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***Oncorhynchus masou* Virus: Pathogenicity of Salmonid Herpesvirus 2 Strains against Masu Salmon Fry (*Oncorhynchus masou*)**

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Abstract. — Pathogenicity of 6 strains of salmonid herpesvirus 2 (SaHV-2) isolated from kokanee salmon, masu salmon, coho salmon and rainbow trout in Japan against 1-month-old masu salmon was compared with immersed artificial infection. Cumulative mortality of masu salmon infected with six SaHV-2 strains ranged 50 to 71%, and no severe difference was observed. Cumulative mortality of the control group was 4% during 60 days.

Introduction

In previous report, we described the serological relationships among salmonid herpesvirus 2 (SaHV-2) strains isolated from Japanese salmonid fish. SaHV-2 strains isolated in Japan showed cross-reactivity to each other and no significant difference was observed (Sung et al., 1996).

There are several reports on the pathogenicity of salmonid herpesvirus (SaHV-2) isolated in Japan from kokanee salmon (*Oncorhynchus nerka*), masu salmon (*O. masou*), coho salmon (*O. kisutch*) and rainbow trout (*O. mykiss*). The reference strain of SaHV-2 (OMV strain 00-7812) isolated from ovarian fluid of masu salmon showed the pathogenicity against several species of salmonid fish. Kokanee salmon was most susceptible following by masu salmon and chum salmon (*O. keta*), but coho salmon and rainbow trout exhibited less susceptibility (Kimura et al., 1983). NeVTA isolated from moribund kokanee salmon exhibited high susceptibility against kokanee and masu salmon, and less susceptibility to chum salmon, pink salmon (*O. gorbuscha*) and rainbow trout but no mortality was reported against charr (*Salvelinus fontinalis*) (Hasobe and Sano, 1989). CSHV isolated from pen cultured coho salmon had infected with coho salmon, masu salmon, and rainbow trout. The virulence of CSHV against coho salmon was higher than other species. On the other hand, NeVTA, YTV, and OMV showed high

virulence against masu salmon, but all these strains showed low virulence against rainbow trout (Kumagai et al., 1994). Suzuki (1993) reported that the fry of rainbow trout and masu salmon was more susceptible to RKV, isolated from diseased rainbow trout. Comparison of the pathogenicity belonging to SaHV-2 strains under the same conditions has not been reported until now. At first, we try to compare the pathogenicity of 6 representative SaHV-2 strains derived from different 4 salmon species against masu salmon fry and observed the mortality under the same conditions.

Materials and Methods

Virus strains and cell lines

Salmonid herpesvirus strains used in this experiment were shown in Table 1 with host species and passage number. SaHV-2-1(NeVTA) (Sano, 1976) was isolated from moribund kokanee salmon and passage number was 5, SaHV-2-2 (OMV reference strain 00-7812) (Kimura et al., 1981) was isolated from the ovarian fluid of mature masu salmon and passage number was 16, SaHV-2-5 (YTV) (Sano et al., 1983) was isolated from tumor tissue of yamame and passage number was 5, SaHV-2-6 (CSTV) (Sano et al., 1988) and SaHV-2-9 (COTV) (Kimura and Yoshimizu, 1991) was isolated from tumor tissue and both passage number was 5, and SaHV-2-10 (RKV) (Suzuki, 1993) was isolated from rainbow trout kidney and passage numbers were 4. These strains were propagated to CHSE-214 line until complete CPE was observed. Infectivity of each strain was mea-

Table 1. Salmonid herpesvirus 2 strains provided for artificial infection against masu salmon fry.

Virus strain	Original name	Fish species	Passage number
SaHV-2-1	NeVTA	Kokanee	5
SaHV-2-2	OMV	Masu	16
SaHV-2-5	YTV	Yamame	5
SaHV-2-6	CSTV	Coho	5
SaHV-2-9	COTV	Coho	5
SaHV-2-10	RKV	Rainbow trout	4

Table 2. Virus infectivity of salmonid herpesvirus 2 strains used for artificial infection.

