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PATHOGENICITY OF RHABDOVIRUS OLIVACEUS (HIRAME RHABDOVIRUS; HRV) TO HIRAME AND SALMONID FISH

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Abstract

Pathogenicity of the Rhabdovirus olivaceus (hirame rhabdovirus; HRV) to hiramé (Japanese flounder) and salmonid fish (rainbow trout, masu, chum, and coho salmon) was studied. Cumulative mortalities of HRV infected hiramé reared at 5, 10, 15 and 20 °C were 40, 60, 20 and 0 %, respectively. The highest virus titer in mixed samples of kidney and spleen was obtained from fish reared at 5 °C. Cumulative mortalities of HRV infected rainbow trout, masu, chum, and coho salmon reared at 12 - 14 °C were 55, 30, 25 and 5 %, respectively. Mortality of rainbow trout weighing 0.2, 0.5 and 3.0 g was 100, 55 and 40 %, respectively. Mortality of rainbow trout infected with HRV and IHNW was 40 and 100 %, respectively. Histopathologically, in both hiramé and rainbow trout, the kidney exhibited necrotic changes such as nuclear degeneration of hematopoietic cells and hemorrhage in the interstitial tissue; the spleen showed necrosis and hemorrhage in the ellipsoid and pulp. In rainbow trout infected with HRV or IHNW similar changes were observed in the kidney and spleen, but in HRV infected fish, no pathological changes were found in the liver of early stages, and necrosis was not observed in the intestine.

Introduction

Hiramé rhabdovirus (HRV) is a fish rhabdovirus discovered from moribund hiramé (Japanese flounder, Paralichthys olivaceus) cultured in Hyogo Prefecture, Japan, in 1984. Characteristics of HRV have been found to distinguish in sufficiently from other known fish rhabdoviruses. This virus has been named Rhabdovirus olivaceus, after the scientific name of the host species (Kimura et al. 1986). This virus is pathogenic to hiramé (Oseko et al. 1988a, 1988b), black sea bream (Mylio macrocephalus), red sea bream (Chrysophrys major) and black rock fish (Sebastes inermis) among oceanic fishes (Sorimachi 1987), and also rainbow trout (Salmo gairdneri). HRV has been found widely distributed in Japan.

Natural outbreaks of HRV infections disappeared when the water temperature increased to 15 °C. First, we studied the effect of the temperature on HRV infection

and then examined the pathogenicity of HRV to salmonid fish by intraperitoneal injection. For the rainbow trout especially, the effect of size on HRV infection was studied and the histopathological changes were compared with those of infectious hematopoietic necrosis virus (IHNW) infected fish.

Material and Method

Fish used

One year old hiramé (weighing about 44.3g), fingerling rainbow trout (Salmo gairdneri, weighing about 6.5, 3.0, 0.5, 0.3g, respectively), masu (Oncorhynchus masou), coho (O. kisutch), and chum salmon (O. keta), weighing about 0.5g were used. The hiramé was cultured in 50 L seawater tanks and salmonid fish were cultured using dechlorinated city water.

Virus employed:

HRV strain 8601-H and IHNW strain ChAb were cultured on rainbow trout gonad cells (RTG-2) using Eagle's minimum essential medium (MEM) supplemented with 10 % fetal bovine serum and 100 IU/ml penicillin and 100 µg/ml streptomycin incubated at 15 °C for 7 days. Culture fluid was filtered and stored at -80 °C until used.

Artificial infection

The hiramé were used for the study of the effect of water temperature on artificial infection of HRV. Four groups of 10 fish each were infected by intraperitoneal injection of $10^{3.10}$ TCID₅₀/fish of HRV and reared at 5, 10, 15 and 20 °C in a 50 L aquarium. The condition of the fish was observed for 15 days. These hiramé were also used for histopathological study. Pathogenicity of HRV to salmonid fish was tested. Each 20 rainbow trout, masu, chum, and coho salmon (weighing about 0.5g) were infected with HRV by I.P. injection of $10^{4.8}$ TCID₅₀/fish and reared at 12 to 14 °C. To study the effect of size on HRV infection, three groups of 20 rainbow trout weighing 0.2, 0.5 and 3.0 g were infected with HRV by I.P. injection of $10^{3.75}$, $10^{4.8}$, $10^{4.8}$ TCID₅₀/fish, respectively and reared in running water at 12 to 14 °C. For comparison of the pathogenicity of HRV and IHNW to rainbow trout, 20 rainbow trout (body weight 3.0 g) were infected with HRV by I.P. injection of $10^{4.8}$ and $10^{4.8}$ TCID₅₀/fish, respectively, and reared in running water at 12 to 14 °C. Mortality was recorded daily for 15 days in the hiramé and 30 days in the salmonid. For histopathological study, 2 groups of 10 rainbow trout each (body weight 6.5 g)

were employed and infected with HRV and IHNV by I.P. injection of $10^{3.0}$ and $10^{3.0}$ TCID₅₀/fish and reared in running water at 12 to 14 °C.

