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REVIEW

Viral hemorrhagic septicemia virus (VHSV), past, present and future: a review

Parisa Mohammadisefat . Mohammad Jalil Zorriehzahra . Milad Adel . Zahra Allahbeygi Chamjangali . Mahya Jabbari . Atieh Eftekhari . Hamzeh Farzipour . Sana Yousefian Jazi

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Abstract VHS is an extremely epizootic virus that causes high morbidity in cold-water fish. Nearly 100 species of fish in the northern hemisphere are affected by VHS disease, which has significant economic consequences for the aquaculture industry. It is crucial to understand how this virus affects fish. The spread of virus depends on environmental factors and the age of fish species. As the temperature rises, the virus's spread increases. In general, salmonid fish are particularly susceptible to VHSV and juvenile fish are also more sensitive to it. The virus is transmitted from infected water to various host species. Various methods of diagnosing and preventing diseases are presented. This review provides a comprehensive overview of VHS disease, including the species affected, diagnosis and detection of the virus, morphology, phylogeny and pathogenicity.

Keywords VHS virus . Morphology . Phylogeny . Pathogenicity . Diagnosis . Vaccine . Prevention

Introduction

One of the major pathogenic viral diseases that infects marine and freshwater fish is the VHS virus which causes high fatality in young fish in areas throughout the Northern Hemisphere (Kim et al. 2019). This virus is considered a crucial economic and social threat to fish cultur with important impacts on fish species and their environment. Furthermore, the high mortality varies depending on environmental and physiological parameters, such as the condition of rearing, fish age, fish species, water temperature, virus structure, and stress. VHS is a primary virus disease that occurs in farmed fishes, especially in Rainbow trout. However,

Parisa Mohammadisefat
Department of Fisheries Science, Urmia University, Urmia, Iran

Mohammad Jalil Zorriehzahra (✉)
Iran National Viral Diseases Strategic Network, Department of Aquatic animal Health & Diseases, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran
e-mail: m.zorriehzahra@areeo.ac.ir

Milad Adel
Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

Zahra Allahbeygi Chamjangali
Shahrekord University, Department of Fisheries Science, Shahrekord, Iran

Mahya Jabbari
University of Tehran, Fisheries Science Department, Tehran, Iran

Atieh Eftekhari
University of Tehran, Department of Microbiology and Immunology Science, Tehran, Iran

Hamzeh Farzipou
University of Kurdistan, Fisheries Science Department, Kurdistan, Iran

Sana Yousefian Jazi
University of Isfahan, Department of Cell and Molecular Biology and Microbiology Science, Isfahan, Iran

VHS caused about 31 mortalities of freshwater fish species in North America (WOAH 2022). Commonly, the target organs, such as the kidney, heart, and spleen are infected by a viral hemorrhagic septicemia virus (WOAH 2022). As fish are infected, a series of signs reveals such as edema and petechiae in muscle, brain, visceral organs, and exophthalmia, skin darkening and pale gills (Munro et al. 2015). This disease is highly contagious, causing high mortality in juvenile cultured and native fish. It is considered a significant economic threat to fish species, and, it is lethal for fry and juveniles (WOAH 2022).

Morphology and structure of virions

VHS is a virus with RNA strand form, from the order of Mononegavirales belonging to Novirhabdovirus genus (Kim et al. 2019). The causative agent is bullet-shaped, enveloped, negative single-strand RNA, with a length and width respectively of 60-70 nm and 170-180 nm, and the size of the linear genome is about 11 kb (Walker et al. 2022). Rhabdoviruses have two main structures, which are the surrounding envelope and the helical ribonucleoprotein core. RNA is enclosed by the nucleoprotein, also polymers and phosphor proteins are related to the helical ribonucleoprotein core structure. The genome of VHS involves six genes each of which is responsible for coding a protein with a specific function (Hwang et al. 2018). Protein N covers the genome and constitutes RNP (ribonucleoprotein) complex together with L and P proteins carrying out the process of genome replication and transcription (Baillon et al. 2020). The viral envelope of the G protein is significant because it causes an infection on the cell membrane. The endosomic membrane of the host at low pH combines with the G protein, and viral nucleocapsids release into the cytoplasm. Protein G is considered a primary target in vaccine design. The P gene resembles phosphoprotein and is used as a bridge for VHSV replication. The M gene is responsible for the matrix protein and its role in replication is to remove cell transcription. Compared to rhabdovirus mammals, the novirhabdovirus genome encodes the NV protein that helps suppress cell apoptosis. The NV gene is related to the interferon response of hosts (Kim 2015).

Virus replication cycle

The virus replication cycle consists of 3 main stages: adsorption, RNA synthesis, and morphogenesis (López-Vázquez et al. 2020). Glycoprotein can bind the virus to cell membrane receptors. Virus entry through endocytosis receptor and means changes in the G protein. Fusion of the VHS virus occurs, and it exudes the RNP to the cell cytoplasm, causing the replication cycle to begin (Dietzgen et al. 2017). For the primary transcription, the process of the M protein is necessary (López-Vázquez et al. 2020). By transcriptase complex, the negative strand genome is done from the genomic 3-end (Walker et al. 2015). Negative-strand viruses pack their RdRp enzyme into the virus particle (Cho et al. 2016). VHSV leaves the host cell with budding, and virions are produced through the plasma membrane (Najib et al. 2016). VHSV has strategies to inhibit the interferon systems via M protein that limit the synthesis of type I interferon (Baillon et al. 2020).

