynchus masou virus disease (OMVD) is characterised by hepatitis and an oncogenic and skin ulcerative condition among salmonid fish in Japan (2, 10, 11, 13, 18), and probably in the coastal rivers of eastern Asia that harbour Pacific salmon.

2. Disease information

OMVD is caused by Oncorhynchus masou virus – an oncogenic virus isolated from masou salmon (Oncorhynchus masou) (8, 9).

2.1. Agent factors

OMV is a herpesvirus and the causative agent of hepatitis and an oncogenic and skin ulcerative condition of masou salmon and coho salmon (O. kisutch), and of hepatitis of kokanee salmon (O. nerka) and rainbow trout in Japan (11, 15, 17, 18).

2.1.1. Aetiological agent, agent strains

Synonyms of OMV include: Nerka virus Towada Lake, Akita and Amori prefecture (NeVTA), Yamame tumour virus (YTV), Oncorhynchus kisutch virus (OKV), coho salmon tumour virus (CSTV), coho salmon herpesvirus (CSHV), rainbow trout kidney virus (RKV), or rainbow trout herpesvirus (RHV) (2, 5, 10, 18, 19, 23).

2.1.2. Survival outside the host (i.e. in the natural environment)

Infectivity of OMV reduces rapidly over the period of 1 week in rearing water, river water or pond water, compared with filter-sterilised rearing water, and bacteria living in environmental water play an important role in virus inactivation (24).

2.1.3. Stability of the agent (effective inactivation methods)

OMV is stable at 5°C for more than 7 days, but unstable at temperatures above 20°C or at partial-freezing temperatures. It is sensitive to ultraviolet irradiation (103 μW second/ cm²), ozone or iodophor treatment, and also inactivated by alcohol and various other disinfectants, as well as heating (50°C, 1 minute) and freezing (3, 6, 16).

2.1.4. Life cycle

Horizontal transmission has been demonstrated, and water-borne transmission can be accomplished in the field. Clinically infected juvenile rainbow trout and carrier adults are the virus reservoir for water-borne transmission. No other virus reservoirs have been identified (20).

2.2. Host factors

2.2.1. Susceptible host species

Epizootics of OMVD have been commonly occurred in juvenile rainbow trout. Yearling rainbow trout and large rainbow trout have also been shown to be susceptible. Masou salmon, chum salmon (O. keta), coho salmon and kokanee salmon have been shown to be susceptible (2).

2.2.2. Susceptible stages of the host

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2.2.3. Species or sub-population predilection (probability of detection)

Amago salmon (O. rhodurus) and iwana salmon (Salveninus pluvius) have not been shown to be susceptible (20).

2.2.4. Target organs and infected tissue

Initial infection by OMV manifests as a systemic and frequently lethal infection associated with haemorrhages. Virus multiplication in the endothelial cells of blood capillaries, haematopoietic tissue and hepatocytes is observed. Four months after these first clinical
signs, a varying number of surviving fish exhibit epithelioma occurring mainly around the mouth, upper and lower jaw and, to a lesser extent, on the caudal fin, operculum and body surface. This neoplasia may persist for up to 1 year post-infection (11, 12, 17, 22).

### 2.2.5. Persistent infection with lifelong carriers

Clinically infected juvenile rainbow trout and carrier adults are the virus reservoir for water-borne transmission and transmission via egg surface association (20).

### 2.2.6. Vectors

No vectors or virus reservoirs have been identified.

### 2.2.7. Known or suspected wild aquatic animal carriers

No carriers of this virus have been identified.

### 2.3. Disease pattern

#### 2.3.1. Transmission mechanisms

The transmission of OMV is horizontal and possibly 'egg-surface associated'. Horizontal transmission may be direct or vectorial, with water being the major abiotic factor. Animate vectors and inanimate objects also act in OMV transmission. Clinically, infected juvenile rainbow trout and carrier adults are the reservoir of virus for water-borne transmission via ovarian fluid and egg-surface contamination to alevins. No other reservoirs of virus have been identified (20).

