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An overview of Betanodavirus and perspective of Viral Nervous Necrosis (VNN) disease in Iranian southern waters

Ziarati M.[1]; Zorriehzahra M.J.[2*]; Kafilzadeh F.[1]; Kargar M.[1]; Ghasemi F.[3]

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Abstract

The Persian Gulf and its shores are important and strategic areas with a large variety of fish species. Betanodavirus infection is known to be a serious threat to susceptible fish and causing economic damages to the fisheries and fishing industry. Concerning to isolation and confirmation of VNN virus in the Mullet fish (*Chelon aurata* and *C. saliens*) in the Caspian Sea and its damages on Mullet stock, probably transmission of VNN could be hazardous in marine fish industry such as cage culture. So, the aim of this article was to characterize the distribution of the Betanodavirus in Iranian southern waters and its transmission. Finally, the issues of the transmission of Betanodavirus infection between the wild and farmed fish of different regions of the Persian Gulf is discussed. The probability of the emergence of viral epidemics and even new and virus-resistant hosts has been investigated whereby the monitoring and surveillance program for tracing the disease and the detection of the Betanodavirus presence is required before clinical signs occur in the near future. Meanwhile, screening of various species of susceptible fish and the identification of the viral carriers as a strategic approach is recommended. In fact, Eco- epidemiological studies are needed and all efforts should be focused on control and prevention of probably virus contamination in the Persian Gulf and Oman Sea waters as one of the strategic points in the world.

Keywords: Betanodavirus, Persian Gulf, VNN, Iran

1-Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
2-Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran
3-Department of Animal Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
*Corresponding author's Email: zorrieh@yahoo.com

Introduction

South of Iran has a long coastline about 4900 Km with the Persian Gulf and Oman Sea (Harlioglu and Farhadi, 2017). The Persian Gulf is the third largest gulf in the world and is one of the most important and strategic areas for its geographical and climatic characteristics (Nematzadeh, 2011). According to the latest list of Persian Gulf fish species, about 907 species are belonging to 157 families are present (Owfi *et al*., 2016). The reason for this species diversity is the high salinity and temperature (Nematzadeh, 2011), which makes fishing and fisheries, as the most important and renewable natural resources in the region after oil industry (Valinassab *et al*., 2011; Harlioglu and Farhad, 2017). Given that the Arabian Sea shares 87.5% of the fish species with the Persian Gulf and is on the other side of the Persian Gulf in the north of the Indian Ocean and is linked by the Strait of Hormuz to the Oman Sea and from there to the open Seas (Nematzadeh, 2011; Owfi *et al*., 2016), therefore, the possibility of transmitting disease from one region to another, is possible. This particularly true for Viral Nervous Necrosis (VNN) especially should be considered because it can be transferred by horizontal and vertical routes by different biological vectors (OIE, 2019). One of the most important emerging infectious diseases that affect fish throughout the world is in fact Betanodavirus infection. Due to the spread of the virus throughout the world, isolation and confirmation of VNN virus in the Mullet fish (*Chelon aurata* and *C. saliens*) in the Caspian Sea and also, the presence of fish susceptible to Betanodavirus in the Persian Gulf, the purpose of this article was to give an overview of Betanodavirus and perspective of viral nervous necrosis in Iranian Southern waters.

Betanodavirus Characteristics

Betanodavirus is an icosahedral virus whose genome consists of two discrete single-stranded positive RNA molecules (Doan *et al*., 2016) with no poly A sequence at the end of 3’ (de la Peña et al., 2011). The RNA1 sequence is about 3.1kb and consists of an ORF coding for the RNA-dependent RNA polymerase (RdRp) with 110kDa, which plays a vital role in the replication of the virus and is also called the Protein A. The RNA2 sequence (1.4kb) encodes the capsid protein with 36KDa (Alpha protein). In addition, during the virus replication, a subgenomic RNA is synthesized from the end region of RNA1 and is named RNA3 (0.4kb) (Toubanaki *et al*., 2015; Valero *et al*., 2015; OIE, 2019). Betanodavirus genome organization are shown in Fig 1. This fragment is 370bp and overlaps with the replicase ORF at the nucleotide positions 2730 and 3100 (Martinez, 2015), and encodes two unstructured proteins called B1 (111 amino acids) and B2 (75 amino acids). B1 boosts the antinecrotic mechanisms of the host cell, while B2 is as an inhibitor protein of host iRNA, and also contributes to the development of mitochondrial and cellular death through the production of hydrogen peroxide suggesting that protein B2 is a necrotic death factor (Doan *et al*., 2016; Low *et al*., 2017). B2 is essential for the proliferation and infection of both Alphanodavirus and Betanodavirus (Mézeth *et al*., 2009).

