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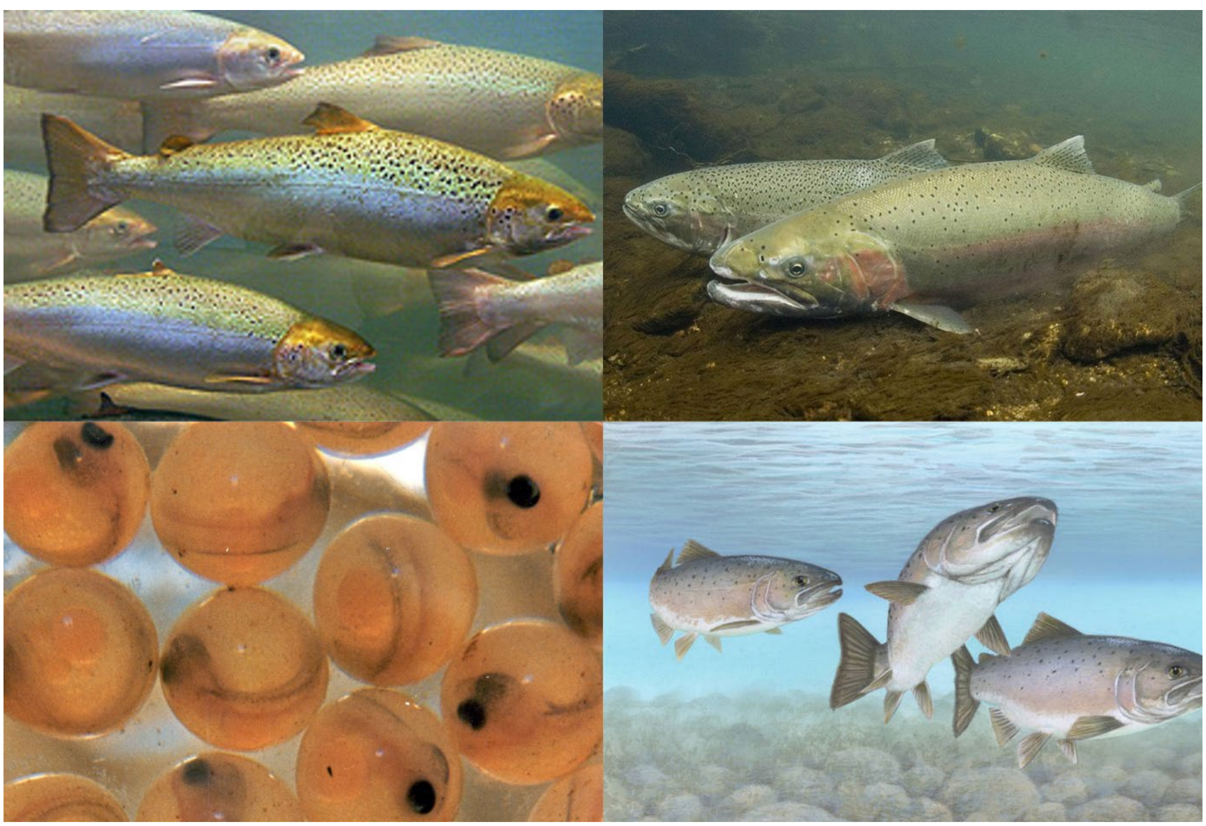
# Hazard Identification: Viral Haemorrhagic Septicemia Virus (VHSV)



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# Introduction

USDA APHIS VS CEAH was asked to generate a Hazard Identification for viral haemorrhagic septicemia virus (VHSV). Hazard identification is a process used to identify hazards (biological, chemical, or physical agents) that may result in adverse consequences in susceptible animal or human populations.[1] The hazard identification process is also used to identify hazards that may be associated with importation of a commodity (live animals, products of animal origin, genetic material, biological products, or pathological material).[1] The hazard must be relevant to the imported and susceptible species, and it must be determined if the hazard is a) present in exporting countries; b) present or absent in the importing country; and c) a notifiable disease or disease that is subject to control or eradication in the importing country.[1]

Subjects within the scope of this document include a description of the hazard (VHSV), identification of susceptible fish species and the geographic distribution of the hazard, and a summary of the epidemiology of the hazard. To conduct this hazard identification, we referenced World Organisation for Animal Health (WOAH) resources, subject matter expert consultation, and available published data and literature relative to VHS epidemiology. Knowledge and data gaps were present that affected complete evaluation of some tenets of this hazard identification.

This document follows:

- The WOAH Handbook on Import Risk Analysis for Animals and Animal Products import risk analysis framework, which is accessible via a link in Appendix, Table 1.[2]
- WOAH criteria for determination of host species susceptibility as described in the WOAH Aquatic Animal Health Code and the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases (Appendix, Table 1).[1, 3]
    - Fish species described in published literature that do not meet these criteria or in which infection was inferred using diagnostic methods that are not validated according to WOAH protocols are not included in this assessment. Briefly, species susceptibility to a pathogen requires that:
        - the experimental transmission is consistent with natural pathways of infection,
        - the pathogen is adequately identified, and
        - the presence of the pathogen in the host constitutes an infection.
- Standards in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (Appendix, Table 1)[4] regarding improvement of animal health welfare, safe international trade in aquatic animals and their products, and diagnostic approaches to disease diagnosis.
- The understanding that epidemiologically, disease occurs as an interaction occurring in environmental spaces (natural and anthropogenically influenced or derived) where host and pathogen tolerance limits for essential biotic (living) and abiotic (nonliving) environmental factors overlap.[5, 6, 7, 8]

- Definitions of animal agriculture biosecurity as:
    - A series of management steps and practices that identify, prevent, control, and mitigate introduction and spread of pathogens in an animal population, and spread of pathogens to other susceptible populations.[9]
    - Measures based on current epidemiological information and understanding of relevant knowledge and data gaps.[10, 11, 12, 13]

Subjects that are not within the scope of this document include an assessment of potential entry and exposure pathways and summaries of likelihood, uncertainty, consequence, and overall risk. This document is intended for internal USDA APHIS VS use and distribution to external stakeholders.

# Key Findings

- Viral haemorrhagic septicemia virus (VHSV) is an aquatic rhabdovirus that can cause severe disease in freshwater and marine fish.
- VHS is a reportable disease in the United States.
- VHS is a WOAH-listed notifiable disease.
- Host susceptibility to infection varies by fish species, and VHSV genotype (I, II, III, IV).
- VHSV genotypes I, II, and III are not present in the United States.
- Rainbow trout are highly susceptible to VHSV genotype I (VHSV-I).
- VHSV-III has recently been detected in farmed steelhead trout in Europe.
- VHSV genotypes IVa and IVb are endemically present in the United States.
    - VHSV-IVa is present in the Pacific Northwest. It has been associated with disease outbreaks in marine fish species. Salmonid fish are susceptible to infection but exhibit low levels of susceptibility.
    - VHSV-IVb is present in the Great Lakes region. Approximately 30 freshwater fish species are susceptible to this genotype. Salmonids can become infected but exhibit low levels of susceptibility.
- VHSV genotype IVc is endemically present along the North Atlantic coast (New Brunswick and Nova Scotia, Canada).
- Some fish species appear capable of serving as long-term reservoir hosts.
- There are no treatments for VHSV.
- USFWS has import regulation requirements for VHSV per Title 50.
- USDA APHIS has no regulatory requirements relative to VHSV.
- State regulations relative to VHSV vary by State.
- Some countries that trade with the United States have import requirements specific to VHSV.

# Virus Haemorrhagic Septicemia Virus

## Introduction

Viral haemorrhagic septicemia (VHS) is a viral disease of wild and farmed marine and freshwater telost (bony) finfish. The etiological agent is viral haemorrhagic septicemia virus (VHSV; genus *Novirhabdovirus*, family *Rhabdoviridae*)(Appendix, Table 1).[4, 14] VHS has been associated with high mortality (greater than 90 percent) disease outbreaks in farmed and wild fish globally.[15] This disease is described in peer-reviewed published literature as a disease with potential to cause serious economic and environmental impacts to aquaculture, indigenous susceptible fish species, and natural resources.[16, 17]

This virus is an enveloped, bullet-shaped, non-segmented, negative-sense, single-stranded RNA virus.[4, 14, 18] The linear genome encodes six proteins (e.g. glycoprotein G, matrix protein M, non-virion protein NV, nucleoprotein N, phosphoprotein P, and RNA polymerase protein L).[4, 18] The genes are separated by conserved gene junctions with di-nucleotide gene spacers.[18] The NV protein is unique to the genus and can suppress apoptosis in early stages of viral infection.[18, 19] The other five proteins are common among rhabdoviruses.[18] The G glycoprotein comprises the neutralizing surface antigen, and is a key component of host cellular receptor adhesion and insertion, viral replication, evasion of host immune responses, infection emergence, and cross-species transmission.[4, 18] Phylogenetic analyses infers that the virus is of marine origin, and that there are four geographically distributed genotypes (I–IV), and several genotype I and IV sublineages (Table 1).