#### 2.3.2. Prevalence

By the beginning of the 1980s, OMV was widely distributed in wild masou salmon in the northern part of Japan. In 1988, OMVD was diagnosed in pond-cultured and net-pen reared coho salmon in the marine environment in the Tohoku district of Japan. However, OMVD of masou salmon and coho salmon was successfully controlled (21).

#### 2.3.3. Geographical distribution

Since 1991, OMVD was found in rainbow trout, and OMVD has become a major problem in pond culture of rainbow trout in central part of Japan (2).

#### 2.3.4. Mortality and morbidity

Observations from both naturally occurring disease and experimental infections indicate that fish from 1 to 5 months of age are most susceptible. In recent years, epizootics have been limited and reported in juvenile, yearling to large rainbow trout (100–500 g), and large-scale losses have been reported. On occasion, morality has reached more than 80% (2).

#### 2.3.5. Environmental factors

Epizootics have been limited to rainbow trout, and reported in juvenile, yearling to large rainbow trout. OMVD occurs in winter or at temperatures below 5°C where well water is used for culture in fresh water (2).
2.4. Control and prevention

OMV is sensitive to ultraviolet irradiation, ozone or iodophor treatment. Since 1983, the OIE Reference Laboratory strongly recommends, as a control strategy, inspection of the ovarian fluid from mature fish and the disinfection of collected eggs with iodine at the early eyed stage. Currently, OMV is no longer detected in most of the hatcheries in the northern part of Japan. Nowadays, all eggs and facilities are disinfected with iodophor just after fertilisation and again at the early eyed stage. As a result, OMV has not been isolated in hatcheries in this area, and outbreaks of OMVD have been avoided, except in the central part of Japan where rainbow trout are cultured (20).

2.4.1. Vaccination

A formalin-killed OMV vaccine produced by an OMV strain isolated from rainbow trout is effective, and has been used successfully to reduce the numbers of OMV replicating in ovarian fluid.

2.4.2. Chemotherapy

Anti-herpesvirus agent, acyclovir: 9-(2-hydroxyethoxymethyl)guanine was effective in inhibiting replication of OMV in both in vitro and in vivo experimental infections of chum salmon fry with OMV. Acyclovir also inhibited the induction of epithelioma (8, 9).

2.4.3. Immunostimulation

No information available.

2.4.4. Resistance breeding

Breeding to establish resistant rainbow trout has been conducted and fish strains are becoming resistant and, after four or five generations, severe mortality no longer occurs.

2.4.5. Restocking with resistant species

No scientific evidence.

2.4.6. Blocking agents

Formalin-killed or attenuated vaccine, and anti-herpesvirus agents such as acyclovir, Ara-A (9-ß-D-arabinofuranosyladenine), IUdR (5-iodo-2'-deoxyuridine) and PA (phosphonoacetate) are effective (8, 9).

2.4.7. Disinfection of eggs and larvae

Disinfection of the egg surface by iodophor at a concentration of 50 ppm (parts per million) for 15 minutes, just after fertilisation and the eyed stage, is effective in preventing OMV infection. OMVD is not reported in alevins originating from disinfected eggs that had been incubated and hatched in virus-free water (23).

2.4.8. General husbandry practices

OMV is sensitive to ultraviolet irradiation, ozone or iodophor treatment. Well water or UV-irradiated river water should be used for hatching and rearing the fry. Inspection of ovarian fluid from mature fish should be conducted, and all eggs and facilities should be disinfected by iodophor just after fertilisation and again at the early eyed stage (20, 21).

3. Sampling

3.1. Selection of individual specimens

Alevins (body length <4 cm) should be collected whole, whereas viscera including liver, kidney and spleen should be collected from fish 4-6 cm long, and skin ulcerative lesions or neoplastic tissues should be taken from larger sized fish. Liver, kidney, spleen and encephalon (any size fish) and/or ovarian fluid from brood fish at spawning time should also be collected (22).