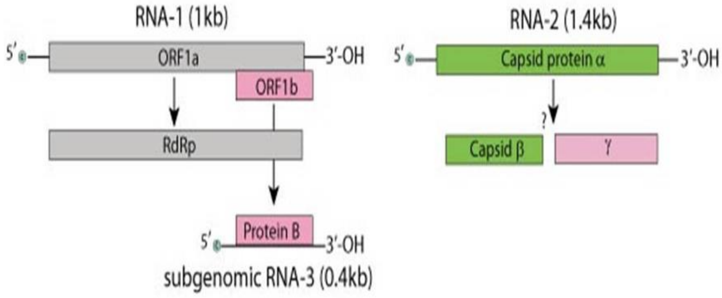

Figure 1: Betanodavirus genome organization
(ViralZone:www.expasy.org/viralzone,
SIB Swiss Institute of Bioinformatics)

Phylogenetic analysis of RNA2 in the variable region T4 classifies Betanodaviruses in four different species: SJNNV, BFNNV, RGNNV, TPNNV. In Norway, a new TNV genotype was also proposed by Johansen, that no isolate was obtained yet (Johansen *et al*., 2004; Korsnes, 2008; Bandin and Souto, 2020).

Betnaodavirus species are known to possess difference in growth temperatures and different hosts: RGNNV genotype is the most abundant and has the widest host range, and are present in the warmest water fish (optimal growth of 25-30°C) such as Asian sea bass, European sea bass and grouper. BFNNV is limited to cold water fish species (optimal growth of 15-20°C) such as Flounders, and TPNNV infects only Tigger Puffer at an average temperature of 20 °C. SJNNV has been detected to Japan in a limited number of warm water species (optimal growth of 25-20°C), but over the past decade, the virus has been found in Southern Europe in fish such as Senegalese sole, gilthead sea bream, European sea bass (Doan *et al*., 2016; Low *et al*., 2017; OIE, 2019).

Classification

The virus belongs to the Nodaviridae family, and recent studies have divided this viral family into three different groups, including Alphanodavirus (infecting insects), Betanodavirus (infecting fish), and a new third group called Gammanodavirus (infecting marine and freshwater shrimp) (Doan *et al*., 2016; NaveenKumar *et al*., 2017). The viral family was named from the village of Nodamura, Japan, where the virus was isolated from the mosquito for the first time in 1956 (Scherer and Hurlbut, 1967).

The disease caused by Betanodavirus in fish has so far been named SVE (Sea bass Viral Encephalitis) (Bellance and Gallet, 1988), VNN (Viral Nervous Necrosis) (Yoshikoshi and Inoue, 1990), FVE (Fish Viral Encephalitis) (Comps *et al*., 1994) and VER (Viral Encephalopathy and Retinopathy)

(Bovo *et al*., 1999; Tanaka *et al*., 2004), but now the World Organization for Animal Health (OIE) officially named it VER on the basis of infection pathology (Costa and Thompson., 2016).

Hosts susceptible to Betanodavirus infection

VNN has been isolated in different species (OIE, 2019). Since 1985, this virus has affected at least 120 species belonging to 30 families, 11 order of marine and freshwater fish species in different geographic areas except South America (Curtis *et al*., 2001; Costa and Thompson, 2016; Doan *et al*., 2016).

