NOTE

The risk associated with vertical transmission of viral haemorrhagic septicaemia virus (VHSV) in rainbow trout (Oncorhynchus mykiss) eggs

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Abstract

To assess the risk posed by vertical transmission of viral haemorrhagic septicaemia virus, VHSV, in rainbow trout ova, an experimental challenge study was conducted. Prior to the fertilisation process samples of milt and/or ova were infected with VHSV. Virus was not detected from either the fertilised ova or hatched fry. These results indicate that the risk associated with VHSV vertical transmission is low.

Risk analysis of the introduction of VHSV to the UK has identified the importation of eggs for the rainbow trout (Oncorhynchus mykiss) industry as a potential route (Gregory, 2008). Although the risk of introduction of VHSV on the surface of eggs is extremely low when iodine disinfection is used prior to, and following import, there is little data on whether VHSV could be transmitted within the egg. The only study on vertical (true intra-ovum) transmission of VHSV to date was conducted over 40 years ago, before the advent of molecular diagnostic techniques (Vestergaard Jørgensen, 1970). A recent comprehensive project on fish egg trade sponsored by the European Commission identified priority areas for research, including investigating the presence of VHSV inside experimentally exposed eggs using modern diagnostic techniques, and investigating attachment of VHSV to sperm (Bovo et al., 2005).

To better understand the risk of transmission through ova it is important to conduct experiments to investigate whether VHSV can be internalised within ova to provide data for risk analysis. This study set out to investigate the risk associated with vertical transmission of VHSV by bathing eggs and milt prior to fertilisation with a known quantity of VHSV. In addition to viral isolation, a highly sensitive real-time PCR method was used to screen samples for VHSV post challenge.

Immediately prior to stripping, mature adult rainbow trout weighing approximately 1.5Kg,
were kept in 2m tanks with a flow rate of 800L/h and a temperature of 6°C at the Marine Scotland stock facility in Aultbea, Wester Ross.

Two mature males and 1 mature female were stripped of their milt and ova respectively and their gonadal products were screened for the fish pathogens VHSV, IPNV and IHNV by virus isolation.

Isolate DK3592B, which was previously confirmed as VHSV genotype Ia (Einer-Jensen et al., 2005) was grown on the FHM cell line (European Collection of Cell Cultures, ECACC 88102401). The viral dose used in this study to infect ova and milt was calculated at 1 x 10^7 TCID₅₀ml⁻¹. A number of different fertilisation combinations were performed using positive and negative ova/milt (Table 1). Prior to fertilisation the milt extracted from the 2 males was mixed to give one milt batch. To infect the ova with VHSV, 3mL of VHSV was added to 30mL ova and in the positive milt batches, 1mL of VHSV was added to 10mL milt. Positive ova/milt batches were incubated and mixed by placing on a plate shaker for 15min. Following fertilisation all eggs were rinsed 3 times and left in water to harden for 1h. After the ova had hardened they were laid into the appropriate hatching trays with each tray having a separate flow through (50L/h, 10°C) water supply. In total five treatments were conducted including the controls and each treatment consisted of approximately 300 eggs.

Eyed eggs were screened for VHSV 4 weeks after fertilisation and hatched fry were screened for VHSV 5 weeks post-fertilisation. Samples for real-time PCR were placed into RNALater® (Qiagen) and stored at -80°C prior to processing. RNA was extracted from pools of 5 fry on a Qiagen M48 BioRobot using the M48 RNA Tissue mini kit also from Qiagen following a homogenisation step utilising the Qiagen tissue lyser system. The RNA was converted to cDNA using ABI Taqman multiscribe kit as per manufacturers instructions. A region of the N-gene was amplified using a universal primer and probe set as described by Matejusova et al. (2008) on an ABI Prism 7000 Thermal cycler. An endogenous control, ELF-1α was used to ensure the integrity of the RNA being employed.

