Virulence of Viral Hemorrhagic Septicemia Virus (VHSV) Isolates from Japanese Flounder *Paralichthys olivaceus* in Rainbow Trout and Several Species of Marine Fish

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(Received July 22, 2003)

ABSTRACT—Two Japanese isolates of viral hemorrhagic septicemia virus (VHSV), JF00Ehi1 (Genogroup I) and KRRV-9601 (Genogroup III), from Japanese flounder *Paralichthys olivaceus* were examined for the virulence to rainbow trout *Oncorhynchus mykiss* and 4 species of marine fish. Rainbow trout injected intraperitoneally with each isolate at a dose of $10^{7.5}$ TCID₅₀/fish showed neither death nor hemorrhage in the skeletal muscle, while the mortalities in Japanese flounder were both 100%. The mortalities by intramuscular injection with JF00Ehi1 at a dose of $10^{7.0}$ TCID₅₀/fish were 95.0%, 75.0% and 97.5% in black rockfish *Sebastes inermis*, red sea bream *Pagrus major* and yellowtail *Seriola quinqueradiata*, respectively.

Key words: VHSV, virulence, *Paralichthys olivaceus*, *Oncorhynchus mykiss*, experimental infection

Viral hemorrhagic septicemia (VHS) is a serious disease among cultured rainbow trout *Oncorhynchus mykiss* in Europe. VHS is listed as a notifiable disease by the Office International des Epizooties (OIE). Viral hemorrhagic septicemia virus (VHSV), the causative agent of VHS, has been isolated from wild marine fish in Western North America, the North Sea, and the east Atlantic Ocean^{1–3}.

Recently, VHSV has been isolated from farmed and wild Japanese flounder *Paralichthys olivaceus* in Japan^{4,5}. Nishizawa *et al.* classified these isolates as Genogroup I and Genogroup III based on a phylogenetic

analysis⁶. In addition, several properties of these VHSV, including optimal culture conditions, have been reported by Mori *et al.*⁷. As epidemiological study found that only Genogroup III was isolated in 1996, and after 1998, all isolates from Japanese flounder were identified Genogroup I using the polymerase chain reaction method (unpublished data).

In the present study, the susceptibilities of rainbow trout and 4 species of marine fish cultured in Japan, Japanese flounder, black rockfish *Sebastes inermis*, red sea bream *Pagrus major* and yellowtail *Seriola quinqueradiata* to VHSV was examined.

Materials and Methods

The isolates of VHSV used in this study were JF00Ehi1 (Genogroup I)⁶ and KRRV-9601 (Genogroup III)⁴ both obtained from farmed Japanese flounder. The virus was cultured using FHM cells at 20°C, and stored at –85°C prior to use. Rainbow trout (average body weight: 31.5 g), Japanese flounder (16.1 g), black rockfish (14.1 g), red sea bream (9.2 g) and yellowtail (10.2 g) were used in the study.

Twenty rainbow trout were kept in two 40 L circulation tanks (10 fish/tank) with a creature filtration system. A dose of $10^{7.5}$ TCID₅₀/0.1 mL/fish (JF00Ehi1 and KRRV-9601) for experimental fish and 0.1 mL of MEM for control fish was administered by intraperitoneal injection. Following the injection, fish were reared at 12.6°C for 35 days. After this period, the kidneys of surviving fish were processed for virus isolation using FHM cells.

Forty Japanese flounder were kept in two 60 L tanks (20 fish/tank). The viral suspension or MEM was administered by intraperitoneal injection as for the rainbow trout. After the injection, fish were reared at 13.5°C for 14 days. Viral antigens in kidney imprints were detected with an indirect fluorescence (IF) test using a polyclonal antibody against VHSV (JF00Ehi1).

For the experiments using black rockfish, red sea bream and yellowtail, each 40 fish were kept in two 90 L tanks (20 fish/tank). A dose of $10^{7.0}$ TCID₅₀/0.1 mL/fish (JF00Ehi1) was introduced into all three species by intramuscular injection. The negative control group was injected with MEM. Afterwards, fish were reared for 14 days at between 13 and 15°C. The virus was isolated from the kidneys of dead fish with FHM cells.

Results and Discussion

In Japanese flounder, the cumulative mortalities for JF00Ehi1 and KRRV-9601 reached 100% (Table 1). Hemorrhage in muscle was observed in some dead fish (Fig. 1-a). No difference in symptoms was observed between the fish injected with JF00Ehi1 and with KRRV-9601. Viral antigens were detected in the kidneys of all dead fish using the IF test with antiserum

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Table 1. Cumulative mortalities of rainbow trout, Japanese flounder, black rockfish, red sea bream and yellowtail infected with VHSV and isolation of virus and detection of IF-positive cells from the kidneys of inoculated fish

Fish	VHSV strains	No of sample	AV	Body weight (g)	Mortality (%)	Isolation of virus (No positive/ No tested)	Indirect fluorescence antibody test (No positive/ No tested)
Rainbow trout ^{*1}	JF00Eh1	20		31.5	0	0/3	—
	KRRV9601	20		31.5	0	0/3	—
Japanese flounder ^{*1}	JF00Eh1	40		16.1	100	— ^{*3}	40/40
	KRRV9601	40		16.1	100	—	40/40
Black rockfish ^{*2}	JF00Eh1	40		14.1	95	10/10	—
Red sea bream ^{*2}	JF00Eh1	40		9.2	75	10/10	—
Yellowtail ^{*2}	JF00Eh1	40		10.2	97.5	10/10	—

^{*1} Infection with intraperitoneal route at a dose of $10^{7.5}$ TCID₅₀/fish

^{*2} Infection with intramuscular route at a dose of $10^{7.0}$ TCID₅₀/fish

^{*3} not done

Fig. 1. Experimental fish injected with VHSV JF00Eh1 isolate. Bar = 1 cm. (a) Japanese flounder showing hemorrhage in muscle, (b) Rainbow trout showing no symptoms, (c) Black rockfish showing hemorrhage in muscle.

against JF00Eh1. On the other hand, neither death nor symptoms of VHS such as darkness, exophthalmia or hemorrhagic muscle were observed in rainbow trout injected with JF00Eh1 or KRRV-9601 (Table 1, Fig 1-b). No histological change was noted in the fish injected with the virus (data not shown). Also, no virus was isolated from 3 fish examined at 35 days post-challenge (Table 1). These results indicate that both JF00Eh1 and KRRV-9601 are little virulent against rainbow trout. Snow *et al.*⁸⁾ reported that the mortality for rainbow trout infected with VHSV isolated from wild Atlantic cod *Godus morhua* was 10%. VHSV is thought to be of marine origin, and to increase its virulence against rainbow trout by successive passages through the fish.⁹⁾ VHSV-isolates in Japan have not become virulent for rainbow trout so far. We have to seclude Japanese strain of VHSV isolated from marine fish from salmonids.

The cumulative mortalities were 95.0, 75.0 and 97.5% for black rockfish, red sea bream and yellowtail, respectively (Table 1). Diseased black rockfish showed an expanded abdomen due to ascites, and internal hemorrhaging of muscle (Fig 1-c). Virus was recovered from the kidneys of all dead fish examined (Table 1). Because marine fish cultured in Japan are suggested to be susceptible to VHSV, surveillance for outbreak of VHSV infection in cultured marine fish must be strengthened.

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