Phylogeny

Based on phylogenetic analysis and research (Ahmadivand et al. 2016), it is believed that the serotypes found in Iran can be traced back to their European counterparts (Fig.1). The primary driver behind this infection outbreak is believed to be the importation of infected eggs.

Virus resistance in the environment

The viral membrane is sensitive to many disinfectants. This virus is sensitive to fatty solvents such as ether and it is inactivated at room temperature and in 50% glycerol and chloroform. It is also very sensitive to acidic environments and heat; at 60 ° C, it disappears within 15 minutes. Alcohol and hydrogen peroxide are not very effective against the virus. It survives at least a week when dry, but about 28 to 35 days in cold water at 5° C, about 3 months at 0 °C but at 20° C or below, or by freeze-drying for long periods (up to several years). The virus remains infectious in filtered fresh water at 4°C for up to 1 year.

Also, at a pH of 5-10, it is resistant to multiple stages of freezing and thawing. The virus is most active at a temperature of 12-14 °C (Olesen and Skall 2013). In freshwater without organic matter and at a temperature of 15 °C, about 99.9% of the virus is inactivated after 13 days, and in salt water after 4 days. In salt water after 10 hours at 15°C, the spread of the virus increases to 50%, however, it is possible to isolate and infect the virus up to 40 hours later. Commercial freezing of fish infected with VHS reduces pathogenicity, or virus titer by 90% or more, but does not completely kill and remove the virus (WOAH 2021).

Antigenic and phylogenetic characteristics

Geographical distribution

In recent years, knowledge of viral hemorrhagic septicemia has increased and the spread of this virus in the Pacific, Atlantic and Baltic Seas has increased. VHSV also affects farmed fish, particularly rainbow trout (Ito et al. 2018).