Table 1. Viral haemorrhagic septicemia virus (VHSV) genotypes and sublineages. [4, 14, 16, 18, 20, 21, 22, 23, 24, 25]

| Genotype 1 | |
|---|---|
| This genotype is found in Europe and is comprised of six sublineages that correspond to specific geographic regions. This genotype is capable of infecting multiple species of fish. It is highly virulent in freshwater farmed rainbow trout and is often associated with high mortality disease outbreaks (up to 100 percent in fry). Phylogenetic data suggests this genotype originated from wild marine fish, with several host species jumps prior to adaptation to rainbow trout. | |
| Sublineage Ia | Found in terrestrial freshwater bodies in continental Europe. It was the first sublineage associated with the European aquaculture industry (freshwater farmed rainbow trout), and it continues to be the primary isolate associated with disease outbreaks in that species. This sublineage can be further divided into VHSV-Ia-1 and VHSV-Ia-2, each with distinct geographic distributions. |
| Sublineage Ib | The prevalent genotype in marine environments in Northern Europe (e.g., the Baltic Sea, Kattegat, Skagerrak, the North Sea, and the English Channel, and as far north as latitude 70°N in Norway). This sublineage is detected in wild marine fish but has not been associated with clinical disease outbreaks. In 1998 and 2000, evidence of transfer between wild fish and farmed steelhead trout was reported in Sweden. Historically, there was a single foreign introduction in Japan. |
| Sublineage Ic | Is found in freshwater bodies in continental Europe, including in mainland lakes of Germany, Austria, and Denmark. |

| | |
|---|---|
| Sublineage Id | Found in marine and freshwater bodies extending from Scandinavia to the Baltic Sea. Has been detected in farmed rainbow trout reared in fresh- and brackish-water in Norway and Finland. Experimentally, this sublineage is pathogenic but less virulent than sublineage VHSV-Ia to rainbow trout. |
| Sublineage Ie | Is described in the literature as a marine isolate from the Baltic Sea. It has been isolated from farmed rainbow trout in freshwater and marine environments. |
| Genotype II | |
| This genotype is present in wild marine fish in the Baltic Sea, including the Gulf of Bothnia and Gulf of Finland. It has also been detected in lamprey in Gulf of Bothnia tributary rivers. This genotype has not been associated with disease outbreaks or mortalities. | |
| Genotype III | |
| This genotype has been detected in wild and farmed marine fish in the North Atlantic Sea from the Flemish Cap near Newfoundland to Norway, and the North Sea (the British Isles, Skagerrak, and Kattegat). In 2007, this genotype was associated with a disease outbreak in farmed steelhead trout in Norway. | |
| Genotype IV | |
| This genotype is found in North America (Atlantic Ocean coastal areas, the Great Lakes region, and the Pacific Northwest). Detections have also been reported in Asia (Japan, South Korea). This genotype contains four sublineages, which can be highly virulent in susceptible marine and freshwater fish species (mortalities ranging from 20 percent to 80 percent in some outbreaks). Salmonids are susceptible to infection; however, the virulence of this genotype and its sublineages are low and clinical disease is rare. | |
| Sublineage IVa | Has been detected in wild marine finfish in the Pacific Ocean waters of western North America from Alaska to California, and in Japan and South Korea. Virulence is variable among fish species. Infected fish may be asymptomatic or may exhibit clinical signs of disease. Pacific and Atlantic salmon, rainbow trout and steelhead trout are susceptible to infection; however infection rates are low and pathogenicity is minimal.  This genotype is sporadically detected in Pacific salmon and has historically been detected in farmed Atlantic salmon in the Pacific Northwest. |
| Sublineage IVb | Is endemically present in freshwater fish in the Laurentian Great Lakes and associated lakes and rivers in North America. It has a broad host range and has been associated widespread epidemics and large die-offs in numerous fish species. |
| Sublineage IVc | Is present in North American Atlantic coastal and brackish estuarine waters of New Brunswick and Nova Scotia, Canada. It does share some genetic sequence homology with genotype IVb. |
| Sublineage IVd | Was recently identified in Iceland in wild and sea-farmed lumpfish. |

VHS is a reportable disease in the United States, is a WOAH listed notifiable disease[4, 26] and is included on the USDA APHIS National Animal Health Reporting System (NAHRS) list and the USDA APHIS National List of Reportable Animal Diseases (NLRAD)(Appendix, Table 1).[26, 27] State and Federal authorities should be contacted upon suspicion or detection of VHS (Appendix, Table 1). Information relevant to the importation of fish species susceptible to VHSV is located in the Appendix, Regulatory Information Associated with Salmonid Aquaculture.

## Susceptible Fish Species

Fish species identified by WOAH as susceptible to VHSV are summarized in Table 2.[4, 20]

Table 2. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to viral haemorrhagic septicemia virus (VHSV).

| Genus and Species | Common Name | Associated Genotypes | | | | |
|---|---|---|---|---|---|---|
| *Coregonus lavaretus* | Common whitefish | Ia | | | | |
| *Esox Lucius* | Northern pike | Ia | | | | IVb |
| *Oncorhynchus mykiss* | Rainbow trout | Ia-e | | III | | IVb |
| *Oncorhynchus mykiss X Oncorhynchus kisutch hybrids* | Rainbow trout X coho salmon hybrids | Ia | | | | |
| *Salmo marmoratus* | Marble trout | Ia | | | | |
| *Salmo trutta* | Brown trout | Ia-b | | | | |
| *Salmo salar* | Atlantic salmon | Ia-b | II | III | IVa | |
| *Salvelinus namaycush* | Lake trout | Ia | | | IVa | IVb |
| *Thymallus thymallus* | Grayling | Ia | | | | |
| *Clupea harengus* | Atlantic herring | Ib | | III | | |
| *Gadus morhua* | Atlantic cod | Ib | | III | | |
| *Limanda limanda* | Common dab | Ib | | | | |
| *Micromesistius poutassou* | Blue whiting | Ib | | III | | |
| *Platichthys flesus* | European flounder | Ib | | | | |
| *Pomatoschistus minutus* | Sand goby | Ib | | | | |
| *Scophthalmus maxima* | Turbot | Ib | | III | | |
| *Sprattus sprattus* | European sprat | Ib | | | | |
| *Trisopterus esmarkii* | Norway pout | Ib | | III | | |
| *Alosa immaculata* | Pontic shad | Ie | | | | |
| *Engraulis encrasicolus* | European anchovy | Ie | | | | |
| *Gaidropsarus vulgaris* | Three-bearded rockling | Ie | | | | |
| *Mullus barbatus* | Red mullet | Ie | | | | |
| *Merlangius merlangus* | Whiting | Ie | | | | |
| *Raja clavate* | Thornback ray | Ie | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Trachurus mediterraneus* | Mediterranean horse mackerel | Ie | | | | | | |
| *Uranoscopus scaber* | Atlantic stargazer | Ie | | | | | | |
| *Lampetra fluviatilis* | River lamprey | | II | | | | | |
| *Centrolabrus exoletus* | Rock cook wrasse | | | III | | | | |
| *Ctenolabrus rupestris* | Goldsinny wrasse | | | III | | | | |
| *Labrus bergylta* | Ballan wrasse | | | III | | | | |
| *Labrus mixtus* | Cuckoo wrasse | | | III | | | | |
| *Pleuronectes platessus* | European plaice | | | III | | | | |
| *Solea senegalensis* | Senegalese sole | | | III | | | | |
| *Symphodus melops* | Corkwing wrasse | | | III | | | | |
| *Ammodytes hexapterus* | Pacific sand lance | | | | IVa | | | |
| *Clupea pallasii pallasii* | Pacific herring | | | | IVa | | | |
| *Cymatogaster aggregata* | Shiner perch | | | | IVa | | | |
| *Danio rerio* | Zebra fish | | | | IVa | | | |
| *Gadus macrocephalus* | Pacific cod | | | | IVa | | | |
| *Oncorhynchus kisutch* | Coho salmon | | | | IVa | | | |
| *Oncorhynchus tshawytscha* | Chinook salmon | | | | IVa | IVb | | |
| *Paralichthys olivaceus* | Bastard halibut | | | | IVa | | | |
| *Sardinops sagax* | South American pilchard | | | | IVa | | | |
| *Scomber japonicus* | Pacific chub mackerel | | | | IVa | | | |
| *Thaleichthys pacificus* | Eulachon | | | | IVa | | | |
| *Ambloplites rupestris* | Rock bass | | | | | IVb | | |
| *Ameiurus nebulosus* | Brown bullhead | | | | | IVb | | |
| *Aplodinotus grunniens* | Freshwater drum | | | | | IVb | | |
| *Coregonus artedii* | Lake cisco | | | | | IVb | | |
| *Coregonus clupeaformis* | Lake whitefish | | | | | IVb | | |
| *Dorosoma cepedianum* | American gizzard shad | | | | | IVb | | |
| *Esox masquinongy* | Muskellunge | | | | | IVb | | |
| *Lepomis gibbosus* | Pumpkinseed | | | | | IVb | | |

| | | | | |
|---|---|---|---|---|
| *Lepomis macrochirus* | Bluegill | IVb | | |
| *Micropterus dolomieu* | Smallmouth bass | IVb | | |
| *Micropterus salmoides* | Largemouth bass | IVb | | |
| *Morone americana* | White perch | IVb | | |
| *Morone chrysops* | White bass | IVb | | |
| *Morone saxatilis* | Striped bass | IVb | IVc | |
| *Neogobius melanostomus* | Round goby | IVb | | |
| *Notropis atherinoides* | Emerald shiner | IVb | | |
| *Notropis hudsonius* | Spottail shiner | IVb | | |
| *Perca flavescens* | Yellow perch | IVb | | |
| *Pimephales notatus* | Bluntnose minnow | IVb | | |
| *Pimephales promelas* | Fathead minnow | IVb | | |
| *Pomoxis nigromaculatus* | Black crappie | IVb | | |
| *Sander vitreus* | Walleye | IVb | | |
| *Fundulus heteroclitus* | Mummichog | | IVc | |
| *Gasterosteus aculeatus* | Three-spine stickleback | | IVc | |
| *Cyclopterus lumpus* | Lumpfish | | | IVd |