Most susceptible hosts are marine fish and some species are considered resistant due to lack of clinical signs. However, some species such as Zebrafish and Goldfish, which were considered to be resistant to VNN from the beginning (Furusawa *et al*., 2007), have been described as susceptible to VNN in recent years (Zorriehzahra *et al*., 2013b). The highest susceptibility to the disease was reported in fishes such as groupers, flatfish, striped jack, and European sea bass. The list of ornamental species of freshwater susceptible to this infection is increasing (Bandin and Souto, 2020). In the future, by introducing newer aquatic species into aquaculture farming system, there is the possibility of creating new hosts (OIE, 2019).

In Iran, this disease was identified for the first time in the Caspian Sea about 15 years ago (Zorriehzahra *et al*., 2005), and in recent years, losses from this virus on the Mullet fish (*C. aurata* and *C. saliens*) have continued in northern waters (Zorriehzahra *et al*., 2005; Ghasemi *et al*., 2013; Zorriehzahra *et al*., 2013a; Nazari *et al*., 2014; Ghiasi *et al*., 2016).

The first VNN suspected case was reported in *Liza klunzingeri* in Persian Gulf and Oman Sea Also, some severe necrosis was observed in the eye and brain of affected Mullet and typical vacuolation were recognized in affected fish (Koohkan *et al*., 2014, 2015).

Types of Betanodavirus infections

Acute infections: Severe clinical signs and high mortality, Betanodavirus infected cells have spread throughout the brain and retina (Martinez, 2015) without pathological changes in nervous tissues.

Chronic infection: Typical histological changes (Low *et al*., 2017)

Late stage infection: Macrophage cells are found in the brain and retina (Martinez, 2015).

Geographic distribution

The disease has been officially reported throughout the world, with the exception of South America (Doan *et al*., 2016), and includes countries in Southeast Asia (India, Indonesia, China, Japan, Korea, Malaysia, Philippines, Thailand, Vietnam), Oceania (Australia, Tahiti), the Mediterranean Basin (France, Greece,Italy, Malta, Portugal, Spain, Tunisia), the UK, Norway, the Caribbean Islands and North America (USA and Canada) (OIE, 2019). Although so far, the virus has not been isolated from South America but it has been detected from Amazon's healthy imported fish (Martinez, 2015).

Viral entrance portal and histological tropism

Researchers believe that the virus is a neuropathogenic agent that has a particular tendency towards central and retinal neural tissues, and several tissues have been proposed as the portal of entry. One assumption is that due to the proximity of the nose to the brain, the virus first infiltrates the nasal epithelium, then reaches the brain through the olfactory nerve and ultimately reaches the retina and then spinal cord (Martinez, 2015; Costa and Tompson, 2016). Another hypothesis is that the gastrointestinal epithelium is a primary propagation site of the virus and in fact, this region is in direct contact with water and food contaminated with the virus and from there it easily reaches to the brain and retina through the cranial nerves (Grotmol *et al*., 1999; Maltese and Bovo., 2007). In general, the virus is transmitted through the sensory or motor neuronal axon to the Central Nervous System (CNS), although there is still no evidence of the presence of the virus in these places (Nguyen *et al*., 1996). Although VNN has been diagnosed through its tropism to neural tissues, some researchers have found evidence of its presence in other tissues such as liver and digestive tract (Johansen *et al*., 2002; Martinez, 2015). Despite the various theories of researchers, Chi (2006) raised a theory based on empirical experiments and challenges of fish. She said that during naturally acute infection, small amount of NNV was first detectable in the spinal cord at the position above swim bladder in 3 out of 39 larvae examined at 0-day old, then all the nervous tissues and the epithelial layer of the gill operculum, the oral cavity and the skin of 1-day old larvae. The intensity of Immunofluorescence (IFA) staining increased in nerve tissues of 2-day old larvae while it remained moderate in the skin. By TEM observation, virions were found either in the nerve tissues or in the hyperplastic epithelial cells of the skin of 1-2 day old larvae. It is suggested that the initial multiplication site of NNV is in the spinal cord, particular the area above swim bladder. From this area, the virus spread backward to the end of the spinal cord and forward to the brain, and ended in the retina. It is possible that the virus enters the host via the skin or gastrointestinal epithelium, and virus in the epithelium is a transient infection instead of productive infection. The presence of NNV in epithelium could be a result of systemic infection or alternatively, epithelial cells of the skin may be susceptible to NNV only in the very early development stage. NNV exhibits neuron tropism suggesting that the virus entered the spinal cord via sensory and/or motor nerve cells linked to the epithelium. However, IFA positive staining was not found in the skin of 3-6 day old larvae during naturally subacute infection, nor in 12-72 hr. old larvae after NNV bath challenge. Therefore, the role of skin as a portal of entry of SJNNV remains unclear (Chi, 2006).