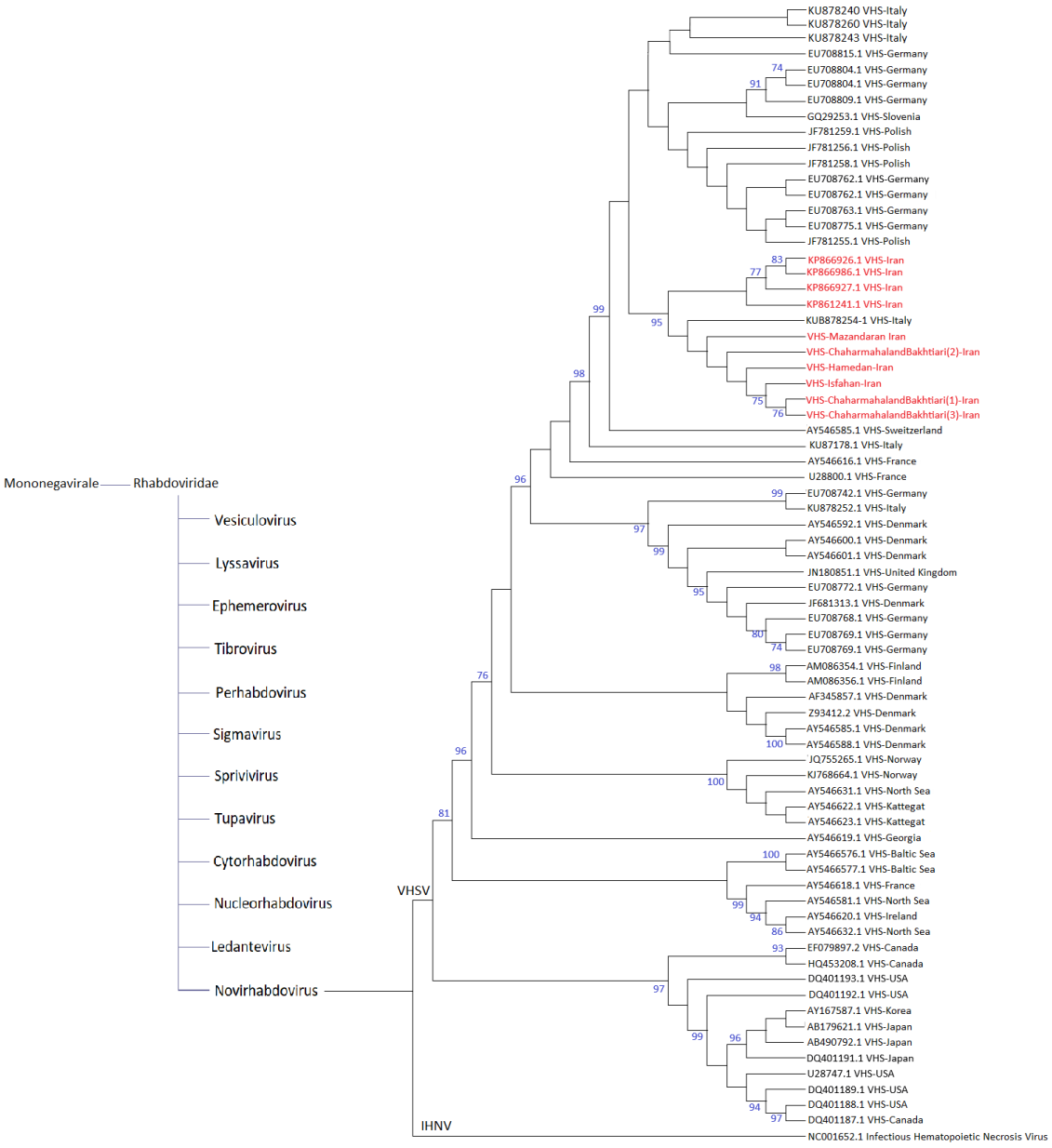

Fig. 1 The phylogeny tree of VHS virus (Fattahi et al. 2020; Marsella et al. 2022)

Viral transmission

Transmission of the VHS virus occurs through direct contact or water. Swallowing the virus can also lead to infection in young rainbow trout. The virus can enter the aquatic environment via sperm and ovarian fluids and urine, or it can accumulate in filter-eating organisms and infect sensitive species (Kim et al. 2019). The secretion of the virus in carriers is intermittent, and these species like blue mussel (*Mytilus edulis*) the virus in their body (Getchell et al. 2013). VHS transmission to other fish is mainly due to horizontal transmission. When the virus enters the water through the urine of an infected fish, it sticks to the epithelium of its new host (Baillon et al. 2020). Fish-eating birds can also be mentioned as a further transmission path of the VHS virus. Therefore, predatory species by eating infected fish have higher virus titers than the waterborne method, and amphipods and leeches also deliver the VHS virus to hunters (Kim and Faisal 2011). Virus transmission occurs at a temperature of 1 to 15°C and a maximum of 20°C. At a temperature of about 1-5°C, the disease occurs more often and with less daily mortality, but more cumulative death. When the water temperature starts to rise, the disease spreads, so the best time of year for the disease to develop is spring (Kim and Faisal 2011).

Pathogenicity

eThe VHS virus enters the fish's body by attaching itself to the gill epithelium and then travels through the blood to the main internal organs. There is also evidence that the skin may be an entry route for the virus (Tamer et al. 2021). During the first one to four days of the VHS outbreak in rainbow trout, the virus' first targets are the kidney and spleen endothelial cells. The VHS virus produces its early pathological effects on the cells lining the circulatory system 48 hours after infection by attachment, penetration and proliferation in the endothelial cells of small veins and sinusoids. Then the virus spreads to the front part of the kidney and causes degeneration. Cellular and necrotic renal hematopoietic tissue as well as macrophages and melanoma macrophages appear 2-4 days after infection. The infection then spreads to the liver, and finally, there is necrosis of the acinar cells of the pancreas (6 days) (Cano et al. 2021).

Disease mechanism

The disease mechanism begins with the transfer of virus particles to various cell receptors of the virus infection, which brings the nucleic acid to the cytoplasm or cell body. Most viral infections begin by attaching to a variety of cellular receptors located outside the cell matrix. This penetrates the cell's plasma membrane and initiates viral infection. Therefore, the virus enters the cytoplasm, and where the virus is released from the colloidal seed (Jeong et al. 2015). In the case of mammalian rhabdoviruses, there is this an idea that it directly affects the type I interferon (IFN) and other mechanisms that affect immunity to escape the host's immunity, which disrupts the host's defense function. In a series of studies, isolates of the rainbow trout virulent virus (VHSV) inhibited the expression of the GTP-binding protein gene of interferon Mx in vitro. This shows that the malignant strains of the virus (VHSV) are likely to disrupt the IFN signaling pathway, and this disruption causes more pathogenicity. Another report states that the VHS Nv protein can negatively modulate the host antiviral response by downregulating the transcription factor NF-kappa B phospholipid kinase 1 (tbki), thereby regulating the IFN response after viral infection (Cano et al. 2021). By culturing the VHS virus on the gill epithelium of fish, researchers infected marine and freshwater fish with VHSV strains. They found that the propensity of some VHS strains to replicate in gill tissue depends first on the source of the virus and then on the fish species. While some studies have shown that the main entry point of the virus is the genital papillae, in another study, a bioluminescence method showed that fins were the portal of entry for the virus (Kim and Faisal 2011).

Pathophysiology

The kidneys, heart, and liver are the target organs of the virus. Complete cell and tissue necrosis are observed in these tissues. In the case of the kidneys, the excretory function and the osmotic regulation of the body are quickly lost. This naturally causes edema and a bloated appearance, and hepatic necrosis

causes loss of glycogen and energy reserves. Heart failure is implicated in damage to myocardial cells, and bleeding from the damaged capillary endothelium leads to anemia and death (Guðmundsdóttir et al. 2019).

