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Short communication

Phylogenetic analysis of the glycoprotein gene of viral hemorrhagic septicemia virus from Iranian trout farms points towards a common European origin

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ABSTRACT

Viral haemorrhagic septicaemia virus (VHSV), a member of family *Rhabdoviridae* and genus *Novirhabdoviridae*, causes mortality in numerous marine and freshwater hosts located in northern hemisphere. To evaluate the genetic diversity of VHSV from the North and South West of Iran, the sequences of a 1483 bp nt region of the glycoprotein gene were determined for four Iranian isolates. These sequences were analysed to evaluate their genetic relatedness with 86 worldwide isolates representing the four known genogroups of VHSV. Phylogenetic analysis by nucleotide sequences showed that all the VHSV isolates studied were closest related to the 19 fresh water strains from Germany grouped within the European genogroup Ia-2. This finding indicates that Iranian VHSV most likely was introduced to Iran by the movement of contaminated fish fry from a source in Europe.

1. Introduction

The fish rhabdovirus viral haemorrhagic septicaemia virus (VHSV) is an important fish pathogen which causing mortality in numerous marine and freshwater hosts, but has a huge economic impact especially on farming of rainbow trout (Basurco et al., 1995; Skall et al., 2005; Thiery et al., 2002; Choi et al., 2011). VHSV is an enveloped RNA virus of the genus *Novirhabdovirus* of the family *Rhabdoviridae*, with a single molecule of linear, negative-sense ss-RNA (approximately 11.1 kb) which contains 6 genes in the order 3′-N-P-M-G-NV-L-5′ (Einer-Jensen et al., 2014; Ammayappan and Vakharia, 2011; Kim and Kim, 2013). In European fish farming, the rainbow trout, is the species were VHSV has most economic impact (Reichert et al., 2013).

Phylogenetic analysis based on the nucleotide sequences of G and N genes of VHSV isolates from marine and freshwater fishhes revealed four genogroups (I–IV) and a number of sublineages (Ia–Ie, IVa–IVb) (Einer-Jensen et al., 2004, 2005; Snow et al., 2004; Nishizawa et al., 2002). Te genotypes appear to be more associated with geographical distribution rather than host species (Kahns et al., 2012; Kuzmin et al., 2009; Stone et al., 1997). Thus, genotype I, II and III isolates have been identified in Europe, whereas genotype IV isolates have been sampled in North America and Asia. Until 1987, viral haemorrhagic septicaemia (VHS) disease was thought to be limited to continental Europe in freshwater farmed rainbow trout and sea water-reared rainbow trout (Castric et al., 1992; Thiery et al., 2002). However in 2005, VHSV was isolated for the first time in Iran (Haghighi Khiabanian Asl et al., 2008). By the early 2011, geographic range of the virus in Iran was found to extend from Gilan province to another province and mass mortality events of rainbow trout were attributed to VHSV (Haghighi Khiabanian Asl, 2011). Despite the long-standing historical presence of VHSV in this region, very little is known regarding the genetic diversity of these isolates and the genetic relatedness and molecular taxonomy of Iranian VHSV isolates to the American, Asian and European isolates has not been

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investigated. This study analyses the nucleotide sequences of four novel Iranian VHSV isolates based on nearly full-length (1483 nt) G-gene sequences isolated from farmed rainbow trout by phylogenetic analysis, including 86 American, Asian and European isolates of VHSV.

2. Materials and methods

2.1. Fish sampling and study site

From October 2013 till May 2014, moribund rainbow trout fry were collected during suspected outbreaks of VHSV in fish farms located in the Northern and Southwest provinces of Iran. Four farms from each of the two provinces of Mazandaran, and Chaharmahal va Bakhtiari with 60% and 40% mortality respectively, were tested (Fig. 1). The farms were run on flow-through system of fresh water with a temperature range of 10–12 °C. From each farm, 30 moribund fish (approximately 1.0 g) showing typical clinical signs of VHS such as darkening of the skin, abdominal swelling, and loss of appetite, were selected and transferred to laboratory. In general, pools of whole ten fry were processed according to the virus isolation procedure stipulated by the OIE with minor modification. (http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.3.09_VHS.pdf)

2.2. Cell culture

Chinook salmon embryo (CHSE-214) cell line was used for propagation of viral strains as described previously (Dadar et al., 2013). Briefly, cells were maintained at 15 °C with Eagle's MEM supplemented with 10% fetal bovine serum, 100 IU/mL penicillin G and 100 μg/mL streptomycin sulphate. Each pool usually contained material from ten fry fishes and was grown in CHSE-214.

2.3. RNA extraction

When a sample was identified as virus positive, the media was removed and subjected to RNA extraction, using the Roche high pure viral kit (Roche, Mannheim, Germany), according to manufacturer's recommendations. Concentration and purity of the RNA was obtained and estimated by measuring absorbance at 260 and 280 nm in a spectrophotometer (Nanodrop spectrophotometer ND-1000, Germany).

2.4. RT-PCR and sequencing

Since there was no adequate information about the Iranian VHSV isolate genome sequence, the existing VHSV sequences were extracted from NCBI and aligned with Mega version 6 software (Dadar et al., 2014; Kumar et al., 2008). Two VHSV specific primers, FVHSV (5′ ATGGAATGGAAYACTTTYTTC 3′) and RVHSV (5′ TCAGACCATCTGACTTCTGG 3′), were designed to target the 5′ and 3′ region of the full-length G gene. The extracted RNA was amplified using a one-step reverse transcriptase-polymerase chain reaction (RT-PCR) kit in accordance with the manufacturer's protocol (Qiagen, Germany).

The FVHSV and RVHSV primers were used to amplify the G gene. The thermal RT- PCR steps were 1 cycle 50 °C for 30 min, 95 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing temperature at 50 °C for 30 s and extension at 72 °C for 90 s for G gene. This was followed by a final extension of 10 min at 72 °C. The RT-PCR products were separated by 1% agarose gel electrophoresis, purified with gel extraction kit (Qiagen, Germany), and subjected to nucleotide sequence analysis by dideoxy chain termination method (Applied Biosystems, CA). Sequence data was edited using Mega version 6 software and the full-length G gene consensus sequences including the primer sequences were

Fig. 1. Collection of VHSV isolates from the Northern and Southwestern provinces of Iran. Of the eight sites analysed, (four in each province), three isolates (IRVHSV, IR-M-VHSV, IR-M2-VHSV) were obtained from three trout hatcheries located in the Mazandaran provinces (1), while a single isolate (IR-SH-VHSV) was obtained from a trout hatchery located in the Chaharmahal va Bakhtiari province (2).