Ways of transmission

Various studies have shown that VNN can be transmitted both horizontally and vertically (NaveenKumar *et al*., 2017). The transmission routes of Betanodaviruses are shown in Fig 2 .

Vertical transmission

It was first proposed by Breuli 1991 from Sea bass and also by Arimoto 1992 in a striped jack and in the following years also by other researchers.

Typical vertical transfer methods for Betanodaviruses include:

VNN parental transfer through infected eggs

It is noteworthy that, for the first time, the experimental method of the presence of VNN in testis, the European Sea bass and Sea bream, two hosts highly susceptible to VNN and sometimes asymptomatic, suggested that the virus could be transmitted by males too (Suebsing *et al*., 2012). Of course, it should be noted that there is no possibility of vertical transmission of all species susceptible to VNN (Martinez, 2015).

Horizontal transmission

The infected fish are an important reservoir of infection and viral particles into the water environment (Azad *et al*., 2006; Kuo *et al*., 2012).

By the water of an infected fish or infected population (Le Breton *et al*., 1997; Hick *et al*., 2011).

Squids and subclinical infectious fish that are consumed as aquatic food (Gomez *et al*., 2010).

Use of raw infectious fish for feeding broodstock (OIE, 2019).

The use of wildlife remnants in food aquaculture systems (de la Peña *et al*., 2011).

Fish-eating birds transmit a number of viruses from around to farmland (Kuo *et al.*, 2012).

Water circulating around the fish that is infected with the virus (Arimoto *et al*., 1993).

Wild and breeding invertebrates, such as snail and oysters (Gomez *et al*., 2008; Panzarin *et al*., 2012).

Artemia, rotifers and shrimp are also important feed for larvae in the marine environment and can be carriers of the virus (Skliris and Richards, 1998).

Marine migratory fish transmits Betanodaviruses to distant geographic areas (de la Peña *et al*., 2011)

It has been determined that some larvae of infectious fish with VNN have the ability to keep infection and transmit the disease virus to the next generation trought the horizontal and vertical transmission of the (Nerland *et al*., 2007).