Epidemiology

Molecular epidemiology is a method of investigating at the molecular level, and it plays an effective role in researching the cause of the disease, the factors that affect the disease, and disease prevention (Honardoost et al. 2018; Yuan and Wang 2021). The glycoprotein, NV protein, and L protein play significant roles in determining the pathogenicity of this virus (Garver and Hawley 2021). VHS strains that cause disease in freshwater fish originate from VHS in salt water (Gadd 2013). Genotypes I and IV are found in freshwater and seawater, but genotypes II and III are only in the sea (Cieslak et al. 2016). Not all species may be susceptible to all virus strains (Garver and Hawley 2021). In an experiment on 17 different fish species in Finnish brackish waters, it was found that the spread of the VHS-Id virus from wild fish to farmed fish is less than 4%. They also found that whitefish is not sensitive to this virus and can survive the disease without any significant signs, although Danish researchers have found that whitefish is sensitive to some strains adapted to rainbow trout. Furthermore, wild herring in Sweden is likely to be the cause of VHS Ib infection in *Oncorhynchus mykiss* (Vennerström et al. 2020). The study by (Ahmadivand et al. 2021), examined farmed rainbow trout in Iran from 2015 to 2019 and found that the strain of VHS virus in these farms was Ia and was of European origin and likely entering Iranian fish farms through imported eggs. The prevalence rate of VHSV in Lake Ontario was 28% (Escobar et al. 2017). Comparing the annual report on HSV infection from 1962 to 2015, an average of three new infected fish were identified. Almost half of them were infected with VHS genotype IV, and genotype II was least affected. The species Perciformes, Salmoniformes and Gadiformes had the highest number of infected cases and all infected cases were also shown to be associated with the Northern Hemisphere (Escobar et al. 2018). This virus has high adaptability, as a 2007 report infected rainbow trout in Norway with a strain of this virus previously diagnosed as non-pathogenic. In addition, it was found that VHS virus genotypes III, II and Ib of marine origin have a low infection for *Oncorhynchus mykiss*, but may be pathogenic for turbot, and herring, but may become pathogenic under dense fish farming conditions; It has been reported that the potent factor in the pathogenicity of VHS virus is polymorphisms of 38 single amino acid located in the VHS genome coding region (Baillon et al. 2020).

Hosting domain

More than 100 species of fish have been infected with VHS disease, including marine and freshwater species. The list of host species (WOAH 2021) is given in Table 1.

The geographical extent of the disease

Currently, there have been no reported cases of the VHS virus in the southern hemisphere (Skall and Olesen 2015). According to various studies (Escobar et al. 2017; Niner et al. 2020; Vennerström et al. 2020; WOAH 2021), genotype Ia is more prevalent in Europe and affects species such as *Oncorhynchus mykiss, Esox Lucius, Scophthalmus maximus, Thymallus thymallus.* Genotyp Ib is more common in the Baltic and North Seas, the English Channel, and north to near Norway (Nordkapp). It is also present in Japan and South Korea. Genotype Ic exists in Germany and Austria, while genotype Id It is distributed in Finland. Genotype Ie has spread to Georgia and Turkey in the Black Sea and affects species such as *Oncorhynchus mykiss*, turbot, sea bass, and whiting. Genotype II has been observed in the regions of Bothnia and Finland and affects *Clupea harengus* and *Lampetra fluviatilis*. Genotype III has been found in the UK, Ireland and the North Atlantic and affects turbot and rainbow trout. Genotype IVa is more common in North America and Canada and has also been distributed in South Korea and Japan in species such as *Clupea pallasii*, *Salmo salar,* and *Paralichthys olivaceus*. Genotype IVb is more common in the Great Lakes of North America, genotype IVc in New Brunswick and Nova Scotia (Canada) and the Great Lakes regions, while genotype IVd has been observed in Iceland affecting *Cyclopterus lumpus*. VHS has been diagnosed in *Oncorhynchus mykiss* in Iran (Ahmadivand et al. 2016).

Table 1 Fish species susceptible to VHSV disease (WOAH 2022)