Samples for virus isolation consisted of 5 ova/

<table>
<thead>
<tr>
<th>Hatching Tray</th>
<th>Fertilisation conditions</th>
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<tbody>
<tr>
<td>1</td>
<td>Positive ova x Positive milt</td>
</tr>
<tr>
<td>2</td>
<td>Positive ova x Negative milt (untreated)</td>
</tr>
<tr>
<td>3</td>
<td>Negative ova x Positive milt</td>
</tr>
<tr>
<td>4</td>
<td>Negative ova x Negative milt (untreated)</td>
</tr>
<tr>
<td>5</td>
<td>Negative ova x Negative milt (E-MEM media only)</td>
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Table 1. Details of the fertilisation conditions of each ova batch laid into hatching trays 1-5. Hatching trays 4-5 were classified as control batches.
fry per pool and followed the protocol detailed in the Office International des Epizooties (OIE) Manual of Diagnostic Tests for Aquatic Animals (2009). Samples were inoculated onto the BF-2 (European Collection of Cell Cultures, ECACC 87032603) and FHM cell lines. After the 14 day incubation period all samples were screened for VHSV by Test-line kit ELISA. The gonadal fluid sampled from the female and both the male broodfish prior to fertilisation, screened VHSV, IHNV & IPNV negative by virus isolation. Experimentally infected milt and ova that were not used in the fertilisation procedure were screened for VHSV by virus isolation and were confirmed VHSV positive by ELISA. All ova and fry (n=1695) tested by either real-time PCR or virus isolation screened VHSV negative.

In this study intra-ovum transmission of VHSV was investigated by experimentally infecting ova and milt samples with a high dose (1 x 10^7 TCID₅₀ ml⁻¹) of VHSV prior to fertilisation. There were no fertilisation problems associated with the experiment, as demonstrated by successful hatching in all treatment groups (hatching percentage 98-99.5%). The virus isolate used in this study had previously been demonstrated to be highly pathogenic to freshwater rainbow trout (Lorenzen et al., 1993). VHSV was not detected in any of the ova or fry screened even when using the highly sensitive real-time PCR molecular method. To the authors knowledge the only VHSV vertical transmission trial prior to this study was conducted by Vestergaard Jørgensen (1970). At that time, viral isolation using cell culture was the only diagnostic tool available. Vestergaard Jørgensen (1970) found that VHSV could only be detected by cell culture isolation in the naturally infected eggs 3-5h after the water circulation was switched on and from the batch of eggs which were experimentally infected with VHSV, the virus could only be isolated up to 10 days post-infection from ova and was not detected from the fry originating from either egg batch. It was concluded that intra-ovum transmission was unlikely to occur. Since that study, VHSV has been isolated from both ovarian and milt samples obtained from wild coho salmon (Oncorhynchus kisutch) from rivers in the state of Washington, USA (Eaton et al., 1991), indicating a potential for vertical transmission of VHSV in some salmonids.

Recent risk assessments (Peeler et al., 2005; Gregory, 2008) have indicated that a lack of scientific evidence on intra-ovum transmission of fish pathogens including VHSV, makes it difficult to assess the risk associated with this route. It is well established that disinfection is very effective in removing external VHSV contamination from eggs (Vestergaard Jørgensen, 1973) but clearly would be ineffective if VHSV was transmitted intra-ovum. Tuttle-Lau et al. (2010) investigated the efficacy of iodophor disinfection of VHSV (genotype IVb) experimentally infected walleye (Sander vitreum) and northern pike (Esox lucius) eggs. The study revealed that VHSV was not isolated from iodophor disinfected ova, but that virus was isolated from a VHSV positive (untreated) control group of northern pike eggs experimentally challenged at a 10^8 PFU ml⁻¹ titre, for a period of 0-4 days post-infection. However, the fry of both species tested VHSV negative and all ova challenged at 10^5 PFU ml⁻¹, screened VHSV negative 1 day post-infection.
This study has added further evidence, using modern, sensitive diagnostic methods, to support the original hypothesis proposed by Vestergaard Jørgensen (1970); that VHSV is unlikely to be vertically transmitted in rainbow trout. It is very important to support risk assessments with scientific evidence and the findings from this study can be used to better inform qualitative risk assessments but can also be used in larger scale risk models that aim to provide a more quantitative estimate of risk. These results give further weight to the theory that the risk associated with VHSV vertical transmission, and therefore importation into the UK via ova, is low.

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References


Vestergaard Jørgensen PE (1970). The survival