Virus Research 229 (2017) 17–23

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Infectious hematopoietic necrosis virus (IHNV) outbreak in farmed rainbow trout in Iran: Viral isolation, pathological findings, molecular confirmation, and genetic analysis

Sohrab Ahmadivand[a], Mehdi Soltani[a,b,*], Karim Mardani[c], Sara Shokrpoor[d], Reza Hassanzadeh[e], Mehran Ahmadpoor[a], Hooman Rahmati-Holasoo[a], Saeid Meshkini[c]

[a] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, P.O. Box: 14155-6453, Tehran, Iran
[b] Centre of Excellence of Aquatic Animal Health, University of Tehran, Tehran, Iran
[c] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[d] Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, P.O. Box: 14155-6453, Tehran, Iran
[e] Iran veterinary organization, Tehran, Iran

ARTICLE INFO

Article history:
Received 24 November 2016
Received in revised form
16 December 2016
Accepted 16 December 2016
Available online 21 December 2016

Keywords:
Iran
Trout
IHNV
Histopathology
Clinical signs
Genotyping
Viral isolation

ABSTRACT

Infectious hematopoietic necrosis virus (IHNV) is the etiological agent of a contagious disease (IHN) mainly in salmonid fish. In the present study, we isolated and identified IHNV in trout fry from Iranian trout farms with unexplained high mortality in 2016. The affected fry showed cumulative mortality of 90% with the gross pathological signs including exophthalmia and hemorrhage of the eye, skin darkening, abdominal distension, ulceration of the snout, and the visceral pallor and yellowish fluid in the intestine. Histopathological examination revealed marked necrosis in the anterior kidney, liver and spleen with the intracytoplasmic inclusion bodies in the liver sections. Also, intranuclear inclusion body and marginated chromatin were observable in the hematopoietic cells of the kidney. The homogenates tissues of infected fry induced IHNV-positive cytopathic effects (CPE) in EPC cells and confirmed by RT-PCR reactions and sequencing. Phylogenetic analysis revealed the Iranian IHNV isolates belonged to the European (E) genogroup with 100% identity to some Italian isolates. This is the first report of IHNV infection in farmed trout fry in Iran describing the viral isolation, clinical symptoms, histopathological findings, molecular confirmation, and genetic analysis suggestion of the specific country of origin.

1. Introduction

Infectious hematopoietic necrosis virus (IHNV; family *Rhabdoviridae*, genus *Novirhabdovirus;* Dietzgen et al., 2012) is a negative-sense single stranded and non-segmented RNA virus, the causative agent of infectious hematopoietic necrosis (IHN) a disease notifiable to the world organization for animal health (OIE, 2016). The virus was first described in Sockeye salmon (*Oncorhynchus nerka*) fry hatcheries in western north America in the early 1950s (Rucker et al., 1953), from where it also quickly spread to Europe and Asia causing high epizootics in trout and salmon species worldwide (Winton, 1991).

The virus is highly contagious and may cause up to 100% mortality at water temperatures of 10–12 °C (Bootland and Leong, 1999; OIE, 2016). Nonetheless, susceptibility to the virus depends on several factors including fish species, fish strain, life stage as well as environmental and rearing conditions, especially water temperature and stress level (Pilcher and Fryer, 1980; Bergmann et al., 2003; Garver et al., 2013). Furthermore, fish susceptibility to the virus varies with viral strain as differences in virulence for rainbow trout to IHNV isolates from different electropherotypes and genogroups have been reported (Lapatra et al., 1993; Garver et al., 2006; Peñarand et al., 2009).

Isolates of IHNV differ in the molecular weight of major structural proteins by electrophoresis method that enables to separate the viral strains into four types (termed electropherotypes). In addition, five major genogroups of IHNV isolates (E, J, L, M and U) were described according to their geographic occurrence based on phylogenetic analysis of partial or complete G gene sequence (Enzmann, 2000; Kurath et al., 2003; Nishizawa et al., 2006; He et al., 2013).

Iran is one of the leading countries in rainbow trout production in freshwater with the most production in five provinces in the north and west regions of the country. Over the past decade,

* Corresponding author at: Faculty of Veterinary Medicine, University of Tehran, P.O. Box: 14155-6453, Tehran, Iran.
E-mail addresses: s_ahmadivand@ut.ac.ir (S. Ahmadivand), msoltani@ut.ac.ir (M. Soltani).