Hosts		
Order	Family	Species scientific name
Acanthuriformes	Sciaenidae	*Aplodinotus grunniens*
		Larimichthys polyactis
Anguilliformes	Anguillidae	*Anguilla anguilla*
Argentiniformes	Argentinidae	*Argentina sphyraena*
Pleuronectiformes	Paralichthyidae	*Paralichthys olivaceus*
Beloniformes	Belonidae	*Belone belone*
Beloniformes	Adrianichthyidae	*Oryzias dancena*
		Oryzias latipes
Carangiformes	Carangidae	*Seriola dumerili*
		Trachurus mediterraneus
Carcharhiniformes	Scyliorhinidae	*Scyliorhinus torazame*
Clupeiformes	Clupeidae	*Alosa immaculata*
		Alosa pseudoharengus
		Sardina pilchardus
	Engraulidae	*Engraulis encrasicolus*
Cypriniformes	Catostomidae	*Catostomus commersonii*
		Moxostoma anisurum
		Moxostoma macrolepidotum
	Cyprinidae	*Danio rerio*
		Notropis atherinoides
		Semotilus corporalis
Cyprinodontiformes	Fundulidae	*Fundulus diaphanus*
		Fundulus heteroclitus
Gadiformes	Gadidae	*Gadiculus argenteus*
		Theragra chalcogramma
		Trisopterus esmarkii
		Trisopterus minutus
	Lotidae	*Enchelyopus cimbrius*
		Gaidropsarus vulgaris
		Lota lota
	Merlucciidae	*Merluccius productus*
	Oxudercidae	*Rhinogobius* sp. (undescribed species)
Ophidiiformes	Ophidiidae	*Hoplobrotula armata*
Osmeriformes	Osmeridae	*Thaleichthys pacificus*
		Hypomesus pretiosus
Ovalentaria	Embiotocidae	*Cymatogaster aggregata*
Perciformes	Ammodytidae	*Ammodytes hexapterus*
		Ammodytes personatus
	Liparidae	*Liparis tessellatus*
	Moronidae	*Dicentrarchus labrax*
		Morone americana
		Morone chrysops
		Morone saxatilis
	Mullidae	*Mugil cephalus*
		Mullus barbatus
	Percidae	*Perca flavescens*
		Sander vitreus
	Trichiuridae	*Trichiums lepturus*
	Uranoscopidae	*Uranoscopus scaber*
Percopsiformes	Percopsidae	*Percopsis omiscomaycus*
Petromyzontiformes	Petromyzontidae	*Lampetra fluviatilis*
		Petromyzon marinus
Pleuronectiformes	Pleuronectidae	*Glyptocephalus stelleri*
		Hippoglossus hippoglossus
		Limanda limanda
		Platichthys flesus
		Pleuronectes platessus
		Reinhardtius hippoglossoides
	Scophthalmidae	*Scophthalmus maxima*
	Soleidae	*Solea senegalensis*
Rajiformes	Rajidae	*Raja clavata*
Salmoniformes	Salmonidae	*Coregonus artedii*
		Coregonus clupeaformis
		Coregonus lavaretus
		Oncorhynchus kisutch
		Oncorhynchus mykiss
		Oncorhynchus mykiss X Oncorhynchus kisutch hybrids
		Oncorhynchus mykiss X *Salmo trutta hybrids*
		Oncorhynchus mykiss X *Salvelinus alpinus* hybrids
		Oncorhynchus mykiss X *Salvelinus namaycush* hybrids
		Oncorhynchus tshawytscha
		Salmo marmoratus
		Salmo salar
		Salmo trutta
		Salvelinus alpinus

Table 1 Continued

		Salvelinus fontinalis
		Salvelinus namaycush
		Thymallus thymallus
Scombriformes	Scombridae	*Scomber japonicus*
	Stromateidae	*Pampus argenteus*
Scorpaeniformes	Cottidae	*Cottus pollux*
	Cyclopteridae	*Cyclopterus lumpus*
	Scorpaenidae	*Scorpaena porcus*
		Scorpaena izensis
	Triglidae	*Eutrigla gurnardus*
Siluriformes	Ictaluridae	*Ameiurus nebulosus*
		Ictalurus punctatus

Age

Young fish are more sensitive to VHSV infection, but adult fish exposed to VHSV for the first time also experience high mortality rates (Zorriehzahra et al. 2019). Rainbow trout weighing less than 3 grams are most vulnerable to VHS, with over 80% of affected fish dying. Mortality in juvenile fish are still high, ranging from 10% to 50% (Vennerström et al. 2020). Adult rainbow trout can also experience mortality rates ranging from 5% to 90%. Pacific herring can have up to 100% mortality when exposed to VHSV genotype Iva (WOAH 2021). Age is a critical factor, and mortality in rainbow trout is entirely dependent on the fish's age and water temperature (Zorriehzahra et al. 2019).

Temperature

VHS is a cold-water disease that infects both freshwater and marine ecosystems, but it maintains its pathogenicity for one year in water at 4 °C (WOAH 2021). The temperature affects the fish's immune system response and the virus replication at low temperatures, with low immune system response leading to higher rates (Vennerström et al. 2020). The optimal temperature for virus survival and multiplication is between 9°C and 12 °C and decreases at temperatures above 15 °C (Vennerström et al. 2020; WOAH 2021). This disease results in a short period of mean mortality between 15 °C and 18 °C (WOAH 2021). With water temperature fluctuations, especially in spring, fish mortality increases (Vennerström et al. 2020; WOAH 2021). All-cause mortality increases at low temperatures between 1 °C and 5 °C Virus transmission occurs at as low as 1 °C and up to 20 °C, but virus viability decreases at temperatures above 20 °C (WOAH 2021). In a study on *Pimephales promelas* conducted by (Fellman 2012), fluctuating temperature were reported to increase VHS infection compared to constant temperature; and the Virus transmission rate also increased with decreasing temperature. *Paralichthys olivaceus* becomes infected with VHS at temperatures between 8 °C and 15 C; with the mortality rate at 10 °C higher than at 13 °C (Kim et al. 2016). One reason for the virus's survival at low temperatures is that low temperature coincide with low UV radiation in cold seasons, as VHSV is sensitive to UV radiation (Vennerström et al. 2020).