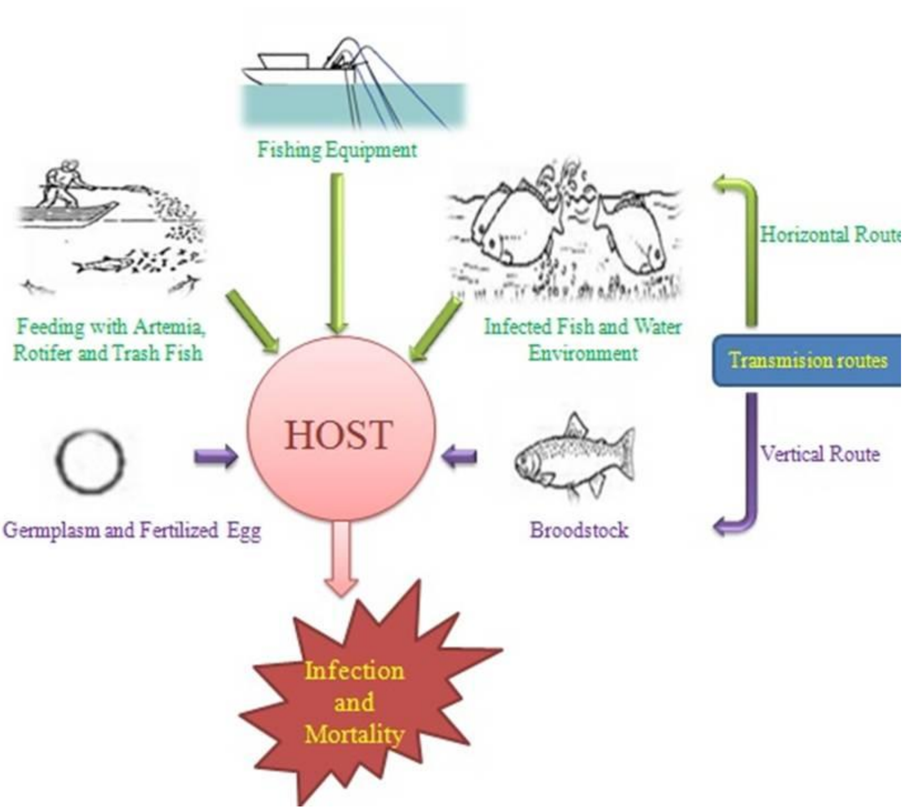

Figure 2: The transmission routes of Betanodaviruses.

Viral vectors

Water is considered the most important abiotic vector. In the fields under cultivation during a clinical outbreak from a farm section to the other part of the farm directly through water or with contaminated persons, boots, fishing trips and other tools, the Betanodavirus can be easily spread. In the open sea, the transmission of infection from one location to another is carried out by dominant currents, farm boats and wildlife migration. Due to the high resistance of the virus to acidic conditions and temperature of 37°C (Frerichs *et al*., 2000), the birds are considered to be a suitable vector. On the other hand, due to the high level of trade, particular attention should be paid to infected areas. The virus was identified from sand worms from the Nereidae family near an infected farm. There is a lot of international trade, worms should also be considered as a risk of spreading Betanodavirus from one region to another (OIE, 2019).

Clinical signs of the disease and histological lesions

Clinical signs of VNN in the acute and chronic stages of the disease are due to the proliferation of the virus and its release throughout the life cycle (Low *et al.*, 2017). Given that VNN is induced by a neuropathological agent, the main clinical signs of the disease are a variety of nervous disorders such as unusual swimming (spiral, swirling or lying down water with swollen abdominal) and other neurological symptoms such as fast and sudden swim. Specific characteristic involved in the abnormal positioning of fish in the water column is that the fish remain on the water surface. Consequently, non-specific signs such as loss of balance, loss of appetite, weight loss, and body color changes are observed. Probably species-specific behavioral changes are present and different species behave differently (Munday *et al*., 2002). Some clinical signs are shown in Fig 3. Other pathological lesions such as swim bladder inflammation, exophthalmia, skin darkness, and anemia are also observed (Adachi *et al*., 2008; Martineze, 2015; Low *et al*., 2017; OIE, 2019).

Figure 3: Some clinical signs of VNN in farmed Asian sea bass. A, swollen abdominal and exophthalmia; B, skin darkness.

Although the disease primarily affects the larval and young stages, serious mortality has also been reported in adult fish (OIE, 2019). In general, younger fish are described to be more susceptible to the disease, as even smaller younger fish with the same age are more susceptible to Betanodavirus, and larger larvae are less susceptible to the disease as compared to their smaller breeds (Banerjee *et al*., 2014).