http://dx.doi.org/10.1016/j.virusres.2016.12.013
0168-1702/

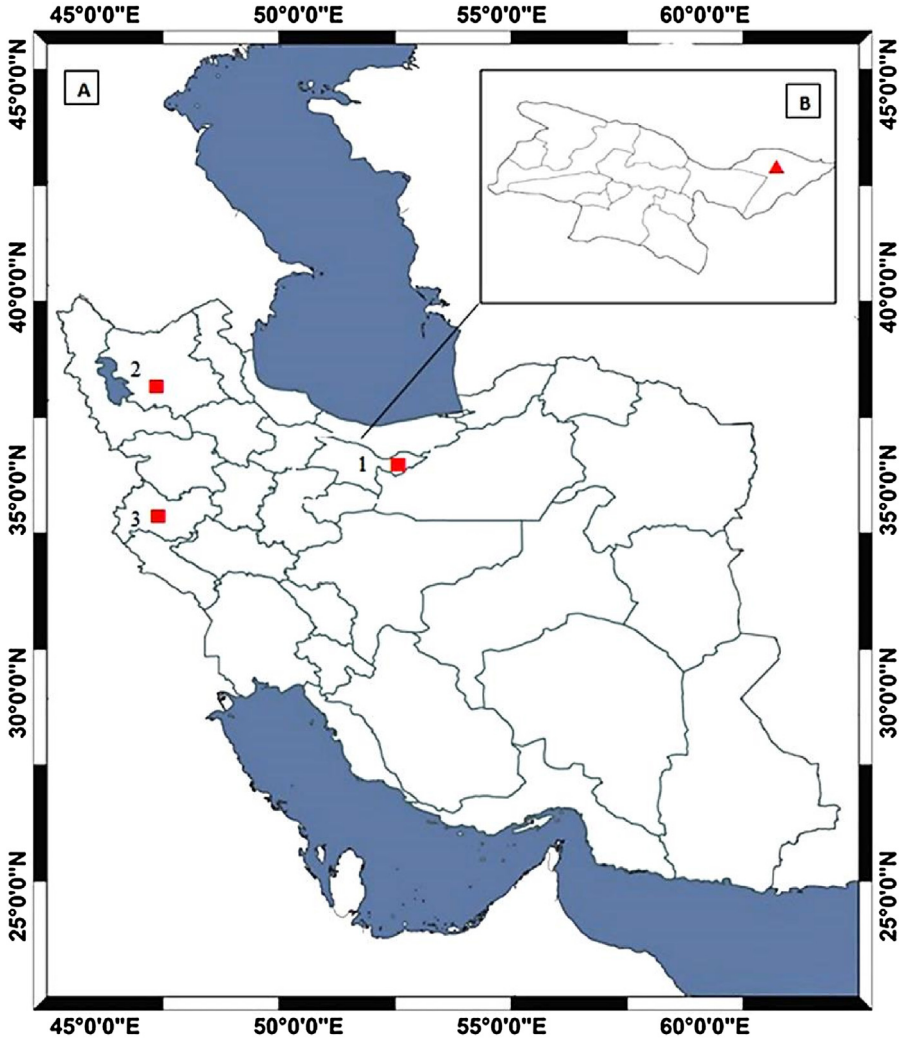

Fig. 1. Map of Iran showing sampling locations of trout hatchery (■) experiencing mass mortality (A). 1: Tehran; 2: West Azerbaijan; 3: Kermanshah. Samples from hatchery (▲) being positive for IHNV in Tehran province (Firuzkuh city), (B).

Iranian trout production remarkably decreased due to outbreaks of some viral diseases including VHS and IPN (Soltani et al., 2015; Ahmadivand et al., 2016). For instance, the recent incidence of VHS and IHN-like disease in the Iran trout farms have reduced the total trout production from 140,000 t/year to about 100,000 t/year as many trout farms were eradicated by Iran veterinary organization and many farmers lost their hatcheries and the growing fish. Regarding the IHN incidence, there are some documented reports of IHN-like disease with inadequate data to confirm of the disease outbreak in Iran farmed rainbow trout. For example, the outbreak of the IHNV-like virus was recorded in juvenile rainbow trout in some farms in the northern area of the country (Fallahi et al., 2003). Also, the phylogenetic relationship of a couple of IHNV isolates has been recently reported based on the partial glycoprotein (G) gene with no further confirmation and any description (Adel et al., 2016). In the present study, we described the isolation and CPE positive characterization, clinical symptoms, histopathological findings, molecular confirmation, and genetic analysis of the IHNV in trout fry from some rainbow trout farms experiencing high morbidity and mortality in Iran.

2. Materials and methods

2.1. Fish source and laboratory examination

From February to May 2016, three outbreaks with mass mortality occurred in rainbow trout hatcheries in Tehran, East Azerbaijan, and Kermanshah provinces of Iran with 90%, 50% and 60% mortality, respectively (Fig. 1a). The fish farms were run on a flow-through system of fresh water at a temperature ranges 10 to14 °C. Samples of the moribund fish weighing 1–2 g were collected and transferred to the diagnostic laboratory, Iran national veterinary diagnostic center (Tehran) for parasitological, bacteriological and virological examinations as well as molecular studies. Wet mount of the gills and mucus scrapings from the skin were prepared for parasitological examinations under a compound microscope. Bacterial cultures from the spleen and kidney tissues were carried out on blood agar incubated at 20 °C for up to 7 days. Furthermore, pools of whole ten fry samples were used for virus isolation and molecular detection. Histopathological examinations were also undertaken using the whole fry (with open gut) fixed in 10% neutral buffered formalin.