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

Wi-Sik Kim, Jeong-Ho Kim*, Toyohiko Nishizawa**, and Myung-Joo Oh***†

*The Fisheries Science Institute, Chonnam National University, Yeosu 556-901, Korea*

**Department of Marine Bioscience, Gangneung-Wonju National University, Gangneung 210-702, Korea

***Department of Aquailfe Medicine, Chonnam National University, Yeosu 550-749, Korea

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 septicemia (VHS) is an infectious disease causing extensive losses in rainbow trout *Oncorhynchus mykiss* farms in European countries (Wolf, 1988; Smail, 1999). Viral hemorrhagic septicemia 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
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 salmon.

**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°C 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 µg/mL of streptomycin.

A total of 60 masu salmon were reared in three aquaria with 200 L (n=20) at 11-13.5°C 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} TCID₅₀/50 µl/fish. As a control, 20 fish were injected with 50 µl/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°C), the supernatant was filtered through a 0.45 µm 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 (TCID₅₀).

**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} TCID₅₀/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} TCID₅₀/fish and in mock-challenged fish. The dead and affected fish showed darkening of the body, expanded abdomen, pale
Pathogenicity of viral hemorrhagic septicemia virus (VHSV) isolated from olive flounder *Paralichthys olivaceus* to masu salmon *Oncorhynchus masou*

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

![Cumulative mortality of masu salmon fry](image)

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

**Table 1.** Virus isolation from dead masu salmon by VHSV infection

<table>
<thead>
<tr>
<th>Dead fish</th>
<th>Virus titer (TCID_{50}/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>7.6</td>
</tr>
<tr>
<td>3</td>
<td>7.55</td>
</tr>
<tr>
<td>4</td>
<td>7.35</td>
</tr>
<tr>
<td>5</td>
<td>7.3</td>
</tr>
<tr>
<td>6</td>
<td>7.3</td>
</tr>
<tr>
<td>7</td>
<td>7.05</td>
</tr>
<tr>
<td>8</td>
<td>6.85</td>
</tr>
<tr>
<td>9</td>
<td>6.55</td>
</tr>
<tr>
<td>10</td>
<td>6.35</td>
</tr>
<tr>
<td>11</td>
<td>6.3</td>
</tr>
<tr>
<td>12</td>
<td>6.3</td>
</tr>
</tbody>
</table>

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

**Acknowledgments**

This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0087136).

**References**


Schlotfeldt, H.J., Ahne, W., Jørgensen, P.E.V. and Glende, W.: Occurrence of viral haemorrhagic septicemia in turbot (Scophthalmus maximus) - a natural out-
Pathogenicity of viral hemorrhagic septicemia virus (VHSV) isolated from olive flounder Paralichthys olivaceus to masu salmon Oncorhynchus masou