The tissue lesions associated with the infection of the Betanodavirus are due to primary neurotropism of the virus, such as the CNS and the retina. During the Betanodavirus infection, the vacuolation and CNS necrosis, e.g. telencephalon, diencephalon and cerebellum is observed (Maltese and Bovo, 2007). The number and size of vacuoles depend on the host age and its species. Most serious lesions occur in larval and younger stages and in large areas of the CNS (Glazebrook *et al*., 1990), therefore, different degrees of vacuolations are seen in the brain, eye, and spinal cord, while pyknosis and karyorrhexis appear in single cells of these tissues (Munday *et al*., 1992). In general, intracellular vacuolation in the metencephalon and in the granular layers of the deep retina are abundant (Galeotti *et al*., 1999), and pyknosis and cellular basophilia in the spinal cord ganglia of *Oplegnathus fasciatus* has been observed (Yoshikoshi and Inoune, 1990). On the other hand, inflammatory processes and the presence of macrophages are likely to assist in the cellular vacuolation (Maltese and Bovo, 2007; Koohkan *et al*., 2014). At later stages brain and eye tissues can show a lot of vacuolating and necrosis, and in the brain of vast areas of vacuoles, they showed a sponge-like appearance. According to researches, brain tissue lesions vary from fish to other fish and the vacuolation is scattered in white matter and grey matter. Microscopic observations also show picnotic and nuclear vacuolation of brain grey matter, as reported by Azad (2006) (Azad *et al*., 2006; Koohkan *et al*., 2014).

The pathogenic mechanism of Betanodavirus in the host body

The entrance to the host cell

The necessary condition for the virus to enter the host cells and create a full-fledged infection is to enter into susceptible cells. There is no complete understanding of the biological pathway of their binding and their cellular receptors and the entry of the viruses to the host, but some authors believe that binding to the acid sialic of the host cell surface and entry through the endothelium occurs (Ito *et al*., 2008). Recently, there are other findings suggesting the involvement of thermal shock protein (Hsc70) in VNN binding to the host cell, which serves as a receptor or co-receptor to react with VNN capsid and plays a key role in the early stages of infection (Chang and Chi, 2015; Costa and Tompson, 2016; Low *et al*., 2017). The main source of endocytosis of Betanodaviruses to host cells is macropinocytosis, which uses two mechanisms: inward pit formation and ruffling of the outer membrane (Liu *et al*., 2005).

The replication and release of the virus from the host

Upon successful entry of the virus into the host cells and release of its genomic material, the host cell biological mechanism is used and increases the expression of genes of virus (Eckerle and Ball, 2002; Sommerset and Nerland, 2004). The pathway and mechanism for the release of the genomic material of the endosom in Betanodaviruses remain unknown (Goldfarb *et al*., 2006). The virus +RNA genome can act as mRNA and allow the expression of viral genes and the translation of viral proteins (Bandin and Souto, 2020). A non-structural protein of virus called B1, coded by RNA3, is identified as an antinecrotic agent of cell death and increases cell viability during the initial stages of virus replication (Chen *et al*., 2009). So, B1 protein function is very important during initial infection and virus replication (Ou *et al*., 2007).

The virus replicase, also known as protein A, accelerates genome synthesis along with mitochondrial membranes and also increases the viral protein synthesis (Su *et al*., 2011; Bandin and Souto, 2020). In the initial stage of virus replication, protein A must be sufficiently synthesized to produce the replication complex and RNA2 is then used to translate capsid protein and to regulate virion packaging (Bandin and

Souto, 2020). The B2 is another non-structural protein that binds to dsRNA, which inhibits the activity of the iRNA and protecting the viral RNA from the host defense mechanism (Ou *et al*., 2007).

The release of new virions is called shedding and is the last stage of a vital virus replication cycle. Since Betanodaviruses are nonenveloped viruses, the mechanism for the release of the virus is through to be the induction of apoptosis in the late-stage infection of the virus, which ultimately leads to lysis and cell death (Guo *et al*., 2003).

Factors influencing viral disease

VNN infection occurs in a variety of forms, from subclinical infection to an acute infection with 100% mortality. Various factors can affect the pathology of VNN infection, which include:

Host species

Age and size in some species of fish (OIE, 2019)

A genetic difference between viral strains and their virulence (Vendramin *et al*., 2013)

Co-infection with other viruses as an exacerbating disease (Kokawa *et al*., 2008)

Environmental factors such as water temperature (Iwamoto *et al*., 2000).