Clinical signs and physical lesions

VHS affects several species; but it is particularly severing in in Salmonidae, and clinical signs depend on the disease stage (Escobar et al. 2018). In severe cases, VHS virus can exist in the fish's body and the clinical signs may not be apparent (Getchell et al. 2019). VHS disease has three types of signs consisting of internal lesions: lesions in the muscles and liver, petechiae in the back muscle and heart, scattered petechiae in the muscles and pyloric area, severe kidney congestion and lesions on the kidneys, bleeding in muscles, brain and internal organs, swollen spleen and liver, serous or blood-containing edema, hyperemia in kidneys and gonads and punctual hyperemia in the liver, pale heart and pale liver; external lesions: petechial hemorrhages around the eyes and on the lower jaws of trout, exophthalmia, melanosis, swollen abdomen, pale gills; and behavioral signs: lethargy, impaired swimming, anorexia (Tamer et al. 2021).

Forms of disease

There are three forms of VHS infection in rainbow trout: acute, chronic, and nervous, although infection

can occur in any form (Vennerström et al. 2020). In some cases, infected fish exhibit systemic signs during acute phase, including anemia, bleeding in the gills, infections in the submucosal tissues of the digestive tract, intestines, and brain, and darkening of the skin. The acute phase occurs despite the time. Most systemic signs disappear in the chronic phase, except for dark skin and severe anemia. The mortality rate is low in this phase. Finally, fish in the last-stage show neurological signs such as swimming in circles or near the bottom of the water, erratic swimming, and corkscrew-like swimming (Gorgoglione 2014).

Immunostimulants

Immunostimulants are natural or chemical compounds that can balance or activate non-specific immune responses of the immune system in the body, increasing overall immunity and natural resistance to pathogens in fish (Leal et al. 2019). Although a substance that can increase resistance to VHS infection has not been reported, several immunstimulants with beneficial effects have been identified, such as IL-1 derived peptides, -glucan from yeast, algae or plant extracts, oligo-elements, vitamins, cytokines, and curcumin (Jeong et al. 2015). Consumption of foods containing vitamins C and E, beta-glucan, and zinc increased serum immune response factors (Leal et al. 2019). Some immune system stimulants such as specific oligosaccharides, LPS, CpGs, -glucan or flagellin in fish can be used orally to improve the status of the innate immune system (Munang'andu et al. 2020). No commercial vaccine is available to increase resistance to VHSV infection, although in vitro studies suggest that the vaccines have a protective function against VHSV infection (Dadar 2020). Recently, a new vaccine containing VHSv genotype IVb glycoprotein that can boost the immune system against VHSV-IVb was investigated (Standish et al. 2016). This vaccine can also act on B cells (Munang'andu et al. 2020).

Pathology

Gross pathological studies have shown that generalized petechiae bleeding in the skin and skeletal tissues, as well as internal organ petechiae bleeding in the back muscles, are important signs of VHS infection (WOAH 2021). Most fish samples had no gastric or intestinal contents other than a yellow sticky gut substance (Sandlund et al. 2021). Histopathological changes show strong fluctuations in muscle, kidney, and liver. Hemorrhage was evident in skeletal muscle, and renal tissue exhibited interstitial hematopoietic necrosis, fragmentation of melanoma macrophages, large central hemorrhage, and renal tubules degeneration. Hinged hepatocellular necrosis with pyknotic and chaotic nuclei was also possible (Ahmadivand et al. 2016).

Diagnosis

There are several methods to detect VHSV such astransmission electron microscopy, histopathology, antibody-based assay, RT-PCR, and isolation in cell culture (Table 2). The last method was introduced as the Golden test by WOAH (2021).

Cell culture

VHS virus isolation is performed by culturing many fish cell lines. However, to detect carrier fish, inocu-

Table 2 Current methods for diagnosing of VHS virus (WOAH 2021)

Methods	First Recognition	Definitive diagnosis
Appearance signs	Standard	-
Pathology	Standard	-
Cell culture	+	+
Based on antibodies	Standard	+
Real-time RT_PCR	+	+
Conventional RT-PCR	+	+
SEM	It is used in some situations	-