## Geographic Distribution

VHSV has been reported in fish present in marine and freshwater bodies throughout the Northern Hemisphere (Northern Europe, North America, and North Asia). Distributions of VHSV and the various sublineages are summarized in Table 1. Countries reporting presence via the WOAH World Animal Health Information System (WAHIS, Appendix, Table 1) database for years that data is available (2005–2022) include Austria, Belgium, Canada (British Columbia, Newfoundland, Nova Scotia, Ontario, and Quebec), Croatia, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Iceland, Iran, Italy, Japan, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, South Korea, Sweden, Switzerland, Turkey, the United Kingdom (England and Scotland), and the United States.[28] VHSV has never been reported in the Southern Hemisphere.[4]

### North America

VHSV-IVa was first identified in the 1980s in Washington State in asymptomatic adult Coho salmon (*Oncorhyncus kisutch*) and Chinook salmon (*Oncorrhynchus tshawytscaha*) returning to hatcheries.[14, 18, 29, 30] Subsequently, the virus has been detected in multiple marine and anadromous fish species (Table 2), including other Pacific salmon (wild and hatchery reared) and farmed Atlantic salmon, that exhibit varying degrees of susceptibility to the virus and clinical signs ranging from asymptomatic infection to epizootic disease outbreaks.[29] Presently, VHSV-Ia is endemically present at low prevalence and intensity throughout the Pacific Northwest from Alaska to California (including British Columbia).[29, 31] The origin of VHSV in this region is unclear. In British Columbia, there are historical reports dating to the 1940s of VHSV-like mortality events in pacific herring and pacific sardine.

VHSV-IVb was first identified in Lake St. Claire, Michigan in 2003. The virus has subsequently been detected in all major water bodies of the Great Lakes system, including the St. Lawrence River and inland lakes in Michigan, New York, Ohio, Ontario, and Wisconsin, and has caused high mortality outbreaks in multiple wild fish species.[14, 17, 18, 32, 33] There have been no detections of VHSV-IVb in freshwater farmed or managed fish in this region. The route of introduction into the Great Lakes has not been definitively identified. Ballast water, natural fish migrations, translocations of fish (including baitfish), and recreational fishing have all been proposed as potential pathways of entry.[34, 35] In the years following introduction, the virus has undergone genetic divergence and diversification among the Upper, Central, and Lower Great Lakes, resulting in declining virulence and occurrence of outbreaks.[25, 36] Presently, it is estimated approximately 30 wild fish species in the Great Lakes region are susceptible to VHSV (Table 2).[35, 37, 38, 39] In 2000, VHSV-IVc was detected in four wild fish species (Table 2), including brown trout (*Salmo trutta*) in the northeastern Atlantic coastline of North America (e.g., New Brunswick and Nova Scotia, Canada).[21, 40]

# Public Health

VHSV is not a zoonosis. There are no threats to human health.

# Epidemiology

In this section, the epidemiology of VHS is summarized. Many factors associated with the epidemiology are not fully described, and there are differences among VHSV genotypes relative to the geographic distribution and host susceptibility to disease. Therefore, generalizations on host susceptibility, environmental characteristics, and pathogen virulence and pathogenicity should interpreted with caution.[41]

## Host Characteristics

Seventy fish species are recognized by WOAH as susceptible to infection with VHSV (Table 2).[4, 20] Comparatively, the number of fish described as susceptible in the peer-reviewed literature approaches 140 species. However, this estimate must be interpreted with caution because it includes species of fish experimentally challenged with VHSV using methods that do not meet WOAH criteria for natural infection (e.g., intraperitoneal injection).[1, 4]

Host factors affecting susceptibility to VHSV infection include fish species, age, overall physiological condition (e.g., immune status, nutritional status, general health, presence of concomitant infection or parasitism, population density), and the presence of certain behaviors (e.g., spawning).[16, 17, 25, 29, 31] Susceptibility has been observed to decrease with age in some fish species, potentially due to acquired immunity from prior VHSV exposures.[29, 31]

According to WOAH, the potential long-term reservoir, carrier, and transmission capacity of susceptible fish species in natural environments are not clearly defined.[4] Subclinical infection at low prevalence rates (1 percent to 17 percent) with host specific genotypes has been observed in some wild marine and freshwater fish species, which suggests that some fish species are capable of serving as long-term reservoir hosts.[16, 31, 42, 43, 44, 45] The duration of the potential carrier status in wild fish is unknown, but has been hypothesized to be lifelong, with virus shedding occurring intermittently.[31, 42, 43, 44] Factors associated with recrudescence of shedding are largely unknown but may be influenced by pathogen, environmental, and host factors.[46]

The duration of viral shedding by experimentally challenged fish varies by species, experimental challenge method (bath immersion *versus* intraperitoneal injection), virus genotype, and challenge dose. Periods of viral shedding in published literature range from 4 days to 60 weeks.[24, 29, 38, 39, 45, 47, 48, 49, 50] Experimentally it has been demonstrated some carrier fish resume shedding after exposure to stress (e.g., handling stress) for up to 15 weeks.[38, 42] Greater rates of shedding have also been documented as water temperatures decreased from 15 °C/59 °F to 8 °C/46.5 °F.[42, 51]

Host susceptibility to infection varies by fish species and VHSV genotype and sublineage (Table 1).[4] Some hosts are susceptible to infection with one genotype, while others are susceptible to multiple genotypes and/or sublineages.[4, 14, 29, 51, 52] Nine salmonid fish are identified by WOAH as variably susceptible to infection by different VHSV genotypes (Table 3).[4, 20]

Table 3. Susceptibility of specific salmonid species to infection with various viral hemorrhage septicemia virus (VHSV) genotypes, as identified by the World Organisation for Animal Health (WOAH)[4, 20]

| Genus and species | Common Name | Associated genotypes | | | | |
|---|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | Rainbow/Steelhead trout* | Ia-e | | III* | | IVb |
| *Oncorhynchus mykiss X Oncorhynchus kisutch hybrids* | Rainbow trout X coho salmon hybrids | Ia | | | | |
| *Salmo marmoratus* | Marble trout | Ia | | | | |
| *Salmo trutta* | Brown trout | Ia-b | | | | |
| *Salmo salar* | Atlantic salmon | Ia-b | II | III | IVa | |
| *Salvelinus namaycush* | Lake trout | Ia | | | IVa | IVb |
| *Thymallus thymallus* | Grayling | Ia | | | | |
| *Oncorhynchus kisutch* | Coho salmon | | | | IVa | |
| *Oncorhynchus tshawytscha* | Chinook salmon | | | | IVa | Ivb |

## *Rainbow trout (freshwater)*

Freshwater rainbow trout are susceptible to infection by VHSV Ia–Ie.[4, 20, 22] The pathogenicity of the subtypes in this species is variable.[14, 18] For example, VHSV-Ia is highly virulent and has been associated with severe disease outbreaks in Europe.[16, 21] Published literature states the pathogenicity of VSHV-Ib–e is low in this species.[16, 23] VHSV-II and VSHV-III are marine isolates. Freshwater rainbow trout are unlikely to be exposed to these genotypes under natural conditions and exhibit low susceptibility to infection experimentally.[23, 53] Rainbow trout are relatively resistant to VHSV-Iva–c; however, low rates of susceptibility to infection and pathogenicity have been observed experimentally.[21, 54]

### *Steelhead trout (marine-farmed and anadromous rainbow trout)*

In general, steelhead trout exhibit the same susceptibility to infection with VHSV genotypes observed in freshwater rainbow trout. However, in 2007, disease outbreaks caused by VSHV-III occurred in marine-farmed steelhead in Norway.[21, 53] Subsequently, this genotype has been detected in net-pen reared steelhead trout in other regions (Finland).[53, 55]

### *Atlantic salmon*

Atlantic salmon are susceptible to infection with VHSV-I–III but are refractory to development of clinical disease.[4, 20, 56] VHSV-IVa has been periodically detected in farmed Atlantic salmon in Canada (British Columbia) since 1996, and the in United States (Washington State, when Atlantic salmon farming was present).[21, 29, 50] Detections have occurred in apparently healthy fish during routine surveillance and in association with low-level clinical disease and mortality.[29] Detections often are concurrent to detections in pelagic fish species found near the affected net pens.[29] Natural infection with VHSV-IVb has not been described. Experimentally, Atlantic salmon demonstrate susceptibility to infection with VHSV-IVb following experimental challenge via intraperitoneal injection (challenge dose $10^6$ pfu (plaque forming units)/fish). Clinical and gross pathological signs of disease and low rates of mortality were observed in some challenged fish (54 percent and 2 percent, respectively). Variable levels of virus ($10^1$–$10^7$ mean viral RNA copies detected/µg total RNA in each fish) were detectable by quantitative reverse transcription polymerase chain reaction (qRT-PCR) up to day 49 post-challenge (termination of the study).[57] However, results of this study should be interpreted with caution, given that the challenge method used does not meet WOAH criteria for natural infection.[1, 4] Review of the literature did not find publications describing detection of VHSV-IVc in farmed Atlantic salmon on the East coast of the United States or experimental studies exploring the susceptibility of Atlantic salmon to this genotype.