Preparation of the specimens for virus isolation and measurement of the virus infectivity

Pooled specimens of kidney and spleen of hiram and the whole bodies of salmonid fish that had died during the observation period were used for virus assay. Surviving fish were also employed. Samples were homogenized by Hank's balanced salt solution (1:10 w/v) using a homogenizer. The homogenates were filtered using a 0.45 µm filter (Millipore HA) and inoculated on the RTG-2 cell using a 96 microtiter plate.

Histopathological study

Kidney, spleen, liver, skeletal muscle and intestine of moribund fishes were fixed in Bouin's fixative and dehydrated with ethyl alcohol. Paraffin-embedded samples were sectioned and stained with hematoxylin and eosin stains.

Results

Pathogenicity of HRV to hiram

The mortality of HRV infected hiram reared at 5, 10, 15 and 20 °C was 40, 60, 20 and 0 %, respectively (Fig. 1). Virus titers were obtained from fish cultured at 5 °C, and at 10 °C. The virus could not be isolated from infected fishes reared at 15 °C and 20 °C.

Pathogenicity of HRV to salmonid fish:

Cumulative mortalities of rainbow

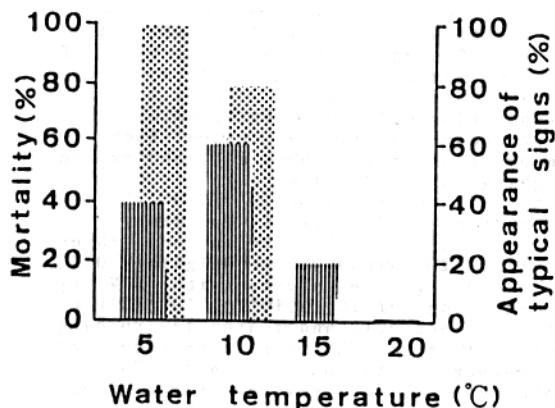


Fig. 1. Effect of the water temperature on the appearance of the typical signs in the hiram after 15 days artificially infected with HRV : dose, I.P. $10^{3.0}$ TCID₅₀/fish.
 ■: Dead fish. ···: Fish showing typical sign.

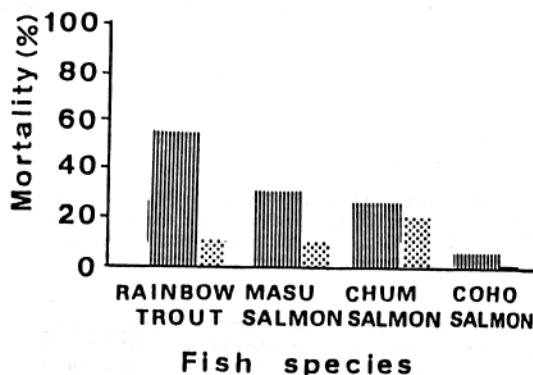


Fig. 2. Comparison of cumulative mortality in rainbow trout, coho salmon, chum salmon, and masu salmon infected with HRV by intraperitoneal injection (dose: $10^{4.0}$ TCID₅₀/fish).
 ■: Test group. ···: Control group.

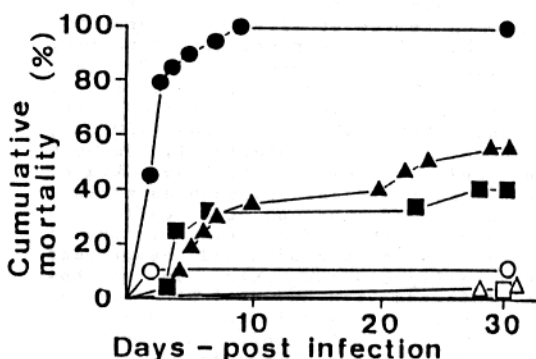


Fig. 3. Comparison of cumulative mortality in different sized rainbow trout infected with HRV by intraperitoneal injection.