SaHV-2 strain	Original name	Infectivity (TCID ₅₀ /L)
SaHV-2-1	NeVTA	10 ^{7.5}
SaHV-2-2	OMV	10 ^{8.5}
SaHV-2-5	YTV	10 ^{9.75}
SaHV-2-6	CSTV	10 ^{9.25}
SaHV-2-9	COTV	10 ^{9.0}
SaHV-2-10	RKV	10 ^{7.75}

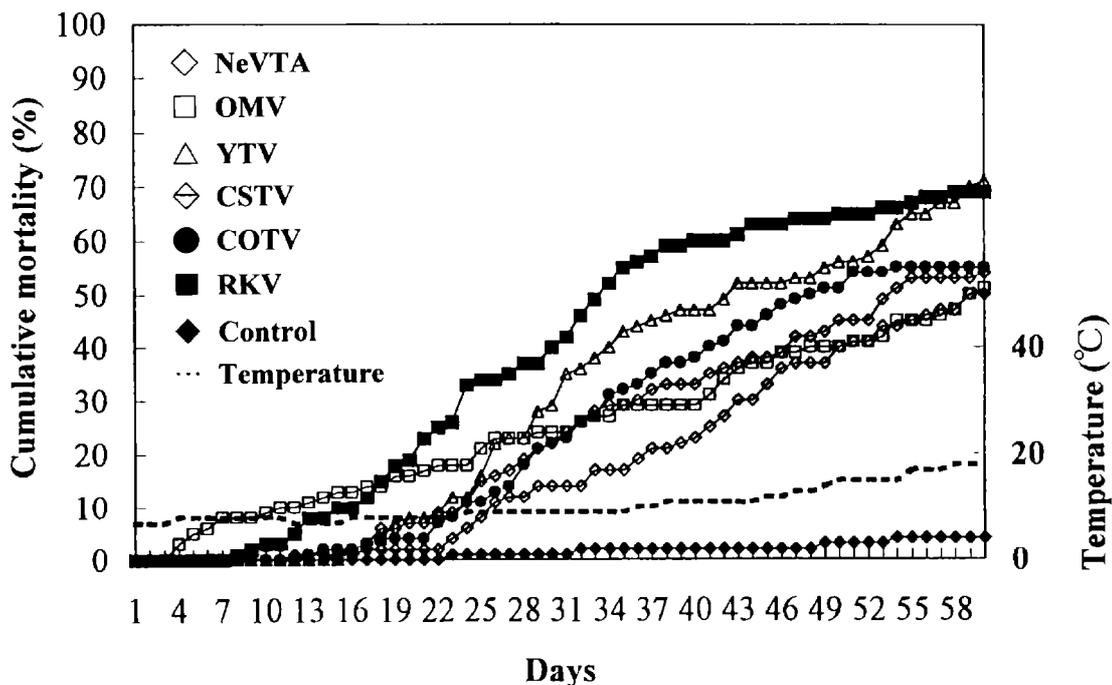


Fig. 1. Cumulative mortality of masu salmon fry exposed to SaHV-2 representative strains. Exposing dose, 10^{2.75} to 10^{3.50} TCID₅₀/L.

sured under the same conditions and used for artificial infection.

Masu salmon

Fertilized masu salmon eggs were provided from Hokkaido Salmon Hatchery and disinfected with iodophore. The eggs and hatched fry were cultured in dechlorinated tap water until showing a feed reaction for the first time. At this time, immersion infection was conducted.

Artificial infection

The Virus infectivity was 10^{2.75} to 10^{3.50} TCID₅₀/L (Table 2). One hundred fish were immersed to the

viral suspension in the dechlorinated city water for 1 hour. Then, the fry was cultured in running water. The water temperature started at 7 °C and gradually increased to 18 °C during 2 months. Every day, dead fry was picked up from aquariums and the cumulative mortality rate was calculated. The virus strains reisolated from dead fry were confirmed by the neutralization test.