+ It is recommended, - Not Recommended

lation of cell lines with tissue is required. To detect VHS virus, the infected fish is appropriate. Moreover, the examination of viral diseases like VHS virus depends on fish size. For instance, for larger fish spleen, kidney, heart, and encephalon would be proper samples. Usually, the fish cell line BF-2 is used. Perhaps FHM or EPC cells are used alternatively (Lorenzen et al. 1999; Olesen and Jørgensen 1992; WOAH 2021). Cell types for virus detection include bluegill fry (BF-2), Chinook salmon embryo (CHSE-214), epithelioma papulosum cyprini (EPC), fathead minnow (FHM) or rainbow trout gonad (RTG-2) (Lorenzen et al. 1999). At a temperature of 20 °C to 24 °C, the cells will grow in MEM medium containing 10% fetal bovine serum FBS along with the standard amount of ammonia. Buffer the culture medium with bicarbonate. It is better in open units for cell culture with 23 mM Tris/HCl and 6 mM Na-bicarbonate or with medium (HEPES=N-2-hydroxyethyl-piperazine-N-2-ethanesulphonic acid). It is buffer, and pH should be in the range of 7.6 ± 0.2. It is possible that cell sensitivity can be increased by reducing the amount of FBS to 2%. Using a solution of 7% (w/v) PEG-20,000 15-30 minutes before helps to detect VHSV. It is noteworthy that the samples must be cool and homogenized with a homogenizer. The volume of the medium should be in the ratio of 10:1, and for liquid samples, it should be in the ratio of 1:1. It should be noted that homogenous or liquid samples should be centrifuged for 15 minutes at a temperature of 2-5°C.We pass the liquid on a 0.45 µM filter centrifuge and put the samples in two cell cultures at 1:100 and 1:1000 dilutions. Cell cultures are incubated at 15°C for 7-10 days. 2 to 3 times a week, and checked for CPE (Batts et al. 1991).

Real time RT-PCR

In this method, the specific RT-PCR gene primers selected for both cDNA transcript production and real-time RT-PCR to strengthen this method. The forward primer is 5'-AAA-CTC-GCA-GGA-TGT-GTG-CGT -CC-3' and the reverse primer is 5'-TCT-GCG-ATC-TCA-GTC-AGG-ATG-AA-3', and the FAM-labeled probe is 6'-FAM-TAG-AGG-GCC-TTG- We use GTG-ATC-TTC-TG-BHQ1 (WOAH 2021). Add 5 µl of the extracted RNA to every 25 µl of RT-PCR reaction. In this method, the Quantitect Probe RT-PCR kit was employed (Jonstrup et al. 2013).

Tow –step real-time RT-PCR

The two-step method has two parts. In the first part, reverse transcription is performed in such a way that the extracted RNA is reverse transcribed into cDNA using random primers. In the second part, the forward primer 5'-ATG-AGG-CAG- GTG-TCG-GAG-G-3' and reverse primer 5'-TGT-AGT-AGG-ACT-CTC-CCA-GCA-TCC are used. We add 2.5 µl of cDNA to 25 µl of PCR reaction solution and under the conditions, we put a thermal spark (Garver et al. 2011).

Indirect antibody fluorescence test (IAFT)

Indirect antibody fluorescence test (IAFT) is another method used to detect VHSV. Cell monolayers are grown in plastic cell culture plates until they reach 80% confluency.

The virus then diluted and detected up to 5000-10,000 plaques to form PFU, then incubated at 15°C for 1 day. The culture medium is treated with a mixture of 30% acetone and 70% ethanol, washed and fixed. VHSV antibody is diluted and added to cell layers, which are then incubated at 37 ^{0}C for 1 hour. The cell layers are washed and treaed with FITC solution before being observed under a microscope with UV light (WOAH 2021).

Definitive diagnosis

Usually, a culture method is proposed to diagnosis disease in cells through immunofluorescence assay and the use of bright antibodies (WOAH 2021). While definitive diagnosis of VHS involves virus separation using cultivation method, recognition by electron microscopy, RT-PCR, genetic sequencing, and genotypic classification are also common. However, the best methods for targeting confirmatory detection includeReverse Transcription and Polymerase Chain Reaction, antibody-based assays, and cultivation method (Table 2). Separation in cultivation method, according to the main detection methods RT-PCR, is the significant

way for confirmatory diagnosis (Hwang et al. 2018).

Vaccine development for fish immunization

For over thirty years, researchers have been working towards developing a vaccine for hemorrhagic septicemia virus (VHS), but there is still no commercially available vaccine. However, some vaccines that have been developed include the killed virus vaccine, attenuated virus vaccine, and new combination vaccines with DNA-based vaccines (WOAH 2021). Other methods that have been developed against the VHS virus include yeast-derived beta-glucans, IL-1 probiotic-derived peptides, and an additive genetic model. One method that has shown promise is reverse genetic method, which was used for rabies virus. This method involves producing recombinant viruses from rhabdovirus species by transcribing the length of virus antigen from the T7 promoter. To construct the complete VHSV antigen vector, the antigens are divided into several parts with limiter enzyme places at two final spots, and each part has a plasmid vector. New VHSVs can be generated by combining cells with plasmids and translocating them with lipid droplet-based reagents (Kim and Kim 2019). Another method is the VHSV attenuation method by reverse genetics, which involves changing the VHSV genome feature. For example, regions of the virus virulence genome can be identified and corrected by reverse genetics of this region. Single-cycle VHSV as a vaccine and the weakened VHSVs for vaccine vectors are also other methods of recombinant viruses (Kim and Kim 2019).

