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단 보

Pathogenicity of viral hemorrhagic septicemia virus (VHSV) isolated from olive flounder *Paralichthys olivaceus* to masu salmon *Oncorhynchus masou*

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The pathogenicity of viral hemorrhagic septicemia virus (VHSV) from olive flounder *Paralichthys olivaceus* was investigated with masu salmon *Oncorhynchus masou* fry. The cumulative mortality of fish challenged with FYeosu05 isolate at $10^{6.5}$ $TCID_{50}$/fish was 60%. No mortality was observed in fish challenged with the isolates at $10^{5.5}$ $TCID_{50}$/fish and in mock-challenged fish. The affected fish showed darkening of the body, expanded abdomen, pale gills and enlarged spleen. VHSV from $10^{6.3}$ to $10^{7.8}$ $TCID_{50}$/g-tissue was re-isolated from the dead fish. These results suggest that the VHSV from olive flounder is pathogenic to masu salmon fry, although with low virulence.

Key words : Viral hemorrhagic septicemia virus, Masu salmon, Pathogenicity

Viral hemorrhagic septicaemia (VHS) is an infectious disease causing extensive losses in rainbow trout *Oncorhynchus mykiss* farms in European countries (Wolf, 1988; Smail, 1999). Viral hemorrhagic septicaemia virus (VHSV), the causative agent of VHS, was not reported outside Europe until 1988, when it was isolated for the first time in North America from returning Chinook salmon *O. tshawytscha* and coho salmon *O. kisutch* (Brunson *et al*., 1989; Hopper, 1989). Thereafter, it was reported from various marine fishes in European countries and North America (Schlotfeldt *et al*., 1991; Meyers and Winton, 1995; Mortensen *et al*., 1999; Hedrick *et al*., 2003). In Far East Asia, VHSV was first isolated from free-living olive flounder *Paralichthys olivaceus* in 1999 (Takano *et al*., 2000). Since then, VHSV-infection with severe mortality has occurred frequently at olive flounder farms in Korea and Japan (Isshiki *et al*., 2001; Kim *et al*., 2009) and VHSV has isolated from several wild marine fish species obtained on both Korean and Japanese coasts (Watanabe *et al*., 2002; Kim *et al*., 2011a).

VHSV is a member of the genus *Novirhabdovirus* from the family *Rhabdoviridae* (Trdo *et al*., 2005), and classified into four genotypes I-IV (Benmansour *et al*., 1997; Stone *et al*., 1997; Nishizawa *et al*., 2002; Einer-Jensen *et al*., 2004; Elsayed *et al*., 2006; Gagné *et al*., 2007; Kim *et al*., 2011a). The Asian isolates (Korean and Japanese isolates) are classified into the genotype IVa, together with North American isolates, but are distinguishable from North American isolates by phylogenetic analyses based on the nucleotide sequences of

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viral genomes. Thus, VHSV in Far Eastern Asia could be an indigenous virus in Korean and Japanese coastal area, but have not been introduced from North America.

So far, VHSV has not been isolated and/or detected from salmonids including rainbow trout in Korea, and mariculture for rainbow trout and masu salmon *O. masou* has been initiated on the Korean coast near olive flounder farms. Although it was previously reported that Asian VHSV isolates from olive flounder were pathogenic to rainbow trout fry (Kim *et al*., 2011b), pathogenicity of VHSV to masu salmon is remained unclear. Thus, in the present study, the pathogenicity of VHSV from olive flounder was validated using masu slamon.

Materials and Methods

Masu salmon eyed eggs were obtained from a culture farm in Gangwon Province, Korea, where there has been no history of fish virus diseases, so far. After hatching, the fish were reared up to around 1 g of body weight (0.74-1.58 g) using tap water at a wet laboratory of the Fisheries Science Institute, Chonnam National University. Prior to the experimental infection, ten fish were inspected for the presence of fish viruses by culture isolation using fathead minnow (FHM) cells and chinook salmon embryo (CHSE-214) cells.

The VHSV isolate used in this study was FYeosu05, which was isolated from moribund juveniles of olive flounder involved in a VHS epidemic at Yeosu, Korea in 2005 (Kim *et al*., 2009). VHSV was cultured at 15℃ using FHM cells maintained in Leibovitz L-15 medium (Gibco) containing 10% (V/V) fetal bovine serum (FBS, Gibco), 150 IU/mL of penicillin G, and 100 ㎍/mL of streptomycin.