### *Pacific salmonids*

Review of the literature did not identify reports of natural infection of Pacific salmonids with VHSV-I–III, and experimental challenge studies examining the susceptibility are generally lacking. Emmenegger et al. (2013) did report that Chinook salmon exhibited susceptibility to VHSV-Ia and VHSV-IVb following experimental challenge by intraperitoneal injection; however, these results should be interpreted with caution.[1, 4, 21] Pacific salmonids are susceptible to infection with VHSV-IVa, but typically do not develop clinical disease.[21, 29, 52] Infections are intermittently detected during routine surveillance of hatchery reared fish returning to freshwater to spawn.[29] Natural infection with VHSV-IVb and VHSV-IVc have not been reported. Limited published research is available describing VHSV-IVb experimental challenge studies in Pacific salmon. Review of the literature did not find publications describing experimental studies exploring the susceptibility of Pacific salmon to VHSV-IVc.

### *Other salmonid fish*

Susceptibility to VSHV has been reported in other salmonid fish. Lake trout are reported susceptible to infection with VHSV-Ia, -IVa, and -IVb.[21, 29, 54] Brown trout are reported as susceptible to infection with VHSV-Ia–b,[4, 20] and Gagne et al. (2007) describes isolation of VHSV-IVb from brown trout mortalities.[40]

### *Other North American fish species*

In the Pacific Northwest, several marine fish species (e.g., pacific hake, pacific herring, pacific sand lance, Walleye Pollock, yellow perch) are highly susceptible to VHSV-IVa infection and can develop clinical disease leading to high mortality outbreaks (Table 1).[29] Published literature states that approximately 30 freshwater fish species in the Great Lakes Region are susceptible to infection with VHSV-IVb (Table 1).[32, 36, 39] VHSV-IVc has been detected in marine/estuarine fish (e.g., mummichog, stickleback, brown trout, and striped bass) in the Atlantic coastal region of North America (e.g., New Brunswick and Nova Scotia, Canada).[21, 40, 58]

### *Other animals*

VHSV has been detected in other aquatic animal species (e.g., amphipods; *Hyalellea* spp. and *Dipporeia* spp., common snapping turtle, leeches, northern map turtle, and a water flea; *Moina macrocopa*).[46, 59, 60, 61] However, capability of these species, piscivorous birds, or terrestrial wildlife that frequent water and/or scavenge fish to serve as transport or transmission vectors in natural environments has not been definitively proven.

## Environmental Characteristics

Environmental factors affecting the length of time that VHSV remains viable in fresh or seawater include, but may not be limited to microbial content, organic load, water salinity and temperature, and exposure to ultraviolet light.[29, 46, 51, 62, 63] The presence of bacteria decreases virus stability. Experimentally, virus survival is reduced when bacteria are present in the water and increases when water is autoclaved and filtered through a 0.22 µm membrane.[4, 62] Experimentally, virus stability improves when organic materials such as aqueous proteins (e.g., ovarian fluids, blood products, bovine serum) are added to water samples.[4, 51] VHSV is capable of surviving for several weeks in soil-based sediments; however, survival is affected by the sediment composition.[64] Experimentally, the virus is capable of surviving on stainless steel surfaces for approximately 42 days at temperatures ranging from 4–25 °C/39–77 °F.[64] On plastic surfaces the virus can survive for 6–21 days at temperatures between 4–37 °C/39.2–98.6 °F.[54, 64] Environmental pH affects virus characteristics. The virus is stable at low pH (5.0); however, replication does not occur until the pH ranges from 7.4–7.8.[14]

All VHSV genotypes appear to be more stable in freshwater *vs* saltwater.[14, 31, 41, 43, 44, 55, 63] Experimentally, in raw freshwater, the virus can persist for 13 and 28–25 days when stored at 15 °C/60 °F and 4 °C/39.2 °F, respectively.[4, 24, 41, 55] In filtered freshwater stored at 4 °C/39.2 °F, viability can be maintained for over one year.[4, 55] In seawater stored at 15 °C/60 °F virus viability is reduced by 50 percent after 10 hours and inactivation (99.9%) occurs after 4 days.[4, 24, 41, 55]

Water temperature affects host factors (e.g., infection rate, development and duration of clinical disease, virus shedding, longer virus persistency in tissues, and mortality) and VHSV characteristics (pathogenicity, replication, and survival in the environment).[29, 51, 65, 66] In marine environments, VHSV outbreaks are most commonly observed in winter and spring months when water temperatures are fluctuating or rising.[51] In inland freshwater environments, most outbreaks occur in spring and early summer when water temperatures are rising and spawning is occurring.[17] The optimal water temperature is consistent among genotypes and ranges from 4–15 °C/39.2–59 °F.[67, 68, 69] Within this range, optimal viral viability and pathogenicity, and replication occurs at 9–12 °C/48.2–53.6 °F and 14–15 °C/57.2–59 °F, respectively.[14, 17, 24, 25] At temperatures below or above the optimal range, virus survival is lower, host transmission and infection rates decrease, and if disease occurs, the course is short with low mortality.[51, 14, 16, 41] Natural outbreaks typically do not occur once water temperatures reach 18–20 °C/64.4–68 °F.[14, 17, 24, 55] VHSV can be grown in cell cultures at temperatures up to 20 °C/68 °F. Virus remains infectious for long periods in cell culture media and fish tissues frozen at -20 °C/-4 °F or lower, and can withstand freeze-thaw cycles.[4, 41, 68] The virus undergoes inactivation when exposed to ultraviolet irradiation (sunlight).[51, 55, 64] Other water quality characteristics (i.e., dissolved oxygen) may affect the occurrence of VHS disease and stability of the virus as well.

## Pathogen Characteristics

VHSV genotypes are cumulatively capable of infecting a large number of marine and freshwater fish species (Table 1). Virulence and pathogenicity is host specific and varies among genotypes.[16, 29, 32, 51, 53, 70] The length of time that VHSV may remain viable in freshwater and marine environments is dependent upon the amount of virus shed into the environment by infected hosts, the density of infected hosts present in the environment, genotype, and environmental factors affecting virus stability.[16, 29, 51]

In general, VHSV isolated from marine species causes low to no mortality in freshwater and anadromous rainbow trout and other salmonids, and vice versa.[4, 51] However, genetic diversification appears to have allowed some genotypes to make species jumps from marine to freshwater and anadromous fish.[16, 22, 71] In Europe, VHSV-Ia is hypothesized to have evolved from a marine ancestor in association with the historical practice of including unpasteurized raw marine fish in the diets of farmed rainbow trout.[22, 72] In 2007, sequence analysis of the VHSV genotype III isolated from a VHS outbreak in farmed steelhead trout showed that the virus responsible for the outbreak was closely related to marine strains that were not considered pathogenic for trout.[16, 73]

The infectious dose and level of exposure required to establish infection in susceptible hosts is dependent upon host susceptibility. For example, in experimental challenge studies, pacific herring exhibited high susceptibility to low challenge doses of VHSV-IVa administered via bath immersion ($10^1$ pfu/mL VHSV for 24 hours in seawater).[29, 47] In other studies, Chinook, Coho, Pink, and Sockeye salmon exhibited resistance to VHSV-IVa infection following high dose experimental challenge via freshwater bath immersion ($10^3$ and $10^5$ pfu/mL for one hour).[29, 74]

## Transmission

The transmission dynamics associated with VHSV are not fully described. VHSV is transmissible to fish of all ages and can occur bi-directionally from wild to farmed fish.[24, 29] Transmission rates are affected by virus genotype, viral shedding rates by individual fish and the fish population per unit time, the prevalence of infection in the affected population over the course of the disease outbreak, the minimum infectious dose required to elicit infection in susceptible fish, virus dilution and movement in the water column, and other host, environmental and pathogen factors.[12, 64]

Direct transmission occurs via exposure to virus shed into the water in the mucus, urine, feces, and reproductive fluids (milt and ovarian fluid) of infected fish.[25, 29, 30, 55, 67] Portals of entry for the virus are thought to include the gills, wounds on the body, and potentially the gastrointestinal tract.[4, 42] Potential sources of waterborne virus include VHSV-infected wild fish, VHSV-positive fish farms and hatcheries, and processing plant effluent, liquid, and other wastes.[10, 12, 34, 55]

