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PATHOGENICITY OF *Rhabdovirus olivaceus* (HIRAME RHABDOVIRUS ; HRV) FOR SALMONID FISH

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Abstract

Pathogenicity of the *Rhabdovirus olivaceus* (hirame rhabdovirus; HRV) against salmonid fish (rainbow trout; *Oncorhynchus mykiss*, masu salmon; *O. masou*, chum salmon; *O. keta*, coho salmon; *O. kisutch* and pink salmon; *O. gorbuscha*) was studied. Artificially infected salmonid fish with HRV by water-born and I.P. injection were reared at 12 to 14 °C. Signs and mortalities of HRV infected fish were observed and also histopathological changes were studied. Cumulative mortalities of HRV infected rainbow trout, chum, coho and pink salmon by water-born infection were 8, 4, 4, and 0 %, respectively. Mortalities of rainbow trout, masu, chum, and coho salmon by I.P. injection were 55, 30, 25, and 5 %, respectively. Highest virus titer was obtain from the rainbow trout infected by both infection methods. Mortalities of rainbow trout weighing 0.2, 0.5, and 3.0g were 100, 55, and 40 %, respectively. And mortalities of rainbow trout infected with HRV and IHNHV were 40 and 100%, respectively. Histopathologically, on the HRV injected rainbow trout, the kidney and spleen indicated necrosis and hemorrhage, skeletal muscle revealed hyperemia and hemorrhage. In rainbow trout infected with HRV or IHNHV 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

Hirame rhabdovirus (HRV) is a fish rhabdovirus discovered from moribund hirame (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

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pathogenic to hirame (Oseko et al. 1988a, 1988b), black sea bream (*Mylio macrocephalus*), red sea bream (*Chryrophrys major*) and black rock fish (*Sebastes inermis*) among oceanic fishes (Sorimachi 1987), and also rainbow trout. HRV has been found widely distributed in Japan.

In this study, we examined the pathogenicity of HRV to salmonid fish by water-born and 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 (IHN) infected fish.

Material and Method

Fish used:

Fingerling rainbow trout (weighing about 0.3, 0.5, 3.0, 6.5g, respectively), masu salmon, coho salmon, pink salmon, and chum salmon, weighing about 0.3g and 0.5g were used. Salmonid fish were cultured at 12 to 14 °C, using dechlorinated city water.

Virus employed:

HRV strain 8601-H and IHN 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:

Pathogenicity of HRV to salmonid fish was tested. On the water-born infection, each 50 rainbow trout, chum salmon, coho salmon and pink salmon (weighing about 0.3g) were exposed for 60 min. to HRV of $10^{2.30}$ TCID₅₀/ml. On the intraperitoneal injection, each 20 rainbow trout, masu, chum, and coho salmon (weighing about 0.5g) were injected HRV of $10^{4.00}$ TCID₅₀/fish. Mortality was recorded daily for 15 days on the water-borne infection and 50 days on the I.P. injection. 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 $10^{3.75}$, $10^{4.00}$, $10^{4.00}$ TCID₅₀/fish, respectively. For comparison of the pathogenicity of HRV and IHN to rainbow trout, 20 rainbow trout (body weight 3.0 g) were infected with HRV by I.P. injection $10^{4.00}$ and $10^{4.30}$ TCID₅₀/fish, respectively. Mortality of infected rainbow trout was recorded for 30 days. These fishes were reared in running water at 12 to 14 °C.

For histopathological study, 2 groups of 10 rainbow trout each (body weight 6.5 g) were employed and infected with HRV and IHN by I.P. injection $10^{5.30}$ and $10^{5.80}$ 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:

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 μ m 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 salmonid fish by water-born infection:

Cumulative mortalities of rainbow trout, chum salmon, coho salmon and pink salmon by water-born infection were 8, 4, 4, and 0 %, respectively (Fig. 1). All dead fish showed no typical signs of HRV infection. But HRV was isolated from infected rainbow trout, highest virus infectivity of diseased fish was $10^{5.80}$ TCID₅₀ /g. No virus was isolated from chum, coho, and pink salmon (Table 1).

