1975

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Ken Wolf
Eastern Fish Disease Laboratory

Tokuo Sano
Tokyo University of Fisheries

Takahisa Kimura
Hokkaido University

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Ken Wolf
Eastern Fish Disease Laboratory
Route #1, Box 17A
Kearneysville, West Virginia 25430

Tokuo Sano
Tokyo University of Fisheries
Konan 4-5-7
Minato-ku, Tokyo

Takahisa Kimura
Hokkaido University
Hakodate, Hokkaido, Japan

FISH DISEASE LEAFLET 44 December 1975

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Division of Cultural Methods Research
Washington, D. C. 20240

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C., 20402-Price $1.00

Stock Number 024-010-00412-6

A newly recognized virus, lethal for fry and fingerlings, has been isolated from brood stock rainbow trout (Salmo gairdneri) at the Winthrop National Fish Hatchery, Washington. A similar if not identical virus has been found in natural epizootics occurring annually since 1970 among fry of landlocked sockeye salmon (Oncorhynchus nerka) on Honshu Island, Japan. More recently, this agent was isolated from moribund and dead adult O. nerka on Hokkaido Island, Japan, but it remains to be determined whether or not the virus causes death in adult salmonids.

The purpose of this leaflet is to bring the new agent to the attention of diagnosticians and researchers. It is recognized that some aspects of the virus and its effect upon host species are still incompletely known.

DIAGNOSIS AND IDENTIFICATION

Behavior Changes

Most affected fry become lethargic as death approaches. During terminal stages some swim erratically, apparently losing motor control, and others are hyperactive.

External Signs

Various degrees of abnormal darkening are common, and exophthalmia--at times extreme--is frequent (Fig. 1). Hemorrhage may be present in the orbit of fish with extreme exophthalmia. Similarly, abdominal distention is common. Gills are abnormally pale, but other external signs are lacking.

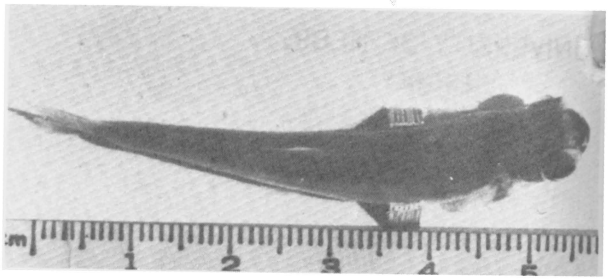

Figure 1. Rainbow trout fry with extreme exophthalmia, prominent distention of abdomen, and darkening associated with infection by Herpesvirus salmonis. The scale is in millimeters.

Internal Signs

Ascitic fluid is abundant, and anemia and edema are evident in the visceral mass. The liver, spleen, and digestive tract are flaccid and the vascular organs are mottled with areas of hyperemia. Kidneys are abnormally pale, though not noticeably swollen. The digestive tract is devoid of food.

Clinical Findings

Stained blood films show 10% or more immature erythrocytes, and some of the cells appear to be dividing. Hematocrit value is greatly depressed and may be as little as 30% of normal.

Histopathological Findings

Blood in infected specimens contains abnormally high numbers of immature erythrocytes and leucocytes. Kidney hematopoietic tissue is greatly increased and mitotically hyperactive. Kidney tubules are filled with serous material; renal tissue is edem-

atous but necrosis is focal and mild where it occurs.

Serous deposits in the orbits are undoubtedly the cause of exophthalmia. Skeletal muscle is edematous and also shows accumulation of serous material. Cardiac muscle is similarly edematous, and hematopoietic activity is present in heart tissue, an organ in which blood cell formation does not normally occur.

The liver is perhaps the target organ for viral effects for it is edematous and shows fatty infiltration and vascular stasis. Hepatic tissue shows areas of mild necrosis--as does pancreatic acinar tissue, but to a lesser degree. Pancreatic necrosis is never as severe or as extensive, however, as it is in cases of infectious hematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN). The spleen lacks red pulp.

Identification

Thus far, antiserum has not been developed for this herpesvirus, and diagnosis and virus identification must be based on internal signs, histopathological changes, and presumptive tests of the agent itself.

The virus is the only salmonid pathogen demonstrably capable of inducing syncytium formation in rainbow trout gonad cells (RTG-2). The rainbow trout fry (RTF-1) cell line is similarly susceptible; but fathead minnow (FHM) cells are refractory, or at least do not show cytopathic effects. In fixed and stained cultures, the syncytia are readily visible, and some nuclei show atypical Cowdry Type A inclusions. May-Grünwald-Giemsa staining reveals prominent cytoplasmic basophilia in the infected cells.

Rabbit antisera against IHN, IPN, and Egtved viruses do not neutralize this salmonid herpesvirus.

The herpesvirus plaque type differs from those of the other salmonid viruses: cell rounding and syncytium development are readily recognizable, and also cellular debris is much reduced (Fig. 2).