Oral transmission of virus in infected prey, bait, baitfish, or feed has been described in the literature as potential transmission pathways.[29, 46, 75, 76] Experimentally, VHSV has been transmitted to naïve fish via feeding of infected fish and fish tissues.[76, 77] Anecdotally, in Denmark in 1985, the number of rainbow trout farms experiencing VHSV outbreaks declined after incorporation of marine fish meal in rainbow trout diets was prohibited, suggesting that VHSV was being orally transmitted via the feed.[18] Vertical transmission has not been definitively proven. However, VHSV is present in the reproductive fluids (ovarian fluid and milt) which can lead to contamination of the surface of eggs during spawning.[4, 25, 41, 51, 78, 79]

Indirect transmission may occur via fomites (e.g., aquaculture equipment, boats, ballast water, fishing tackle, and other materials).[18, 51, 54] Anthropogenic movement and translocation of live fish, eggs, and gametes are considered primary methods of VHSV introduction into Europe, Iran, and the Great Lakes region of the United States.[21] Movement and use of baitfish has been also been suggested as a probable pathway of introduction of VHSV into the Great Lakes and other inland waters.[76] Transmission via animal vectors has been suggested but not definitely confirmed.[24] Experimentally, various freshwater turtle species have been found capable of harboring VHSV for up to 20 days after feeding on fish experimentally infected with VHSV.[24, 69] Virus has been detected in invertebrates such as leeches (*Mzyobdella lugubris*), amphipods (*Diporeia* spp.) and cladocerans (*Moina macrocopa*) as well.[59, 60, 80] Fish-eating birds and wildlife that access areas where VHSV-infected fish are present may be capable of introducing VHSV into areas by acting as mechanical vectors.

## Clinical Signs and Pathogenicity

VHS should always be considered a disease rule out when suspect clinical signs are observed in susceptible fish species found in environmental conditions and geographic areas where VHS occurs.[41] Primary portals of entry are thought be the epithelial cells of the gills, skin, and gastrointestinal tract.[15, 50] Target organs include the brain, endothelial cells of blood vessels and heart, gills, hematopoietic tissues in the spleen and kidney, fibroblasts in the dermis and at the base of fins, liver, and muscle.[14, 24, 41, 51] The incubation period is water temperature dependent. When water temperatures range between 1–12 °C/34–54 °F the incubation period is 1–2 weeks. In colder and warmer water temperatures the incubation may be shortened or extended up to four weeks, respectively.[14]

Clinical signs are not pathognomonic and vary among individual fish and fish species.[17, 18, 50] Some fish exhibit no clinical signs, while others develop acute, chronic, or neurological manifestations of disease.[14, 17, 24, 43, 46] Clinical signs associated with acute infection include abdominal distention, anorexia, darkened skin color, exophthalmia (popeye), lethargy, petechial hemorrhages in the eyes, internal organs, musculature and skin, and rapid onset of high mortality with no clinical signs. Affected fish may exhibit abnormal behaviors (e.g., abnormal swimming, crowding at enclosure edges or water outlets, do not attempt to escape netting, flashing, isolation from schools/shoals, and spiraling).[4, 14, 46] Rainbow trout specifically may present with darkened color, exophthalmia, lethargy, and may stay near the edges or the outlet of enclosures.[4] Skin lesions are frequently described as common clinical signs in cod, haddock, and herring.[72, 81, 82] In freshwater fish and halibut infected with VHSV-IVb pale gills are a common clinical sign.[24, 83] Clinical signs observed in chronic infection may include symptoms noted in acute infection, and anemia (pale gills), uncoordinated and/or spiral swimming, and significant cumulative mortality over time.[14, 24, 46] Fish affected by the neurological form exhibit severe abnormal swimming behavior (flashing and spinning), and low cumulative mortality.[14, 46] Fish that survive VHSV infection develop a strong antibody response.[17, 24]

## Morbidity and Mortality

Mortalities typically begin prior to or shortly after clinical signs appear.[14, 41] Morbidity and mortality rates vary depending on environmental, pathogen, and host factors.[14, 16, 41] For example, juvenile rainbow trout infected with VHSV-Ia develop severe disease with mortality rates approaching 100 percent. Comparatively, mortality rates associated with VHSV-Ia infection and disease in older fish are lower (25 percent to 75 percent).[70, 72] Infection of rainbow trout and other salmonids (Pacific salmonids, Atlantic salmon) with VHSV-IVa and VSHV-IVb results in zero to low (10 percent) cumulative mortality while high mortality (up to or greater than 90 percent) outbreaks may be observed in wild marine and freshwater fish species, respectively.[15, 72, 84, 85] Chronic fish losses resulting in low daily but high cumulative mortality rates are observed at low water temperatures (less than 5 °C/41 °F).[24] When water temperatures range from 9–12 °C/48.2–53.6 °F, mortality rates increase (up to or greater than 90 percent).[14, 86] Mortality rarely occurs once water temperatures reach or exceed 15 °C/59 °F.[14, 24, 86]

## Treatment

There are no treatments for VHS. There is currently no commercial vaccine available. Resistance to VHSV has not been established. However, potential genetic variation in rainbow trout for resistance to VHSV infection has been demonstrated.[87, 88]

## Diagnostic Testing

A presumptive diagnosis can be made based on clinical signs, and gross and histopathological findings.[24] Laboratory confirmation of infection is required for definitive diagnosis.

Gross pathological lesions include ascites, and/or edema in the peritoneal cavity, exophthalmia, gill pallor, hemorrhage in the eyes and under the skin around the pectoral and pelvic fins, hyperemia, lack of food in the gastrointestinal tract, multifocal hemorrhages, swelling or necrosis of the kidney, and the liver may be pale, mottled, or contain multifocal hemorrhages. The dorsal muscles, internal organs, and skin should be examined for petechial hemorrhages. Fish affected by the chronic and neurological forms of the disease may exhibit no gross pathological signs.[4, 17, 24, 41]

Histopathological findings include extensive focal necrosis and degeneration in kidney, liver, and spleen, hemorrhagic myocarditis, hemorrhage and necrosis in the thymus and/or pancreas, and widespread subtle to severe vasculitis in internal organs and the skeletal muscle.[4, 14, 24, 41]

Degeneration of peripheral nerves and optic nerves may be observed in fish affected by the neurological form of the disease.[24] Distribution of the lesions can vary dependent on the VHSV genotype and the fish species. Rainbow trout infected with VHSV-Ia typically have necrotizing lesions in the kidney and liver, but may also have lesions in the brain, heart, spleen, and other tissues.[41] Severe lesions in the myocardial tissues with accompanying changes in the liver and hematopoietic tissues were the most prevalent histopathological findings in Great Lakes freshwater fish infected with VHSV-IVb and turbot in Asia infected with VHSV-Ib.[41, 89, 90] If histological changes are absent, viral proteins may be visualized by immunohistochemical staining.[24, 50, 89]

Available diagnostic assays include antibody-based assays (e.g., enzyme linked immunosorbent assay [ELISA], indirect fluorescent antibody testing [IFAT], reverse transcription loop-mediated isothermal amplification [LAMP]), molecular assays [RT-PCR, qRT-PCR]) followed by sequencing, transmission electron microscopy (TEM), and virus isolation (VI) in cell culture.[41] Because VHS is WOAH-listed reportable disease, specific diagnostic assays, are required for disease confirmation.[4, 20] WOAH recommended protocols for targeted surveillance, presumptive, and confirmatory diagnosis sampling, sample submission and diagnostic testing are described in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (Appendix, Table 1).[4, 20] In the United States, confirmatory testing at the National Veterinary Service Laboratory (NVSL) is required following first detections. Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL.[26] Relevant information, including sample submission instructions, may be accessed via the NVSL links in Appendix, Table 1.

## Prevention and Control

In the context of animal agriculture, "biosecurity" is defined as a series of management steps and practices implemented to identify, prevent, control, and mitigate the introduction of infectious pathogens into an animal population, spread of the pathogen within that population, and spread of the pathogen to other susceptible populations. Measures should be based on current epidemiological information and understanding of relevant knowledge and data gaps.[10, 11, 12] Risk factors associated with the introduction and spread of VHSV in wild and cultured fish populations, and measures to prevent and control the introduction, spread, and impacts of VHSV are well described in published peer-reviewed literature, and include but are not limited to: [10, 11, 32, 34, 55, 91]

- Presence of wild VHSV-infected and/or VHSV-susceptible fish populations in waterbodies near hatcheries and fish farms.
- Hydrologic connectivity between waterbodies and water sources where VHSV-infected and naïve susceptible fish species (wild and farmed) are present.
- Exposure of susceptible wild and farmed fish species to personnel, fomites, boats, equipment, or fish wastes from known VHSV-positive areas.
- Unregulated translocation or import of live fish, eggs, and gametes from VHSV-endemic areas.
- Lack of appropriate farm biosecurity and best management practices.
- Insufficient regulatory infrastructure for fish health oversight.
- Lack of passive surveillance for VHSV (including genotype identification).