Results

Cumulative mortality of masu salmon infected with SaHV-2 strains is shown in Fig. 1, and the percentage of cumulative mortality is shown in Fig. 2 exhibited. Each 10 salmon fry infected by different

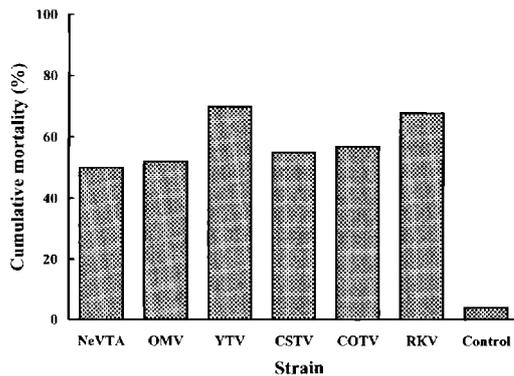


Fig. 2. Cumulative mortality of masu salmon fry exposed to SaHV-2 representative strains. Exposing dose, $10^{2.75}$ to $10^{3.50}$ TCID₅₀/L.

SaHV-2 strains was provided to reisolate the virus, and reisolated virus was observed to be neutralized by specific SaHV-2-2 (OMV-OO7812) serum. In the case of fish infected with SaHV-2-1 (NeVTA), the first dead fry was observed after 11 days post immersion and the cumulative mortality reached 50%. In fish treated with SaHV-2-2 (OMV), the first dead fry was observed after 4 days post immersion and the cumulative mortality reached 51%. In the case of SaHV-2-5 (YTV), the first dead fry was observed after 16 days and the cumulative mortality was 71%. In SaHV-2-6 (CSTV) and SaHV-2-9 (COTV) isolated from tumor of coho salmon, and SaHV-2-10 (RKV) isolated from rainbow trout kidney, the affected fish showed the death after 13, 15 and 8 days post immersion, respectively. On the other hand, the total mortality of the control group immersed MEM5 under the same conditions was only 4% during 60 days.

Discussion

In this experiment, 6 salmonid herpesviruses were provided. This results make it possible that SaHV-2 is pathogenic to masu salmon fry, regardless of deriving salmon species and their organ. Cumulative mortality of masu salmon infected with SaHV-2 reached from 51% to 71% in this experiment. These mortalities are similar to that of Kimura et al. (1983) and Hasobe and Sano (1989). But SaHV-2-6 (CSTV) and SaHV-2-9 (COTV) isolated from coho salmon showed different results from that of Kumagai et al. (1994). They reported that CSHV isolated from coho salmon showed less virulence than other strains against masu salmon. But SaHV-2-6 (CSTV) and SaHV-2-9 (COTV) were showed simi-

lar cumulative mortality against masu salmon in this experiment. In the case of SaHV-2-10 (RKV) isolated from rainbow trout, it showed high virulent against masu salmon. In this experiment, SaHV-2-5 (YTV) and SaHV-2-10 (RKV) showed rapid and acute mortality patterns compared with other 4 strains (Fig. 1). But this results make it possible that mortality is not significantly different with respect to total cumulative mortality. So it is possible that SaHV-2 has no difference of the pathogenicity against masu salmon. However, many things remains to be explored about pathogenicity, especially infection route and virus multiplication organ, another thing is that coho salmon and rainbow trout also need to be explored the pathogenicity.

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***Oncorhynchus masou* virus: サケ科魚類のヘルペスウイルス-2分離株のサクラマス稚魚に対する病原性**

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日本のサケ・マス由来のヘルペスウイルス (Salmonid

Herpesvirus-2: SaHV-2) の代表株6株を用い,同一条件下でサクラマスの浮上稚魚に対する病原性を比較検討した. 感染実験に供試したウイルスの継代数はサクラマス由来のOMVのみが16代であったが, RKVは4代, NeVTA, YTV, CSTV, COTVは5代であった. $10^{2.75}$ から $10^{3.50}$ TCID₅₀/Lに調整した各ウイルスを用い, 1時間浸漬攻撃し, 2ヶ月間流水飼育し経過を観察した. その間の累積死亡率はYTVが71%, RKVが69%, COTVが55%, CSTVが54%, OMVが51%, NeVTAが50%となり, 多少の差は認められたが, 全体として明白な違いは観察されなかった.