3.2. Preservation of samples for submission

Collected specimens must be held and transferred to the laboratory at temperatures under 5°C (22).

3.3. Pooling of samples

Specimens are collected individually but for small alevins, a five-fish pool can be taken.
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3.4. Best organs or tissues

Kidney is the best organ for 1-month-old fish, and liver is the best organ for fish aged 3 months or more.

3.5. Samples/ tissues that are not appropriate

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4. Diagnostic methods

The diagnosis and screening for OMVD is based on direct methods that include isolation of OMV in cell culture followed by its immunological identification, immunological demonstration of virus antigen or virus-specific gene detection in infected fish tissues.

• Check body surface and the data on water temperature, origin of seed, disinfection of egg surfaces, life span of UV lamp, etc.

• Open the body cavity and remove kidney, spleen or liver tissue from diseased fish. The tissue is fixed for histopathological studies, and stamp smears are made for use in the fluorescent antibody test (FAT). Tissue is homogenised in Hanks’ balanced salt solution with antibiotics, and treated overnight in a refrigerator.

• The supernatant is collected and inoculated on to RTG-2 or CHSE-214 cells, and the plates are incubated at 15°C for 10 days with a positive control.

• When a cytopathic effect (CPE) appears, culture the fluid inoculates again on the same cell line and under the same culture conditions.

• Isolated virus is tested for immunological identification by indirect FAT (IFAT), immunoperoxidase (IP) stain and/or the enzyme-linked immunosorbent assay (ELISA), and for specific gene detection by polymerase chain reaction (PCR).

• Immunological antigen detection of OMV or virus-specific gene in infected fish tissues can be demonstrated using the FAT or IP stain, and PCR.

• Blood samples are collected and sera are tested for antibody detection using ELISA.

4.1. Field diagnostic methods

It is very important to collect field information, such as water temperature, origin of seed, egg disinfection, life span of UV lamp, etc.

4.1.1. Clinical signs

In rainbow trout, the diseased fish exhibit almost no external signs except that some fish manifest ulcerative lesions on the skin. Internally, white spots on the liver are observed. Infected fish show darkening of the body colour, pop eye and skin ulcers.

4.1.2. Behavioural changes

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4.2. Clinical methods

General diagnostic tests for parasites, bacteria and virus are carried out. The body surface and internal organs are observed carefully, along with the colour of the liver.

4.2.1. Gross pathology

Diseased fish exhibit almost no external signs, although some fish show ulcerative lesions on the skin. Internally, intestinal haemorrhages and white spots on the liver are observed. In the case of coho salmon and masou salmon, tumour tissues are sometimes observed.

4.2.2. Clinical chemistry

No important information.

4.2.3. Microscopic pathology
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Check for parasites or moulds on the skin and bacteria in the kidney tissue.

4.2.4. Wet mounts
Liver and kidney stamp-smear specimens are tested by FAT using rabbit antiserum or monoclonal antibody against OMV (4).

4.2.5. Smears
Liver and kidney smears are stained with the Gram stain and observed for rickettsia or other bacteria.

4.2.6. Fixed sections
Histopathological sections of liver and kidney are prepared and examined for necrosis and syncytium formation.

4.2.7. Electron microscopy/cytopathology
When virus is isolated, the necrotic area of liver is examined by electron microscopy.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods
Direct detection methods for OMV include isolation of OMV in cell culture followed by its immunological identification, immunological demonstration of virus antigen or virus-specific gene detection in infected fish tissues.

4.3.1.1. Microscopic methods
It is difficult to see a virus particle using a microscope but syncytium formation is observed in the liver.

4.3.1.1.1. Wet mounts
It is very important to distinguish between white spots in the liver caused by rickettsia or bacteria.

4.3.1.1.2. Smears
Stained smears are very important for distinguishing between the causative agents P. rickettsia and R. salmoninarum.