Prevention

According to (www.cfsph.iastate.edu), sources of transmission of the VHS virus include objects or carriers that transmit the disease, sources of contaminated water, and the introduction of infected fish into farms, and vectors that can transmit the pathogen. To prevent the disease prevalence on the farm, some biosecurity practices such as those mentioned are required; contact of farmed fish with wild fish should be prevented, and the sources of new fish and eggs entering the farm should be limited, and fish likely to be vectors should be quarantined. Make sure the water entering the farm is free of pathogens. All tools and equipment and breeding tanks should be disinfected and sharing with other farms should be avoided. Disinfectant solutions should be used, and farm vehicles and personnel should follow farm biosecurity guidelines. Also, by limiting stressors on fish and regularly monitoring and recording the health and behavior of fish, improving water quality and food preservation, checking for the presence of disease signs in fish, and promptly removing dead or diseased stock. Avoid contact of farmed fish with other farmed fish or wild fish or animals, and prevent unnecessary people from entering the farm. If biosecurity principles are not followed, fertilized eggs that transmit the disease can be a factor in transmitting the disease to farms (Karreman et al. 2015). It is also possible to prevent the disease by stimulating the immune system or administering a vaccine to create long-term immunity (Puente-Marin et al. 2018). However, there is no commercially effective vaccine against VHSV to date. Although there is no compelling commercial vaccine against viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN) in European countries, researchers succeeded in producing a new DNA vaccine for both mentioned exotic viral diseases (VHS and IHN). This vaccine has succeeded in protecting and decreasing the mortality rate of Rainbow trout fry in the experimental study (Marsella et al. 2022). Using pathogen-free generators for breeding can also prevent the transmission of pathogens (Zorriehzahra et al. 2019). Passive and active surveillance can be useful for detecting the virus (Gustafson et al. 2014). RT-PCR and qPCR are used for quick diagnosis and prevention of disease transmission (López-Vázquez et al. 2015). There are some additives able to prevent the severity of the VHS virus or lead to an increase in fish resilience against this virus (Table 3).

Future perspective

Since VHS was first isolated from farmed trout in Iran in 2014 (Naderi-Samani et al. 2020), there is a need to adopt procedures to control and eradicate the disease. Phylogenetic analysis has shown that the Iranian isolates are similar those from European ones (Genogroup Ia) (Ghorani et al. 2016), so it should take into consideration in vaccine development. As previously mentioned, glycoprotein G plays the crucial role in viral immunogenesis and binds to fish cell receptors, making it an ideal candidate for vaccine development

Table 3 The effect of some additives on the VHSV

Species	Additive	Effect	Dosage	Administration	Duration	Reference
Olive flounder (*Paralichthys olivaceus*)	*Celosia cristata* and *Raphanus sativus*	The severity of the disease can be prevented	5 liters /per fish	Oral	48h	(Park et al. 2017)
Olive flounder (*Paralichthys olivaceus*)	licorice (*Glycyrrhiza uralensis*)	Excellent performance against VHS virus Inactivating the VHS virus	250 and 500 mg/kg	Oral	14 Days	(Lim et al. 2021)
Olive flounder (*Paralichthys olivaceus)*	Saponin and Chitosan	It causes less stress to the fry With inactive virus	0.29, 1.45, and 2.9 mg/g	Oral	10 days	(Jung et al. 2022)
Rainbow trout (*Oncorhynchus mykiss)*	Chloramine-T	Increasing surveillance from 27% to 76%	10 ppm	Oral	7 days	(Ganjoor 2016)

programs. Conventional vaccines can induce immunity by removing the glycoprotein G coding region, but the virus continues its replication cycle, which may not be favorable for disease control. Yang and Kim (2022), constructed recombinant baculovirus-based VHSVG protein delivery tools for olive flounder immunization. The recombinant vaccine with the highest antibody content against VHS is significant in disease control and eradication, so our focus and efforts should be directed towards the development and manufacture of recombinant vaccines.

Conclusion

This review highlights some important information about VHS disease. As previously mentioned, this virus causes high morbidity rates in aquatic ecosystems, particularly in farmed fish and damages the aquaculture industry. Therefore, all information related to diagnosis, treatment, and care should be prepared. Furthermore, viral hemorrhagic septicemia (VHS) is a serious disease that affects a wide range of fish species and causes significant economic losses in the aquaculture industry. The development of efficient vaccines is crucial in preventing and controlling the spread of VHS. This review manuscript has highlighted the current knowledge on VHS, with emphasis on prediction and vaccine development. Although significant progress has been made in understanding the pathogenesis of VHS, there is still a need for more research to develop practical and efficient vaccines. Further studies are required to identify the most effective antigens, adjuvants, and delivery methods for VHS vaccines. The development of effective vaccines for VHS will not only benefit the aquaculture industry but also contribute to the conservation of wild fish populations. Furthermore, it is important to note that the main reason for VHS occurrence in Iranian fish farms has been identified as the importation of eyed-eggs from European countries. This has been confirmed by researchers through phylogeny analysis and molecular methods. Therefore, stricter regulations and monitoring of imported fish eggs are necessary to prevent the introduction and spread of VHS in Iranian aquaculture.

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