Herpesvirus involvement in infected tissues or cell cultures, as revealed by electron microscopy, is also presumptive identification of the agent.

CAUSE OF THE DISEASE

The etiologic agent is a member of the herpesvirus group. Electron microscopy shows that the replication cycle begins in cell nuclei which contain both empty capsids and capsids containing nucleoids. Typical of herpesviruses, mature virions are enveloped and may be found in vesicles in the cytoplasm and especially in extracellular aggregates. A peculiarity of this herpesvirus is the rather intense osmiophilic nature of the envelope or material within the envelope. Infected cells show paramyelin-like lamellae or fibrils within the nucleus similar to those caused by the channel catfish virus.

A
B
C
D

Figure 2. Plaque characteristics of salmonid viruses in RTG-2 cells.

A. IPN virus (all strains thus far isolated) characteristically produces a stellate plaque with very irregular margins. The cells become somewhat pyknotic but plaque interiors often contain cells that have not been killed. There is little cellular debris. The plaque itself has a lace-like network of RTG-2 cells which, though killed, retain a semblance of normal shape even after death. Killed cells stain only slightly with crystal violet.

B. IHN virus produces a well-defined plaque in which affected cells characteristically round up and accumulate by contraction at the plaque margins. Interiors of the plaque typically have prominently rounded cells and may also be filled with discontinuous, coarsely granular debris. Under higher magnification, cells near the edges of the plaque show margination of chromatin, a diagnostic feature of IPN virus in cell cultures.

C. Herpesvirus salmonis plaques tend to be elongate and to have relatively clean interiors. Plaques often follow whorl lines of typical RTG-2 growth, and living cellular processes extend into the open area. The key features are cellular fusion and syncytium formation.

D. Egtved virus produces a plaque which characteristically has a well-defined margin. The plaques appear to be punched out of the cell sheet, and their interiors are filled with a uniformly distributed finely granular debris. Cells in the plaque interior are usually all dead.

Herpesvirus salmonis, the name proposed for this agent, is heat-, chloroform-, ether-, and acid-labile, but some infectivity persists after standard treatment at pH 10. With a suitable positive control, the agent does not agglutinate human Type O+, rabbit, or rainbow trout erythrocytes at pH 7.3 during incubation at 4 C. Differential filtration shows that infectivity readily passes a membrane filter of 220-nm mean porosity, but is retained by a 100-nm membrane.

Infectivity titers are comparatively low, attaining only about 1 X 10^5 plaque-forming units (PFU) per milliliter.

SOURCE AND RESERVOIR OF INFECTION

Since virus has been isolated from ovarian fluids, it is safe to consider that adults are the prime source of the agent. In this context, it also appears reasonable to assume that this feature ensures generation-to-generation survival, because abundant young host fish are available after the spawning season.

The histopathologic changes in renal tissues indicate that infected fry and fingerlings shed virus during the time disease signs are present, and possibly earlier.

MODE OF TRANSMISSION

Fish-to-fish transmission is assumed--at least during epizootics. Also, one must consider the possibility of vertical transmission--the virus being either in or on eggs Thus far, experimental transmission by holding susceptible young fish in water with virus has not been reported.

INCUBATION PERIOD

After intraperitoneal injection of virus at 10 C, an incubation time of at least a month elapsed before the first death occurred. Fry 5 to 10 cm in total length sustained the first mortality at 33 days and fingerlings began to die at 38 days. Thus far, other methods of infection have not been reported.

PERIOD OF COMMUNICABILITY

It can only be postulated that the disease is communicable while clinical signs are present. A carrier state is obviously present in adults, because Herpesvirus salmonis was originally isolated from ovarian fluid.

SUSCEPTIBILITY AND RESISTANCE

Rainbow trout (fry and fingerlings) and kokanee or landlocked sockeye salmon (fry) are thus far the only known susceptible species. The effect on adults of these two species is not yet known. Atlantic salmon (Salmo salar), brown trout (S. trutta), and brook trout (Salvelinus fontinalis) have been suitably tested by intraperitoneal injection, but have proved to be refractory. Other species of Pacific salmon have not been tested.

RANGE

The newly reported infection is known to occur in Japan and the United States. Because it is best isolated at 10 C, a temperature lower than that usually employed for salmonid virological examinations, the virus might be present elsewhere--having been missed during earlier work.

OCCURRENCE

In Japan, the virus has been implicated with problems that occurred only during the summer. The virus caused epizootic mortality among young kokanee salmon, and it was implicated with high summer mortality among adult kokanee salmon in a freshwater lake.

METHODS OF CONTROL

The only certain method of control is avoidance, and that requires that sources of the virus be known. Since low temperatures seem to be required for the virus to produce disease, it may be possible to minimize mortality by holding infected fish at temperatures of 15 C or higher. There is no assurance, however, that the virus will be eliminated by such a practice.

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