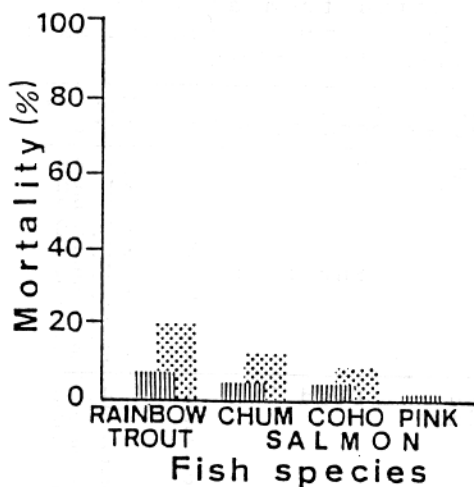


Fig. 1. Comparison of cumulative mortality in rainbow trout, coho, chum, and pink salmon infected with HRV by water-born (exposed for 60 min. in $10^{2.30}$ TCID₅₀/ml).
: Control group. |||||: Test group.

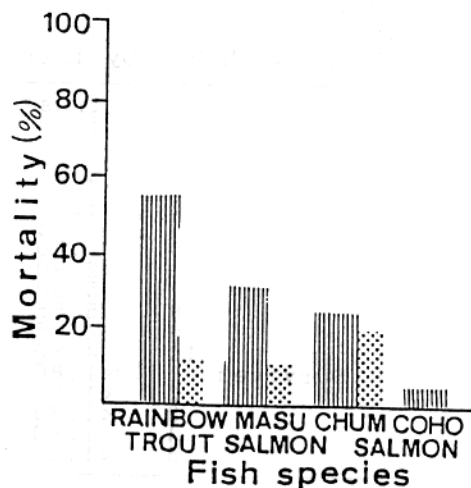


Fig. 2. Comparison of cumulative mortality in rainbow trout, coho, chum, and masu salmon infected with HRV by I. P. injection (dose: $10^{4.00}$ TCID₅₀ /fish).
: Control group. |||||: Test group.

Table 1. Result of virus isolation from artificially infected rainbow trout, coho salmon, chum salmon and pink salmon with HRV by water-born method.

Fish species	Fish condition	No.	Virus* titer	Fish species	Fish condition	No.	Virus* titer
Rainbow trout	Dead	1	3.55	Coho salmon	Dead	1	<1.80
		2	3.30			2	<1.80
		3	5.30		Survived	1	<1.80
		4	2.80			2	<1.80
	Survived	1	5.80			3	<1.80
		2	5.80			4	<1.80
		3	<1.80			5	<1.80
		4	<1.80	Chum salmon	Dead	1	<1.80
		5	<1.80			2	<1.80
Pink salmon	Dead	1	<1.80		Survived	1	<1.80
		1	<1.80			2	<1.80
	Survived	2	<1.80			3	<1.80
		3	<1.80			4	<1.80
		4	<1.80			5	<1.80
		5	<1.80				

* , log TCID₅₀/g

Pathogenicity of HRV to salmonid fish by I.P. injection:

Cumulative mortalities of rainbow 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

Table 2. Result of virus isolation from artificially infected rainbow trout, coho salmon, chum salmon and masu salmon with HRV by intraperitoneal injection.