○△□: Control group. ●▲■: Test group.
 ○: body weight 0.2g. △: body weight 0.5g. □: body weight 3.0g.
 ●: dose: $10^{3.75}$ TCID₅₀/fish. ▲: dose: $10^{4.0}$ TCID₅₀/fish. ■: dose: $10^{4.25}$ TCID₅₀/fish.

trout, masu, chum, and coho salmon were 55, 30, 25 and 5 %, respectively (Fig. 2). Rainbow trout began to die at 4 days after infection and the mortality reached 35 % at 11 day and 55 % at 29 days post-infection. The dead fish showed typical signs of HRV infection such as bilateral exophthalmus, increase in ascetic fluid and hemorrhage in the muscle. Deaths of masu salmon were observed at 6 days with the same signs, and mortality reached 30 % at 12 days post-infection. Rainbow trout had higher infectivity than masu salmon. No virus was isolated from coho and chum salmon.

Effect of size of rainbow trout on HRV infection

Cumulative mortality of rainbow trout weighing 0.2, 0.3 and 3.0 g was 100, 55, 40 %, respectively. Susceptibility of the rainbow trout to HRV decreased as body weight increased (Fig. 3).

Comparison of the pathogenicity of HRV and IHNV to rainbow trout:

Cumulative mortality of rainbow trout infected with HRV and IHNV was 40 and 100 %, respectively.

Histopathological study of hiramé infected with HRV

The kidney of hiramé reared at 5 °C and moribund fish reared at 10 °C indicated necrotic changes and hemorrhage in the interstitial lymphoid tissue (Fig. 4a). The spleen of the 5 °C group showed necrosis and hemorrhages in the ellipsoid and pulp (Fig. 4b). Similar necrosis was also observed in the spleen of the 10 °C group, but was not severe as compared with the fish cultured at 5 °C. Infiltration of macrophages into the spleen of the test groups reared at 15 and 20 °C were noted.

Histopathological study of rainbow trout infected with HRV

Necrosis and hemorrhage were observed in the interstitial lymphoid tissue of the kidney (Fig. 5a) and in the ellipsoid and pulp of the spleen. Hyperemia and hemorrhage in the muscle of the posterior part of the head were recognized. No pathological changes were observed in the intestine of HRV infected trout (Fig. 5c). In the early stages of the HRV infected fish no changes were observed in the liver (Fig. 5b). However, 11 or 12 days post-infection, heavy hemorrhage and necrotic changes were found there.

Histopathological changes of IHNV infected rainbow trout

Necrosis was observed in the hematopoietic cells of the kidney (Fig. 6a) and in the ellipsoid and pulp of the spleen. Heavy hemorrhage was found in the liver (Fig. 6b). Although histopathological change was not observed in the intestine of HRV infected fish, necrosis was recognized in the submucosa and granular cells of the intestine of IHNV infected fish (Fig. 6c).



Fig. 4. Tissue of a moribund hiramé infected with HRV, reared at 10 °C. (a) : Kidney, necrosis of hematopoietic cells and hemorrhage are found in the interstitial lymphoid tissue. (b) : Spleen, necrosis of ellipsoid and pulp hemorrhage are recognized in the tissue. H & E stain. (bar = 20 µm)

Fig. 5. Tissue of a HRV-infected rainbow trout. (a) : Kidney. Necrosis of hematopoietic cells and hemorrhage are observed in the interstitial lymphoid tissue. (b) : Liver, early stage of HRV infection, no histopathological changes are observed in the tissue. (c) : Intestine, no histopathological changes are found in the tissue. H & E stain. (bar = 20 µm)

Fig. 6. Tissue of IHNV-infected rainbow trout. (a) : Kidney, necrosis of hematopoietic cells are found in the interstitial lymphoid tissue. (b) : Liver, necrosis of hepatic cells and heavy hemorrhage are observed in the tissue. (c) : Intestinal necrosis is recognized in submucosa and granular cells. H & E stain. (bar = 20 µm)

Table 1. Histopathological changes observed in HRV and IHNV infected rainbow trout

