Studies on Viral Diseases of Japanese Fishes—V Infectious Pancreatic Necrosis of Amago Trout

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An IPN-like epizootic with high mortality occurred among Amago, *Oncorhynchus rhodurus macrostomus*, reared in a rainbow trout farm. The cumulative mortality was about 28%.

Histopathological examinations showed that intensive congestion, hemorrhage and necrosis in the kidneys were rather more evident than was necrosis in the pancreatic tissue.

Inoculants prepared from the diseased Amago fry showed the isolant to have a titer of about $10^{8.5}$ TCID₅₀/ml. Neutralization tests of the agent with IPN polyvalent antiserum indicated that the agent was an IPN virus. This is the first case of an IPN outbreak among *Oncorhynchus* reared in Japan.

Yamame reared in nearby ponds of the same farm showed no mortality and no agent could be isolated.

During the past several years, culture of native Japanese salmonids of the genus *Oncorhynchus* has developed significantly. Amago, *O. rhodurus macrostomus* is one species which can be reared in the same kind of environment as rainbow trout (*Salmo gairdnerii irideus*), and Yamame (*O. masou ishikawae*).

In a previous report, it was noted that Japanese salmonids can be infected with Infectious Pancreatic Necrosis (IPN) virus; for example, we encountered IPN among Amago reared in a rainbow trout farm located in Nagano Prefecture in May, 1972.

Materials and Methods

Experimental fish were from an epizootic at Minamisawa Trout Farm, Nagano Pref., among 21-week-old Amago fry, weighing 1.2–2.0 g and having a length of 4.0–5.1 cm. Parent stock were from Gifu Pref., and the eggs were taken artificially at Kiso-kawa Hatchery, Nagano Pref., 16th to 18th November, 1971.

The isolation of a suspect agent followed the protocol described in the previous paper.¹⁾ Serum neutralization tests²⁾ for the agent were carried out using rabbit polyvalent IPN antiserum prepared by Wolf at the Eastern Fish Disease Laboratory, W. Va. U. S. A.

The biophysical stability of the agent to ether, chloroform, alcohol, glycerin and heat was also tested.

Specimens were preserved in Bouin's fixative for histologic examination.

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Results

High mortality of Amago fry was observed in ponds having a temperature of 10.5 to 13.0°C during early May to mid June, 1972. The epizootic was acute, and mortality exceeded 0.5% per day for 25 days of the 40 day-epizootic. Cumulative mortality was approximately 28%. The diseased fish were sluggish in their behavior and dark in color. Abdomens were distended and contained ascitic fluid. There was exophthalmus, and in particular congestion at the base of the pelvic and anal fins. Such fish eventually died showing spiral swimming alternating with quiet behavior. The gross anatomy revealed: partial congestion and petechiae in the liver (Fig. 1). Some of the organs showed whitish-gray color, relaxation of stomach and intestine which in some fish contained a transparent or milky mucous, hemorrhagic lesions in kidneys and bloody ascites. Pathological change in the kidney is unusual for Infectious Pancreatic Necrosis; it consisted of congestion or hemorrhage in glomeruli and renal cortex, edema, as well as the destruction or desquamation of the tubular epithelium in kidneys and was found in all specimens (Fig. 2 and 3). Pancreatic necrosis was not observed as frequently as among rainbow trout; it was severe in some but only slight in others (Fig. 4). Partial necrosis was found in liver and hyaline degeneration in striated muscle occurred as it has an incidental finding in rainbow trout with IPN (Fig. 5 and 6).

Some pathogenic protozoa and bacteria were found in a very few cases, but they were not considered to be primary pathogens.

The inoculant prepared from diseased fry produced strong cytopathic effects (CPE) at about 20 hours postinoculation when RTG-2 cells were infected and incubated at 20°C. The titer was $10^{8.5}$ TCID₅₀/ml. The general figure of CPE was the same as has been observed in IPN among rainbow trout.

Among Yamame (*O. masou ishikawae*) reared in nearby ponds unusual mortality never occurred, and inoculants prepared from Yamame never produced CPE in RTG-2 cultures.

The results of serum neutralization tests with the agent from Amago indicated IPN virus. Partial CPE occurred on the 5th day after inoculation, while virus with normal serum was scored complete CPE on the 3rd day. This agent was stable in the presence of ethanol, chloroform, ether, glycerin, and occasionally remained partial infective after heating at 60°C for 30'.

Discussion

From the results of serum neutralization tests, ether sensitivity tests and heat stability tests, we identify this agent as a strain of IPN virus.

Kidney hemorrhage and bloody ascites in Amago are common pathological changes.

These changes have not been observed often in rainbow trout with IPN, though SANO²⁾ described extensive congestion in anterior and posterior kidney on microscopical photographs of his paper on rainbow trout with IPN. These authors would like to think that more severe pathology in Amago's kidney should occur.

WOLF and PETTIJOHN³⁾ reported the isolation of IPN virus from Coho salmon, *Oncorhynchus kisutch* as one of hosts other than rainbow trout. Therefore, it is doubtless that IPN has spread not only in geographical, but in biological wide range of host, *Salvelinus*,^{4,5)} *Salmo*⁶⁾ and *Oncorhynchus*.³⁾

In the latest report of SANO,⁷⁾ fish infectivity trials with IPN virus in three species of *Oncorhynchus* there was no pathogenicity to Yamame. Also in this study, Yamame reared in nearby ponds never showed an IPN outbreak. These facts suggest that Yamame may be a genetically resistant species. The Yamame may be able to produce an interferon-like substance.

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References

- 1) T. SANO: This Bull., **37**, 495-498 (1971).
- 2) T. SANO: *ibid.*, 499-503 (1971).
- 3) K. WOLF, and L. L. PETTIJOHN: *Prog. Fish-Cult.*, **32**, 17-18 (1970).
- 4) E. M. WOOD, S. F. SNIESZKO, and W. T. YASUTAKE: *A. M. A. Arch. Pathol.*, **60**, 26-28 (1955).
- 5) T. J. PARISOT, W. T. YASUTAKE, and V. BRESSLER: *Amer. Fish Soc.*, **92**, 63-66 (1963).
- 6) S. F. SNIESZKO, E. M. WOOD, and W. T. YASUTAKE: *A. M. A. Arch. Pathol.*, **63**, 229-233 (1957).
- 7) T. SANO: This Bull., **39**, 117-120 (1973).

Explanation of Plate

- Fig. 1.** Photograph of dissected Amago infected with IPN showing congestion and petechiae in the liver.
- Fig. 2.** Section of the kidney of the diseased Amago stained with H-E. It shows congestion or hemorrhage in renal tissue, edema, as well as necrobiotic changes of the tubular epithelium.
- Fig. 3.** Section of the kidney of the diseased Amago stained with H-E. showing hemorrhage in renal cortex.
- Fig. 4.** Section of the pancreatic tissue of the diseased Amago stained with H-E. It shows a slight pancreatic necrosis which is observed less frequently in this species compared with rainbow trout.
- Fig. 5.** Section of the liver of the diseased Amago stained with H-E. showing noticeable necrosis and necrobiosis.
- Fig. 6.** Section of the abdominal muscle of the diseased Amago stained with H-E. showing hyaline degeneration.