A total of 60 masu salmon were reared in three aquaria with 200 L (n=20) at 11-13.5℃ without water flowing. After being anaesthetized with AQUI-S® (New Zealand Ltd.), 20 fish each were intraperitoneally injected with VHSV FYeosu05 at $10^{6.5}$ and $10^{5.5}$ $TCID_{50}$/50 ㎕/fish. As a control, 20 fish were injected with 50 ㎕/fish of Hanks' balanced salt solution (HBSS, Gibco). Clinical signs and mortalities of the fish were monitored everyday.

Dead fish in each group and five fish in the control were submitted for titration of VHSV using FHM cells. Briefly, the pooled internal organs including the spleen, kidney, gills, and heart were aseptically collected and homogenized with 9 or 19 volumes of HBSS. After centrifugation (3,000×g, 20 min, 4℃), the supernatant was filtered through a 0.45 ㎛ membrane filter to submit for titration. The titration of viral infectivity was performed using 96-well microplates seeded with FHM cells. After 14 days of culture, the appearance of cytopathic effects (CPE) was evaluated to determine the 50% tissue culture infectious dose ($TCID_{50}$).

Results and Discussion

The time-dependent mortality rates of the infected fish are shown in Fig. 1. The fish challenged with FYeosu05 at $10^{6.5}$ $TCID_{50}$/fish began to die on the 4th day of challenge, and cumulative mortalities reached 60%. In contrast, no mortality was observed in fish challenged with FYeosu05 at $10^{5.5}$ $TCID_{50}$/fish and in mock-challenged fish. The dead and affected fish showed darkening of the body, expanded abdomen, pale

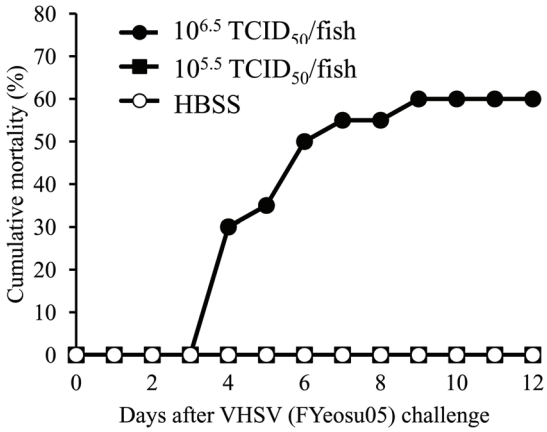

Fig. 1. Cumulative mortality of masu salmon fry experimentally infected by intraperitoneal infection with FYeosu05 isolate from olive flounder.

gills, and enlarged spleen, as well as occasional hemorrhaging in the lateral musculature (Fig. 2). These clinical signs were quite similar to those of rainbow trout affected with VHS (Yasutake, 1970, 1975; Wolf, 1988), but were consistent with those of rainbow trout infected with VHSV from olive flounder (Kim *et al*., 2011b). VHSV was recovered from all of the dead fish, and virus from $10^{6.3}$ to $10^{7.8}$ $TCID_{50}$/g-tissue was re-isolated from the dead fish (Table 1). No virus was detected in the control fish. These results suggest that the VHSV from olive flounder is pathogenic to masu salmon fry, although no mortality was observed in the fish injected with a dose of $10^{5.5}$ $TCID_{50}$/fish. These results were consistent with those of rainbow trout infected with VHSV from olive flounder (Kim *et al*., 2011b). It was reported that rainbow trout isolates of VHSV exhibited high virulence against rainbow trout by challenge with 10^5 $TCID_{50}$/fish (Skall *et al*., 2004), suggesting that the virulence of the present VHSV isolate from olive flounder seems to be lower than the virulence of isolates from rainbow trout.

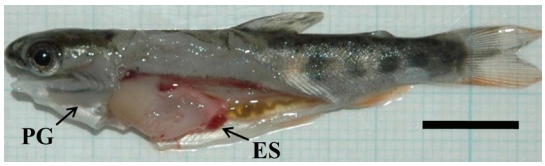

Fig. 2. Diseased masu salmon with pale gills (PG) and enlarged spleen (ES). Scale bar=1 cm.

Table 1. Virus isolaiton from dead maso salmon by VHSV infection

Dead fish	Virus titer ($TCID_{50}$/g)
1	7.8
2	7.6
3	7.55
4	7.35
5	7.3
6	7.3
7	7.05
8	6.85
9	6.55
10	6.35
11	6.3
12	6.3

Based on the present results, it is concluded that VHSV from olive flounder is pathogenic to masu salmon fry, although with low virulence.

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