Virus	Organ						
	Ant.Kid.	Kidney	Spleen	Liver	Muscle	Pancreas	Intestine
HRV	Necrosis	Necrosis Hemorrhage	Necrosis Hemorrhage	Normal Hemorrhage	Hemorrhage Hemorrhage Necrosis	(Necrosis)	Normal
IHNV	Necrosis	Necrosis Hemorrhage	Necrosis	Necrosis Hemorrhage	(Hemorrhage)	(Necrosis)	Necrosis

Discussion

HRV infected hiramé reared at 10 °C showed the highest mortality and fish cultured at 10 °C showed the highest observable rate of the typical signs of HRV infection and highest virus infectivity. The virus was not isolated from the fish cultured at 15 and 20 °C. From these data, the optimal temperature for HRV infection might be 5 to 10 °C. Similar results were reported for IHN (Amend 1970) and VHS (Carlisle et al. 1984; Jørgensen 1982).

Cumulative mortalities of rainbow trout and masu salmon reached 55 and 30 % respectively, and showed typical signs of HRV infection. No virus was isolated from chum or coho salmon. Mortality of rainbow trout (body weight 0.2 g) was 100 %, but mortality decreased in proportion to increasing body weight. In comparison with the pathogenicity of HRV and IHNV, rainbow trout showed the highest susceptibility to IHNV.

In rainbow trout, infected with HRV, necrosis and hemorrhage were observed in the interstitial lymphoid tissue of the kidney, ellipsoid and pulp of the spleen. Hyperemia and hemorrhage in the muscle of posterior part of the head were recognized. No pathological change occurred in the intestine of HRV-infected trout or in the liver of the early stages of the HRV-infected fish. However, 11 or 12 days post-infection, heavy hemorrhage and necrotic changes were found in the liver. In the IHNV-infected rainbow trout necrosis was observed in the kidney and spleen, but hemorrhage was not so severe as in the HRV infected groups. Heavy hemorrhage was found in the liver. Although pathological change was not observed in the intestine of HRV infected fish, necrosis was recognized in this organ.

The results of the comparative histopathological study of HRV- and IHNV-infected rainbow trout are shown in Table 1. HRV or IHNV infected fishes showed similar histopathological changes, that is hemorrhage in the kidney and spleen. Therefore histologically these two viruses are very similar. However the disease of HRV-infection was not the same as IHN, because HRV didn't show the destruction of submucosa and granular cells, that are the characteristic symptoms of IHN described by Amend et al. (1969). But, these signs,

and heavy hemorrhage in the muscle of HRV infected trout, were very similar to VHS (Yasutake et al. 1972).

References

- Amend, D.F. 1970. Control of infectious hematopoietic necrosis virus disease by elevating the water temperature. J. Fish. Res. Board Can. 27:265-270.
- Amend, D.F., W.T. Yasutake, and R.W. Head. 1969. A hematopoietic virus disease in rainbow trout and sockeye salmon. Trans. Am. Fish. Soc. 98: 796-804.
- Carlisle, J. and De Kinkelin. 1984. Experimental study of the susceptibility of two marine fish species, sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) to viral hemorrhagic septicemia. Aquaculture 41: 203-212.
- Oseko, N., M. Yoshimizu and T. Kimura. 1988a. Histopathological study on diseased hiramé (Japanese flounder; *Paralichthys olivaceus*) infected with *Rhabdovirus olivaceus* (Hiramé Rhabdovirus; HRV). Fish Pathology 23:117-123.
- Oseko, N., M. Yoshimizu and T. Kimura. 1988b. Effect of water temperature on artificial infection of *Rhabdovirus olivaceus* (hiramé rhabdovirus; HRV) to hiramé (Japanese flounder, *Paralichthys olivaceus*). Fish Pathology 23:125-132.
- Sorimachi, M. 1987. Hiramé rhabdovirus (HRV) infection of marine fish. FIDIC News 11:41-42.
- Jørgensen, P.E.V. 1982. Egtved virus: temperature-dependent immune response of trout to infection with low-virulence virus. J. Fish. Dis. 5:47-55.
- Kimura, T., M. Yoshimizu and S. Gorie. 1986. A new rhabdovirus isolated in Japan from cultured hiramé (Japanese flounder, *Paralichthys olivaceus*) and ayu (*Plecoglossus altivelis*). Dis. Aquat. Org. 1:209-217.
- Yasutake, W.T. and D.F. Amend. 1972. Histopathogenesis of experimentally induced viral hemorrhagic septicemia in fingerling rainbow trout (*Salmo gairdneri*). Bull. Off. Int. Epiz. 69:977-984.