The dose of the virus (Tanaka *et al*., 1998)

Stressful factors such as population congestion, fish nutritional characteristics and mesh size used in the system

Inoculation route under laboratory conditions (Peducasse *et al*., 1999)

Gentic lineage of the fish (Doan *et al*., 2016)

Virus control

After entering the viral diseases to aquatic environment, control and eradication could be difficult due to the high stability of NNV in the aquatic ecosystem (Bandin and Souto, 2020).

Some strategies for controlling the virus include screening of embryos, water and eggs (Kai and Chi, 2008), monitoring of broodstock and apply biosecurity affaires (Martinez, 2015), washing and disinfection of larvae and fertilized eggs with ozone chlorine and use of vaccination (OIE, 2019) as well as applying the herbal compounds (Saberi et al., 2017).

Virus destruction

There are various ways to distruct the virus, including chemical materials (formalin, binary ethylenimine, BEI, and β-propiolactone), physical ways (heat and UV), and the use of certain antimicrobials and vaccines (Bandin and Souto, 2020). The use of antimicrobial peptides (AMP) has been proposed in the development of host immune response as a destructive method for infected fish (Wang *et al*., 2015). Focus on advanced therapy to stimulate the intrinsic immune system of the fish. The use of antimicrobial peptides (AMP) epinedicin-1 and hepticidin-1 was provided for further survival against VNN infection (Martinez, 2015).

Several vaccines have been recomended such as inactivated, subunit, recombinant-protein, DNA and live vaccines (Bandin and Souto, 2020). Given that sometimes fish can be contaminated with more than one genotype and there is no immune response between them, multivalent vaccines are needed to support fish from different varieties (Costa and Tompson, 2016).

Diagnostic tests

Virulent presence by observation of tissue sections with; Transmision electron microscopy (TEM), Immunohistochemistry (IHC), Indirect immunofluorescence antibody (IFAT), Molecular methods such as: RT-PCR, RT-nPCR, RT-qPCR and Cell culture (Moody and Crane, 2014).

In general, RT-PCR and Nested PCR are the most accessible method for detecting a virus in clinical infectious fish but Real time PCR is also the fastest, most sensitive and most specific method. Overall molecular methods have advantages such as short time, speed, high sensitivity and specificity, and appropriate tools for rapid detection of the virus in clinical and subclinical infectious fish. However, isolating the virus with cell cultures following immunostaining and molecular detection is still considered as the gold standard methods (OIE, 2019). A small number of cell lines have the ability to isolate Betanodaviruses. Two of the most widely used cell lines for the isolation of Betanodaviruses are available through the European cell cultures collection of ECACC: The SSN-1 cell line derived from the Striped Snakehead (Frerichs *et al*., 1996) and the E-11 cell line derived from SSN-1 (Iwamoto *et al*., 2000).

Survival outside the host

Betanodaviruses are very resistant to aquatic environments and can persist for more than 6 months in freshwater and 6 months at 15°C (Frerichs *et al*., 2000), while at 25°C or higher, survival rates are dramatically negatively affected. Following an outbreak of VNN, marine contamination with long-term exposure to the virus and the presence of an infection source for susceptible wild species is probable. Betanodavirus easily loses its virulence outside the water by drying and is inactivated after a period of about 7 days at 21 °C (99%) (Maltese and Bovo, 2007).

Effective disabling methods

Physical disabling by 60°C for 30 minutes and exposed to 440 μW for ten minutes can be obtained while chemical inactivation can be done with sodium chloride (50 ppm for 10 minutes), ozone (3.0 mg Cl_2L^{-1} for 6/7 min) and ethanol 60%. The virus is resistant to formalin-chloroform and ether and is able to withstand pH conditions from 2 to 11 for 24 hours without any loss of infectivity (Arimoto *et al*., 1996). In general, common disinfectants such as sodium hypochlorite, calcium hypochlorite, iodine, hydrogen peroxide, and benzalkonium chloride are very useful for the inactivation the virus. While formalin, ether, ethanol, methanol, chloroform is not feasible to deactivate the virus. Ozone is also used to prevent or reduce the infection of the eggs, and on the other hand, water contaminated with the virus is disinfected by exposure to UV (Frerichs *et al*., 2000; Grotmol and Totland , 2000; Costa and Tompson, 2016; OIE, 2019).