Fish species	Fish condition	No.	Virus* titer	Fish species	Fish condition	No.	Virus* titer
Rainbow trout	Dead	1	2.55	Chum salmon	Dead	1	<1.80
		2	3.30			2	<1.80
		3	5.55			3	<1.80
		4	3.55			4	<1.80
		5	4.30			5	<1.80
		6	2.80		Survived	1	<1.80
		7	5.30			2	<1.80
		8	5.55			3	<1.80
		9	4.05			4	<1.80
		10	4.80			5	<1.80
	Survived	1	<1.80	Masu salmon	Dead	1	4.05
		2	<1.80			2	3.80
		3	<1.80			3	4.55
		4	<1.80			4	2.55
		5	<1.80			5	2.80
Coho salmon	Dead	1	<1.80		Survived	6	3.05
		1	<1.80			1	<1.80
		2	<1.80			2	<1.80
		3	<1.80			3	<1.80
		4	<1.80			4	<1.80
		5	<1.80			5	<1.80

* , log TCID₅₀/g

days post-infection. Rainbow trout had higher infectivity than masu salmon. No virus was isolated from coho and chum salmon (Table 2).

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 and 40 %, respectively. Susceptibility of the rainbow trout to HRV decreased as body weight increased (Fig. 3).

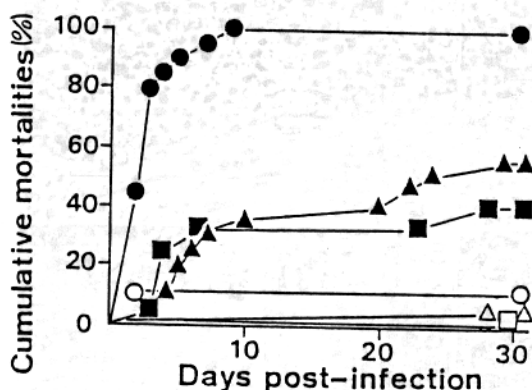


Fig. 3. Comparison of cumulative mortality in different sized rainbow trout infected with HRV by I. P. injection.
 ○△□: Control group.
 ●▲■: Test group.
 ○●: 0.2g, □■: 0.5g, △▲: 3.0g (body weight).
 ●: $10^{3.75}$, ▲: $10^{4.00}$, ■: $10^{4.00}$ TCID₅₀/fish (dose).

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 rainbow trout infected with HRV:

Necrosis and hemorrhage were observed in the interstitial lymphoid tissue of the kidney (Fig.4a) and in the ellipsoid and pulp of the spleen (Fig.4b). Hyperemia and hemorrhage in the muscle of the posterior part of the head were recognized. In the early stages of the HRV infected fish no changes were observed in the liver (Fig.4c). However, 11 or 12 days post-infection, heavy hemorrhage and necrotic changes were found there. No pathological changes were observed in the intestine of HRV infected trout (Fig.4d).

Histopathological changes of IHNV infected rainbow trout:

Necrosis was observed in the hematopoietic cells of the kidney (Fig.5a) and in the ellipsoid and pulp of the spleen (Fig.5b). Heavy hemorrhage was found in the liver (Fig.5c). 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.5d).