- Proximity of susceptible wild and farmed fish populations to fish processing plants and associated effluent water and other wastes.
- Release of fish farm effluent water that was not treated as required to inactivate VHSV.

Measures to prevent and control VHS introduction, spread, and impacts should be designed to prevent introduction of the virus to a) areas where the disease is not currently present, and b) naïve wild and farmed fish populations. Such measures may include:[10, 11, 32, 34, 41, 55, 91]

- Identification VHSV endemic areas or zones.
- Identification of areas with aquatic environments that are optimal for VHSV introduction.
- Equipment disinfection requirements for aquaculture, fisheries, and recreational fishing.
- Identification VHSV-susceptible (and/or infected) wild fish species in natural water bodies with potential hydrological connectivity to the hatcheries or fish farms.
- Identification of hatcheries and farms that rear VSHV-susceptible species in areas where VHSV is endemically present.
- Restriction of broodstock collection locations, fish culture and stocking locations, and fish transfers.
- Active and passive surveillance for VHSV of wild and farmed fish populations that includes genotype identification.
- Development of databases that allow disease trace back.
- Establishment of VHSV areas or zones and VHSF-free status designations for bait industries, commercial fish processing, fish farms and hatcheries, and recreational fishing areas.
- Implementation of farm biosecurity and best management practices to prevent VHSV introduction, including but not limited to:
    - Use of specific pathogen free stock, including eggs.
    - Quarantine of shipments of live fish, eggs, or gametes.
    - Testing of live fish, eggs, and gametes for VHSV prior to release from quarantine.
    - Implementation of "all in, all out" policies for fish stocks when possible.
    - Use of appropriate personal protective equipment, foot baths, and other personnel biosecurity measures.
    - Use of disinfectants appropriate for inactivation of VHSV.
    - Fallowing of fish rearing structures after disinfection.
    - Use of spring or well water instead of ground water (rivers, streams, lakes).
    - Use of recirculating aquaculture systems (RAS) rather than flow through (FTS) or open water rearing systems.
    - Avoidance of farming practices that create stress (high population densities, overfeeding, poor water quality management).
    - Avoidance of polyculture, especially with VHSV-susceptible species.
    - Treatment of influent water appropriate for inactivation of VHSV.
    - Treatment of effluent water appropriate for VHSV inactivation prior to release.
    - Development of contingency plans, including eradication and movement restrictions, in the event VHSV introduction occurs.

Disinfectants with VHSV efficacy include chlorine, chloroform, ether, formalin, glycerol, hydrogen peroxide (Peroxigard™, Oakville, Canada), iodophors, potassium bisulfate (Virkon

Aquatic® , Syndel, Ferndale, Washington, USA) sodium hypochlorite, and sodium hydroxide.[4, 29, 79, 92] Heat inactivation temperatures described by Bovo et al. (2005) include 30 °C/86 °F for 24 hours, 50 °C/122 °F for 10 minutes, and 70 °C/158 °F for 1 minute.[79] VHSV can also be inactivated by ultraviolet irradiation (280–200 nm wavelength), desiccation, and exposure to pH levels less than 2.4 or greater than 12.2.[14, 41, 79] Newly fertilized and eyed eggs may be disinfected with iodophors.[4, 41, 79, 92] Disinfectants containing high salt concentrations and concentrated ammonium sulphate solutions may not be effective.[4, 54]

In the United States, VHS is a reportable disease that is endemically present in some wild fish populations.[26, 27] Reporting of VHSV detections is required under USDA APHIS NLRAD and WOAH notifiable disease reporting requirements.[4, 20, 26, 27] At present, individual States are responsible for VHS regulatory oversight which may include measures such as import/export controls, VHSV testing and disease-free certification of hatcheries/farms, wild fish surveillance, and public outreach.[17, 29, 37, 93, 94, 95, 96] These requirements vary by State (Appendix, Table 3).[96]

If VHSV is suspected or detected via diagnostic testing the State veterinarian and Federal veterinary officials should be contacted and samples collected and submitted under the guidelines provided by the NVSL (Appendix, Table 1).[97] Control measures utilized by individual States and/or USDA APHIS may include controlling the movements and humane destocking of infected farmed fish, and cleaning, disinfection, and quarantine of affected premises according to WOAH protocols. Internationally, many countries utilize import/export regulations and recommendations to limit or control the risk of VHSV introduction (Appendix, Table 2). A summary of WOAH import/export guidelines specific to VHS, U.S. regulations, and other regulatory information related to aquaculture in the United States is provided in the Appendix, Regulatory Information Associated with Salmonid Aquaculture.

## Summary

VHS is an economically significant disease of susceptible wild fish, and farmed fish reared for commercial, recreational, and restocking purposes. Farmed fish reared in net pens or in land-based FTS farms that utilize surface water (e.g., lakes, rivers, streams) are at greatest risk of exposure to VHSV. Fish reared in land-based FTS or RAS aquaculture systems that utilize ground water are least likely to be exposed. Stringent biosecurity and influent water treatment measures are recommended for all land-based aquaculture systems to decrease the risk of VHSV introduction.[41]

The significance of VHSV is illustrated by its broad host range across all genotypes and persistent prevalence in apparently healthy carrier fish. The historical adaptation of VHSV-I from marine to freshwater fish, the evolution of VHSV-III as a pathogen in steelhead trout, and the more recent adaptation of VHSV-IVb from marine to freshwater fish species and its rapid dissemination throughout the Great Lakes region demonstrates that VHSV genotypes have the potential for environmental and host adaptation with highly impactful outcomes.[16, 21, 33] Thus, assessment of pathways of movement and introduction for indigenous (IVa, IVb, and IVc) and non-indigenous genotypes (I, II, III, and IVd), programs to monitor the epidemiology of VHSV in the United States, and measures to prevent introduction and translocation of VHSV into new areas are important.[21, 29]

## Limitations

In this hazard identification the characteristics and epidemiology of VHSV in susceptible hosts were summarized using available information collected from WOAH resources, subject matter experts, and available published peer-reviewed materials. Knowledge gaps and limitations identified during the data and literature review process included but are not limited to:

- Experimental challenge studies using challenge methods that approximate natural exposure are generally lacking.
- Factors associated with the epidemiology all VHSV genotypes in Atlantic salmon, rainbow/steelhead trout, other salmonid fish are not fully described.
- Factors associated with the epidemiology all VHSV genotypes in marine and freshwater finfish other than salmonids are not fully described.
- All environmental, viral, and host factors associated with VHSV are not fully known.
- Introductory pathways, transmission routes, and risks for land-based aquaculture systems (FTS and RAS systems) rearing susceptible fish species are not fully described.
- Factors related to virus infectivity, persistence, and viability in marine and freshwater environments are not completely understood.
- Factors related to virus infectivity, persistence, and viability in inland aquaculture environments are not completely understood.
- Data on VHSV detections and disease outbreaks in the United States is not collated and/or publicly accessible.
- Research regarding the home range distribution and movement of key carrier and susceptible wild fish species during seasons when environmental factors are optimal for virus shedding and disease spread are lacking.

# Appendix

## Tables

Table 1. Links to resources relevant to viral haemorrhagic septicemia (VHS).

| Resource | Link |
|---|---|
| National Veterinary Service Laboratory<br>National Animal Health Laboratories | USDA APHIS \| Diagnostic Testing at the NVSL<br>USDA APHIS \| Laboratory Information and Services<br>USDA APHIS \| Laboratories |
| USDA APHIS National Animal Health Reporting System (NAHRS) | USDA APHIS \| National Animal Health Reporting System (NAHRS) |
| USDA APHIS National List of Reportable Animal Diseases (NLRAD) | USDA APHIS \| National List of Reportable Animal Diseases |
| USDA APHIS Veterinary Services and State authorities | Federal and State Animal Health (usaha.org)<br>USDA APHIS \| Contact Veterinary Services |
| 2017 OIE Report of the Meeting of the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases | a-ahg-susceptibility-of-fish-september-2019.pdf (woah.org) |
| World Organisation for Animal Health (WOAH) Aquatic Animal Health Code | Aquatic Code Online Access - WOAH - World Organisation for Animal Health |
| World Organisation for Animal Health (WOAH) Manual of Diagnostic Test for Aquatic Animals | Manual Online Access - WOAH - World Organisation for Animal Health |
| World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS) database | World Animal Health Information System WAHIS - WOAH - World Organisation for Animal Health |
| World Trade Organization (WTO), Sanitary and Phytosanitary Measures | WTO \| WTO Agreements Series: Sanitary and Phytosanitary Measures |
| The United Nations Code of Conduct for Responsible Fisheries based upon UNCLOS and other international laws. | International Agricultural Law and Organizations<br>Aquaculture Overview - National Agricultural Law Center (nationalaglawcenter.org) |
| FAO Aquaculture Regulatory Frameworks | AQUA-CULTURE REGULATORY FRAMEWORKS (fao.org) |
| United States Fish and Wildlife Importation Guidelines | Information for Importers & Exporters \| U.S. Fish & Wildlife Service (fws.gov)<br>CFR-2016-title50-vol1.pdf (govinfo.gov)<br>Help Center Articles - Do I Need a Permit? (servicenowservices.com) |
| USDA APHIS Import permit information | USDA APHIS \| Fish, Fertilized Eggs, and Gametes |
| USDA APHIS International Regulations (IREGS) website | USDA APHIS \| Animal and Animal Product Export Information) |