Discussion

Due to the fact that there are numerous reports of the presence of this viral disease in various species of fish, VNN can be considered as a serious threat to the aquatic industry of the world (Zorriehzahra, 2020). VNN was reported in Iran for the first time, in the Mullet fish from the Caspian Sea (Zorriehzahra *et al*., 2005). Until now, the clinical signs and traces of of this disease have been observed in Iranian southern waters (Koohkan *et al*., 2014; 2015). On the other hand, the available evidence of contamination of fish in different parts of the world and especially countries of around of Iran like Kuwait and Bahrain, as well as countries such as India, can be discussed as a ways of transmission of the virus to Iran or vice versa. In 2012, when severe fatalities occurred in the Persian Gulf and the Oman Sea, suspected samples of *Liza klunzingeri* were collected and examined histopathologically. By observing the vacuolation in the brain and retina tissues, the likelihood of VNN like-virus occurrence was reported (Koohkan *et al*., 2014), similarly to those reported previously by Azad *et al*. (2005) for the first time in India. In 2008, the first mortality from grouper fish due to the VNN was reported in a mariculture in Kuwait, followed by the confirmation of the presence of the virus in 2011, observing similar disease in the fish in the area (Azad *et al*, 2005; Azad *et al*., 2014). In 2014, following an outbreak of disease in Asian Sea bass fish grown in the cage from the western coast of India, fish with clinical signs of VNN disease were tested by Nested PCR and sequencing of PCR reaction products, and then presence of VNN virus was confirmed (Banerjee *et al*., 2014).

Figure 4: The situation of Iran and its neighboring countries in the Middle East and Persian Gulf's relationship with the Indian Ocean and others (https://steelguru.com).

Recent reports also indicate the presence of virus in sea bream fish from the Persian Gulf waters in Bahrain. In fact the histopathological and mortality studies of VNN in different ages of the bream have identified the typical vacuolation in brain and retina tissues. This investigation was the first report of VNN in sea bream fish in Bahrain (NaveenKumar *et al*., 2017).

The Persian Gulf is semi-enclosed and in the adjacent seaside basin and north of the Indian Ocean (Owfi *et al*., 2016). The situation of Iran and its and Persian Gulf's relationship with the Indian Ocean and others are shown in Fig 4. Also, the most important way is the transmission of marine viruses through water and related subjects. Meanwhile, vertical and horizontal transmission of Betanodaviruses has been confirmed, therefore, in the open sea, The migration of wild fish, migratory birds, water flow, farm's boats and the widespread trade of fish could be considered as the most common pathway for disease transmission (OIE, 2019). Other factors that influence the composition of viral communities in marine environments are the transmission of ballast water of ships (Kim *et al*., 2016), and since Iran is linked to neighboring countries and other open waters through the waterway, it can be referred to as a transmission way of the virus. Given that Betanodavirus has a small fragmented RNA genome and with a possibly high mutation rate, it is possible to create a viral reassortant and infect new hosts (Costa and Tompson., 2016). On the other hand, high resistance of virus in the marine environment, as a major threat for fish populations (OIE, 2019). Referring to the above mentioned cases and emphasizing both the horizontal and vertical transmission of Betanodavirus, it is possible for viral transfer in the Iranian southern waters from the Persian Gulf and the Oman Sea to the Indian Ocean and other open waters of the region. Since many marine species of the adjacent countries, such as Saudi Arabia, are common to the Persian Gulf. So in order to prevent the destruction of fish stocks in the region and before the onset of severe diseases, there is a pressing need for the screening of various species of susceptible fish and the identification of the ciral carriers. Also, regarding to develop of some marinfish industry such as Cage Culture in the region and increase of environmental stress, some regional and national control and prevention approach such as Survielense and Monitoring program should be applied through authorized organizations strongly.

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