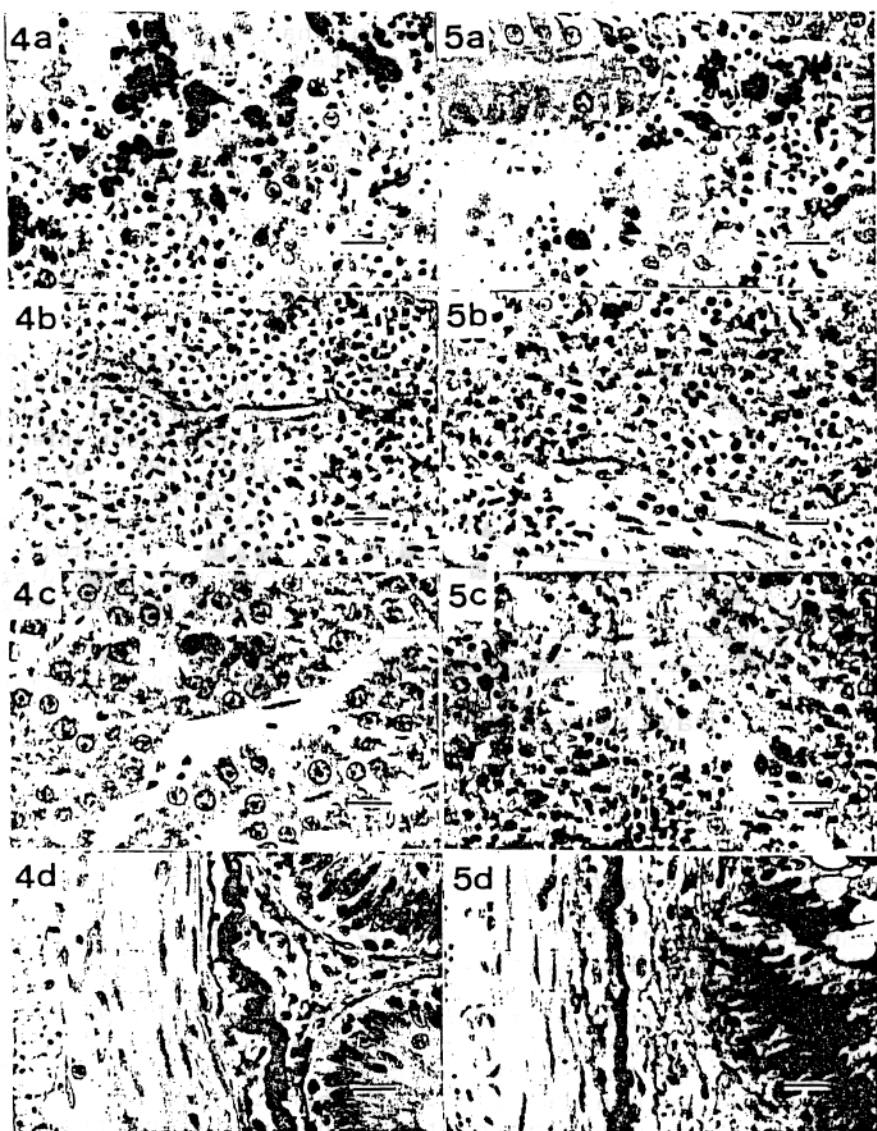


Fig. 4. Tissue of a HRV-infected rainbow trout. (a): Kidney, necrosis of hematopoietic cells and hemorrhage are observed in the interstitial lymphoid tissue. (b): Spleen, necrosis and hemorrhage are shown in the pulp. (c): Liver, early stage of HRV infection, no histopathological changes are observed in the tissue. (d): Intestine, no histopathological changes are found in the tissue. H & E stain. (bar= 20 μ m)

Fig. 5. Tissue of IHNV-infected rainbow trout. (a): Kidney, necrosis of hematopoietic cells are found in the interstitial lymphoid tissue. (b): Spleen, necrosis and hemorrhage are shown in the pulp. (c): Liver, necrosis of hepatic cells and heavy hemorrhage are observed in the tissue. (d): Intestine, necrosis is recognized in submucosa and granular cells. H & E stain. (bar=20 μ m)

Discussion

On the water-born infection, All salmonid fishes didn't show severe decrease. But infected rainbow trout has a little high infectivity of HRV. If observation period were expand or much more viruses were inoculated, mortality of the infected rainbow trout might be increased. On the I.P. injection, 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 0.2g) was 100 %, but mortality decreased in proportion to increasing body weight. In comparison with the pathogenicity of HRV and IHN, rainbow trout showed the highest susceptibility to IHN.

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 IHN-infected rainbow trout necrosis was observed in the kidney and spleen, but hemorrhage was not as severe with 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 IHN-infected rainbow trout are shown in Table 3.

Table 3. Histopathological changes observed in HRV and IHN infected rainbow trout.

Virus	Organ				
	Kidney	Spleen	Liver	Muscle	Intestine
HRV	Necrosis Hemorrhage	Necrosis Hemorrhage	Normal Hemorrhage Necrosis	Hemorrhage Hemorrhage	Normal
IHN	Necrosis Hemorrhage	Necrosis	Necrosis (Hemorrhage) Hemorrhage		Necrosis

HRV or IHN 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).

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