Table 2. Countries for which USDA APHIS has a negotiated export health certificate to ship live salmonid fish, eggs, and gametes, and their requirements for testing for viral haemorrhagic septicemia (VHS) (as of 2023).

| Country | Infectious Viral Haemorrhagic Septicemia (VHS) Freedom Testing Required |
|---|---|
| Argentina | Yes |
| Armenia | Yes |
| Austria | Yes |
| Belarus | Yes |
| Belgium | Yes |
| Bosnia-Herzegovina | No |
| Brazil | Yes |
| Bulgaria | Yes |
| Canada | Yes |
| Chile | Yes |
| China | Yes |
| Croatia | Yes |
| Cyprus | Yes |
| Czech Republic | Yes |
| Denmark | Yes |
| Estonia | Yes |
| Finland | Yes |
| France | Yes |
| Georgia | Yes |
| Germany | Yes |
| Greece | Yes |

| | |
|---|---|
| Hungary | Yes |
| Ireland, Republic of | Yes |
| Isle of Man | Yes |
| Israel | Yes |
| Italy | Yes |
| Kazakhstan | Yes |
| Kyrgyzstan | Yes |
| Latvia | Yes |
| Lithuania | Yes |
| Luxembourg | Yes |
| Malaysia | No |
| Malta | Yes |
| Mexico | Yes |
| Morocco | Yes |
| Netherlands | Yes |
| New Zealand | Yes |
| North Macedonia | Yes |
| Norway | Yes |
| Peru | Yes |
| Poland | Yes |
| Portugal | Yes |
| Romania | Yes |
| Russian Federation | Yes |
| Serbia | Yes |

| | |
|---|---|
| Singapore | No |
| Slovakia | Yes |
| Slovenia | Yes |
| South Africa | Yes |
| Spain | Yes |
| Sweden | Yes |
| Switzerland | Yes |
| Taiwan | No |
| Turkey | Yes |
| Turks and Caicos Islands | Yes |
| Ukraine | Yes |
| United Arab Emirates | Yes |
| United Kingdom/ Great Britian | Yes |

Table 3. Countries that have reported presence of viral haemorrhagic septicemia (VHS) in domestic and wild fish species per the World Organisation of Animal Health (WOAH) World Animal Health Information System (WAHIS) database (Appendix, Table 1) for years that data were available (2005–2023).

| Country | Year | Animal category | Disease status |
|---|---|---|---|
| Austria | 2006–2023 | Domestic | Present |
| Belgium | 2005–2010, 2012, 2014–2021 | Domestic | Present |
| Bulgaria | 2007–2009 | Domestic | Present |
| | 2009 | Wild | Present |
| Canada | 2005–2023 | Domestic, Wild | Present or Suspected |
| Croatia | 2013–2018 | Domestic | Present |
| Czech Republic | 2007–2011, 2013–2016, 2019–2023 | Domestic | Suspected |
| Denmark | 2005–2009 | Domestic | Present |

| | | | |
|---|---|---|---|
| Estonia | 2011 | Domestic | Present |
| Finland | 2005–2012 | Domestic | Present |
| France | 2005, 2007–2010, 2012–2014, 2016–2021, 2023 | Domestic | Present |
| Germany | 2005–2023 | Domestic | Present |
| Ghana | 2019 | Domestic | Suspected |
| Iceland | 2015 | Wild | Present |
| Iran | 2005, 2007, 2013–2022 | Domestic | Present |
| | 2007 | Wild | Suspected |
| Iraq | 2010–2011 | Domestic | Present |
| Italy | 2005–2016, 2018–2023 | Domestic | Present |
| Japan | 2005–2023 | Domestic | Present |
| | 2005–2008 | Wild | Present |
| Latvia | 2023 | Domestic | Present |
| Netherlands | 2005, 2011, 2022–2023 | Domestic | Suspected |
| | 2005 | Wild | Present |
| Norway | 2007–2009 | Domestic | Present |
| Poland | 2005–2019, 2023 | Domestic | Present |
| Romania | 2006, 2016–2017, 2022–2023 | Domestic | Present |
| Slovakia | 2008–2011, 2016–2019 | Domestic | Present |
| Slovenia | 2008–2010 | Domestic | Present |
| South Korea | 2005–201, 2019–2023 | Domestic | Present |
| | 2005–2007, 2022–2023 | Wild | Present |
| St. Helena | 2019 | Domestic | Suspected |
| Sweden | 2015–2016, 2019–2023 | Wild | Present |
| Switzerland | 2006–2010, 2012, 2014–2015, 2017, 2019, 2022–2023 | Domestic | Present |
| Trinidad and Tobago | 2011, 2013 | Domestic | Present |
| Turkey | 2006, 2007 | Domestic | Present |
| Turkmenistan | 2017 | Domestic | Present |

| | | | |
|---|---|---|---|
| United Kingdom | 2007–2008, 2012–2013 | Domestic | Present |
| | 2007–2008 | Wild | Present |
| United States of America | 2005–2011 | Domestic | Suspected |
| | 2005–2023 | Wild | Present |

## Regulatory Information Associated with Salmonid Aquaculture

### International Laws Regulating Seas and Fisheries

A comprehensive summary of all international laws regulating seas and fisheries is beyond the scope of this document. Briefly, the United Nations (UN) plays a significant role in the development of international laws. The 1982 United Nations Conference on the Law of the Sea (UNCLOS) sets offshore territorial boundaries that establish zones of exclusive economic and fisheries rights for coastal nations. This is the de facto set of guidelines for the world's oceans.[98] Some nations have not ratified this convention, resulting in different international laws among nations affecting aquaculture. The UN has also developed a Code of Conduct for Responsible Fisheries based upon UNCLOS and other international laws.[98] The Food and Agriculture Organization of the United Nations (FAO) Legal papers Online: Aquaculture Regulatory Frameworks[99] also provides information summarizing significant issues related to the development and implementation of aquaculture regulatory frameworks.

### Regulatory Information Associated with United States Salmonid Aquaculture

Marine and inland salmonid aquaculture systems are regulated by Federal, State, and when applicable local and Tribal governments.[100] At the Federal level, "aquaculture" is defined in the National Aquaculture Act of 1980 as "the propagation and rearing of aquatic species in controlled or selected environments."[98, 101, 102] This act calls for development of a National Aquaculture Development Plan identifying aquatic species that have significant potential for culturing on a commercial or other basis by the Secretary of Agriculture, Secretary of Commerce, and the Secretary of the Interior.[98, 103] The act also contains recommendation for aquaculture research and development, technical assistance, design and management of facilities, and coordination of national activities and resolution of legal and regulatory constrains affecting aquaculture.[98] The Joint Subcommittee on Aquaculture was created by enactment of the National Aquaculture Act and amended in 1985 with intention to increase effectiveness and productivity of Federal aquaculture research, transfer, and assistance programs.[98]

Federal agencies with aquaculture regulatory oversight include the Department of Health and Human Services (DHHS) Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the National Oceanic and Atmospheric Administration (NOAA), the United States Coast Guard (USCG), the United States Department of Agriculture (USDA) Animal Plant and Health Inspection Service (APHIS), the United States Army Corps of Engineers, the United States Department of the Interior (USDI) Bureau of Ocean Energy Management (BOEA), the United States Fish and Wildlife Service (USFWS).[98, 100]

Marine farms must comply with regulations found in the Clean Water Act,[104] the Endangered Species Act,[105] the Fish and Wildlife Coordination Act,[106] the Magnuson-Stevens Fishery Conservation and Management Act,[107] the Marine Mammal Protection Act,[108] the National Environmental Policy Act,[109] the National Marine Sanctuaries Act.[110] Federal agencies and regulations specific to inland aquaculture include many of the agencies described above, with exclusion of agencies specific to marine aquatic systems.