4.3.1.1.3. Fixed sections
Fixed sections are necessary for the preparation of histopathological sections.

4.3.1.2. Agent isolation and identification
Isolation of OMV in cell culture followed by immunological demonstration of virus antigen or the virus specific gene in infected fish tissues.

4.3.1.2.1. Cell culture/artificial media
OMV can be isolated in the RTG-2 or CHSE-214 cell lines (14). Cells are cultured at 15°C for 7–10 days.

4.3.1.2.2. Antibody-based antigen detection methods
For antibody-based antigen detection methods, IFAT, IP stain and ELISA are available (4).

4.3.1.2.3. Molecular techniques (PCR, ISH, sequencing, etc.)
For molecular techniques, PCR and the in-situ hybridisation test are available (1).

4.3.1.2.4. Agent purification
Agent purification is done using a two-step centrifugation.

4.3.2. Serological methods
Since there is insufficient knowledge of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been recognised as a valuable diagnostic method for assessing the viral status of fish populations. Recently, non-
specific reactions on ELISA plates have successfully being reduced thus making ELISA techniques available for the detection of virus antibody in salmonid fish, flounder, and puffer fish (7); antibody-detection ELISA is available.

5. Rating of tests against purpose of use

To aid in the diagnosis of OMVD, certain key features such as life stage and species of fish, water temperature, disease signs, and disease history of the facility and stock of fish are evaluated. To isolate OMV, tissue and reproductive fluids are examined by standard cell culture techniques. Processed specimens must be inoculated onto the RTG-2 or CHSE-214. CPE includes rounded cell and giant syncytium formation. Plaque assay procedures that use a methyl cellulose overlay are also used for isolation and enumeration of OMV. Disease signs, microscopic pathology, past history of OMVD, and observation of typical CPE provide the best evidence for a presumptive diagnosis.

Table 5.1. Methods for targeted surveillance and diagnosis of OMVD

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<th>Method</th>
<th>Targeted surveillance</th>
<th>Presumptive diagnosis</th>
<th>Confirmatory diagnosis</th>
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<tr>
<td></td>
<td>Larvae</td>
<td>PLs</td>
<td>Juveniles</td>
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<td>Gross signs</td>
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<td>Antibody-based assays</td>
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Table 5.1 continued. Methods for targeted surveillance and diagnosis of OMVD

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<th>Method</th>
<th>Targeted surveillance</th>
<th>Presumptive diagnosis</th>
<th>Confirmatory diagnosis</th>
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<tbody>
<tr>
<td>DNA Probes α <em>in situ</em></td>
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<tr>
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<td>Sequence</td>
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PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from Oncorhynchus masou virus disease

The surveillance procedures for OMV are based on isolation of the virus in cell culture and co-culture of neoplastic tissues with salmonid cell lines. Confirmatory testing is by immunological identification using neutralisation or FAT, and virus-specific gene detection using the PCR reaction.

Isolation of the OMV in cell culture using RTG-2 or CHSE-214 cells, and co-culture of neoplastic tissues with salmonid cell lines are described in Sections 1–4. Confirmatory testing is by immunological identification using neutralisation or FAT, IP stain, antigen-detection ELISA, or virus-specific gene detection using PCR described in Sections 1–4.

7. Corroborative diagnostic criteria

The identification procedures for OMV are based on isolation of the virus in cell culture and co-culture of neoplastic tissues with salmonid cell lines. Confirmatory testing is by immunological identification using neutralisation or FAT, IP stain, antigen-detection ELISA, virus-specific gene detection using PCR reaction, and antibody detection ELISA.

7.1. Definition of suspect case
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The definition of a suspect case is virus isolation and neutralisation, or FAT and PCR.

7.2. Definition of confirmed case

The definition of a confirmed case is a combination of virus isolation and neutralisation, and result of FAT/or ELISA and PCR.

8. References


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25. There is an OIE Reference Laboratory for Oncorhynchus masou virus disease (see Table at the end of this Aquatic Manual or consult the OIE Web site for the most up-to-date list: www.oie.int).