State and within State (county and local) governments regulate aquaculture activities that are permitted or licensed at the community level.[111, 112] Generally, permits address building, community level marketing, processing and trade, fish disease testing and import, fish species certification relative to wildlife management, waste discharge, water use, and zoning.[112, 113, 114] Regulations are not uniform among States and can vary within State based on the geographic location of the aquaculture facility (coastal, inland, wetland, offshore), and associated local environmental impacts.[112, 114] State agencies that provide regulatory oversite include, but may not be limited to, State Departments of Agriculture, Fish and Wildlife, and Natural Resources.[111, 113, 115]

Some States may require development of aquaculture-specific Best Management Practices designed to enhance farm biosecurity, production and minimize environmental impacts.[115, 116, 117] For example, Atlantic salmon farming operations Maine participate in the Global Aquaculture Alliance Best Aquaculture Practices program and the Maine Aquaculture Association Code of Practice, Bay Management and Biosecurity programs.[115, 118] As of October 2021, Maine also provides Aquaculture Operational Standards for Land-based Recirculating Aquaculture Systems (RAS).[119]

## Regulatory Information Associated with International Trade

### *The Word Organisation for Animal Health*

The World Organisation for Animal Health (WOAH) 2022 OIE Aquatic Animal Health Code describes international standards for protecting aquatic animal and public health.[4] Standards related to the establishment of restrictions designed to prevent introduction of animal health hazards by importing countries are included in these provisions. These standards are based on the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures.[120, 121] The SPS agreement outlines several provisions that Member countries must consider when establishing import restrictions. Members must determine the level of transmission risk, animal health measures, and biosecurity standards required to manage disease risks among live animals and animal products within the country. The level of protection deemed appropriate by a Member should be sufficient to protect human, animal, and/or plant health or life within its territory. Member countries must ensure that their sanitary and phytosanitary measures to not arbitrarily or unjustifiably discriminate between Members where identical or similar conditions prevail. Members cannot seek import restrictions that are not equivalent to those established domestically, or apply restrictions in a manner constituting a disguised restriction on international trade.[120, 121]

### *WOAH Import Information Specific to VHSV*

WOAH import/export guidelines specific to VHS vary depending on the status of the exporting country, zone, or compartment, and are found in the WOAH Aquatic Animal Health Code.[4]

Briefly, when:

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with VHS. The importing country Competent Authority should require that the consignment be accompanied by an international aquatic animal health certificate issued by the exporting country Competent Authority. The international aquatic animal health certificate should state that, based upon the procedures described in the WOAH Aquatic Animal Health Code, Articles 10.10.5., 10.10.6., or 10.10.7. (as applicable), and 10.10.8., the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with VHSV.
2. Importing aquatic animals or aquatic animal products from a country, zone or compartment that is NOT declared free from VHS infection. The importing country Competent Authority should assess the risk as described in the WOAH Aquatic Animal Health Code, and consider the following risk mitigation measures:
   a. If the intention is to grow out and harvest the imported aquatic animals, the aquatic animals should be delivered directly to a quarantine facility and held there for the duration of the animals' lifetime. Before leaving quarantine (wither in the original facility or via biosecure transport to another quarantine facility) the animals are killed and processed into one or more products described in the WOAH Aquatic Animal Health Code, Article 10.10.3., or authorized by the Competent Authority. All water (transport, effluent, waste) equipment and waste materials are treated to inactive VHSV as described in the 2022 WOAH Aquatic Animal Health Code, Chapters 4.4., 4.8., and 5.5.
   b. If the intention is to establish a new stock for aquaculture, the exporting country should identify source populations, evaluate their aquatic animal health records, test the identified source populations for VHS in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4., and select a foundation population (F0) of animals with a high health status for infection with VHSV. The importing country should import the F0 population to a quarantine facility and test for VHS as described in WOAH Aquatic Animal Health Code, Chapter 1.4., to determine the suitability of the population for broodstock. A first generation (F1) population should be produced quarantine, cultured under conditions conducive for clinical expression of VHSV infection, and sampled and tested for VHSV as described in WOAH Aquatic Animal Health Code, Chapter 1.4., and WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.5. If VHSV is not detected in the F1 population, it may be defined as free from infection and released from quarantine. If VHSV is detected, the F1 population should not be released from quarantine, and should be killed and disposed of in a biosecure manner as described in WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 4.8.
3. Importing disinfected eggs for aquaculture from a country, zone, or compartment that is NOT declared free VHSV. Prior to importation, the importing country Competent Authority should assess at minimum, the likelihood that water used during disinfection of the eggs may be contaminated with VHSV, and the prevalence of VHSV infection in the broodstock (including evaluation of testing of ovarian fluid and milt). If the risk is acceptable, risk mitigation measures should be applied. The eggs be disinfected prior to import as described in the WOAH Aquatic Animal Health Code, Chapter 4.5. During the interval between disinfection and importation, the eggs should not contact anything that may impact their health status. The importing country Competent Authority should require that the consignment of eggs be

accompanied by an international aquatic animal health certificate issued by the exporting country Competent Authority certifying that the risk mitigation procedures were conducted. The importing country Competent Authority should consider internal measures such as additional disinfection of the eggs upon arrival in the importing country.

### *The United States*

#### National Import Information

The USFWS oversees importation of live and dead salmonid fish, eggs, and gametes (Appendix, Table 1).[106, 122, 123] The USFWS defines fish, including salmonids, as wildlife. This definition describes wildlife as "any wild animal, alive or dead, whether or not bred, hatched or born in captivity, and any part, product, egg, or offspring thereof."[122, 124] Per the Lacey Act of 1900, importation and transportation of salmonid fish (live or dead), eggs, and gametes into the United States and its territories or possessions is injurious or potentially injurious to the welfare and survival of wildlife or wildlife resources of the United States, the health and welfare of human beings, and the interests of forestry, agriculture and horticulture.[122, 124] These designations place importation and transportation of live salmonid fish, eggs, and gametes under the purview of USFWS which issues permits under wildlife laws and treaties at international, national, and regional levels.[122, 123]

All live (or dead) uneviscerated fish, live fertilized eggs, or gametes of salmonid fish are prohibited entry into the United States for any purpose except by direct shipment. Imports must receive prior written approval from the USFWS Director. Requirements for importation are available in detail in the National Archives and Records Administration, Code of Federal Regulations (CFR), Title 50: Wildlife and Fisheries.[124] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[124] Shipments must be accompanied by a United States Title 50 Certification Form completed in the country of origin by a USFWS-certified aquatic animal health inspector. This form is valid for six months after date of issue and certifies that the fish stocks from which the shipments originated have been tested for infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), Oncorhynchus masou virus (OMV), and Viral haemorrhagic septicemia virus (VHSV).[122, 123, 125] Eggs must be disinfected within 24 hours prior to shipment using specific protocols described in CFR, Title 50, and water used for shipping must be derived from pathogen-free water.[122, 123, 124, 125]

The United States Department of Agriculture (USDA) Animal Plant Health and Inspection Service (APHIS) requires import permits for live fish, eggs, and gametes from species susceptible to Spring viremia of carp virus (SVC) and Tilapia Lake virus (TiLV).[126] USDA APHIS does not have regulations or recommendations specific to IHNV and the international import or interstate movement of live salmonid fish, eggs, or gametes.

#### National Export Information

Exporters of fish designated as wildlife are required to obtain export permits from USFWS. Shipments must be declared and cleared by USFWS and USCBP at USFWS designated ports.[124, 128, 129] Many countries of import require documentation of animal health by USDA APHIS. Country specific exportation requirements for Aquaculture/Aquatic Animals may be accessed on the USDA APHIS International Regulations (IREGS) website (Appendix, Table

1).[129, 130] Briefly, the United States has negotiated international export health certificates, completed by an accredited veterinarian and endorsed by a Veterinary Services area office, for shipments of live salmonid fish, eggs, and gametes.[130] Some countries for which USDA APHIS has negotiated an export health certificate applicable for shipment of live salmonid fish, eggs, or gametes require testing for IHNV prior to export from the United States (Appendix, Table 2).

## State Import and Export Information

In 2006, following emergence of VHS in the Great Lakes region, USDA APHIS enacted a Federal Order that prohibited or restricted interstate movement of VHS-susceptible fish species from VHS-affected or at-risk States and importation of those species from Canada (Ontario and Quebec).[32, 34] In 2014, this Federal Order was removed on the contingency that individual States maintain VHS regulations and other practices to reduce risk.[131] At present, State regulations specific to VHS surveillance and management, and live fish, egg, or gamete import and movement (international and interstate) are variable (Table 4).[132] Some States require disease freedom testing for all fish regardless of source of origin as a part of a comprehensive fish health certification requirement. Other States require VHS testing for live fish, eggs, and gametes sourced only from States designated positive for the virus. Some States do not require any testing or disease freedom declaration. Information for Tribal or local governmental regulations specific for VHSV import testing where generally unavailable for review.

Table 4. States that have some viral haemorrhagic septicemia virus(VHSV) requirements for imported farmed and/or wild live fish, eggs and gametes[132]

| State | Requirements |
|---|---|
| Alabama | No |
| Arizona | Yes |
| Arkansas | Yes |
| California | Yes |
| Colorado | Yes |
| Connecticut | Yes |
| Delaware | No |
| Florida | No |
| Hawaii | No |
| Idaho | Yes |
| Illinois | Yes |
| Indiana | Yes |
| Iowa | Yes |
| Kansas | Yes |
| Kentucky | Yes |
| Louisiana | No |
| Maine | Yes |
| Maryland | Yes |
| Massachusetts | Yes |
| Michigan | Yes |

| | |
|---|---|
| Minnesota | Yes |
| Mississippi | No |
| Missouri | Yes |
| Montana | Yes |
| Nebraska | Yes |
| Nevada | Yes |
| New Hampshire | Yes |
| New Jersey | Yes |
| New Mexico | Yes |
| New York | Yes |
| North Carolina | No |
| North Dakota | Yes |
| Ohio | Yes |
| Oregon | Yes |
| Pennsylvania | Yes |
| Rhode Island | Yes |
| South Dakota | Yes |
| Tennessee | No |
| Texas | No |
| Utah | Yes |
| Vermont | Yes |
| Virginia | No |
| Washington | Yes |

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