U.S. DEPARTMENT OF AGRICULTURE

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Strategy and Policy

Center for Epidemiology and Animal Health

April 2025

Hazard Identification: Epizootic Haematopoietic Necrosis Virus (EHNV)

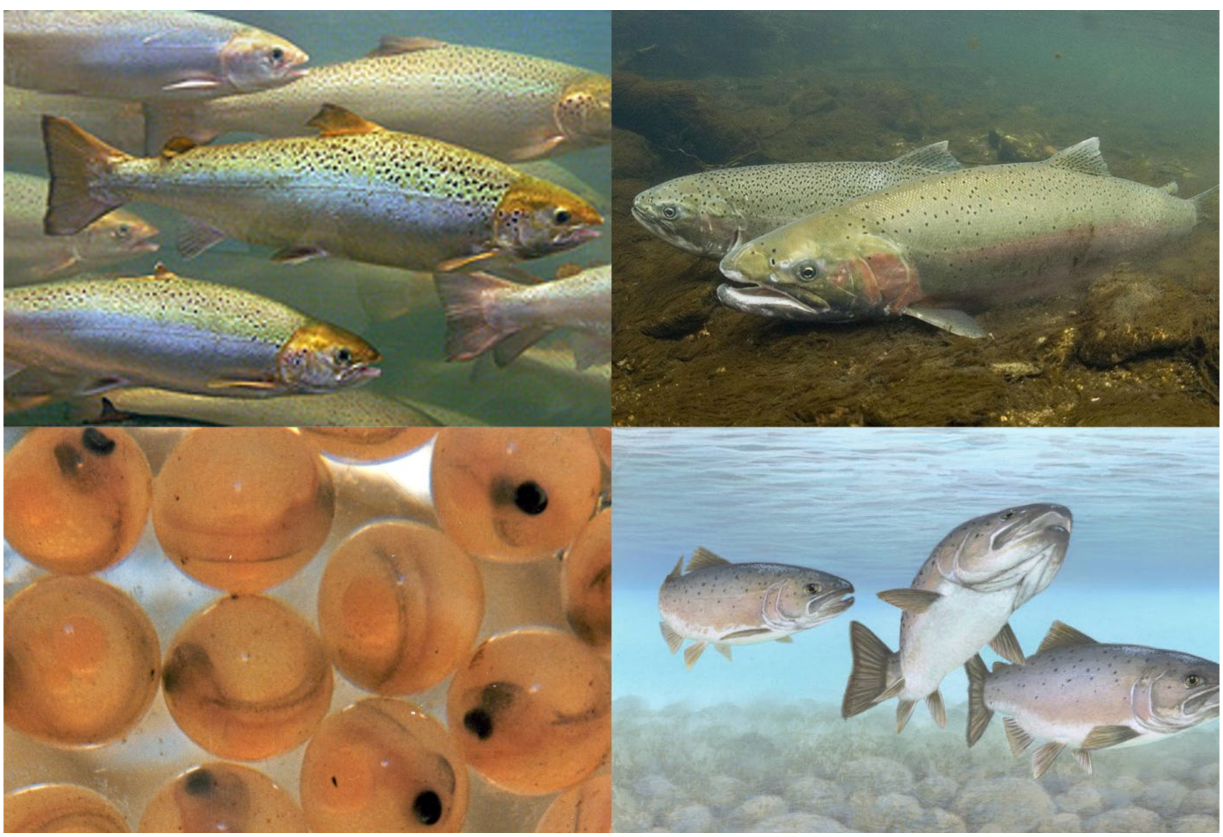

Table of Contents

Introduction

USDA APHIS VS CEAH was asked to generate a Hazard Identification for epizootic haematopoietic necrosis disease (EHN). Hazard identification is a process used to identify hazards (biological, chemical, or physical agents) that may result in adverse consequences in a susceptible animal or animal populations.[1] The hazard identification process is also used to identify pathogenic agents 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 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 subject to control or eradication in the importing country.[1]

Subjects within the scope of this document include a description of the hazard (EHN), 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 EHN epidemiology. Knowledge and data gaps were present that affected complete evaluation of some tenets of this hazard identification document.

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).[3, 4]
  - 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)[5, 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.[6, 7, 8, 9]
- 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.[10]

Measures based on current epidemiological information and understanding of relevant knowledge and data gaps.[11, 12, 13, 14]

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

Key Findings

- Epizootic haematopoietic necrosis (EHN), caused by epizootic haematopoietic necrosis virus (EHNV), is a foreign animal disease in the United States. It is only reported in Australia.
- EHN is a necrotizing disease syndrome in redfin (European) perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*).
- Redfin perch are highly susceptible to EHNV infection.
- Rainbow trout are less susceptible to infection. Morbidity and mortality are often similar to expected rates associated with aquaculture activities.
- Outbreaks of disease are typically associated with increased water temperature.
- EHNV appears capable of persisting for long periods of time in the environment. The virus is highly resistant to drying, desiccation, and many physical conditions and chemical agents commonly used to inactive viral pathogens.
- Transmission appears to be horizontal; however, some transmission factors are not fully described.
- Clinical signs and gross pathological lesions are not pathognomonic. Definitive diagnosis must be confirmed using laboratory diagnostic assays.
- Recommended import biosecurity measures include pre-import certification of live fish, eggs, and gametes, and/or their source(s) for EHNV freedom.
- In the United States, there are no Federal regulations specific to EHNV and the import or interstate movement of live salmonid fish. The U.S. Fish and Wildlife Service (USFWS) does require disinfection of salmonid eggs prior to import; however, there are no validated disinfection protocols for fish eggs specific to EHNV. Information describing State, Territorial, Tribal or other local regulation of live fish, eggs or gametes relative to EHNV is generally lacking.

Epizootic Haematopoietic Necrosis Virus

Introduction

Epizootic haematopoietic necrosis (EHN, Nillahcootie redfin virus, Redfin virus) is a disease present in Australia that affects redfin (European) perch and freshwater rainbow trout. The WOAH Manual of Diagnostic Tests for Aquatic Animals (Appendix, Table 1) defines EHN as disease caused by infection with genomically identified epizootic haematopoietic necrosis virus (EHNV, Family Iridoviridae, genus *Ranavirus*) specific to Australia.[15, 16, 17, 18, 5, 19]

EHN is a foreign animal disease in the United States and is included in the USDA APHIS National List of Reportable Animal Diseases (NLRAD) and National Animal Health Reporting System (NAHRS) lists of reportable diseases (Appendix, Table 1).[20, 21] All animal health professionals, including accredited veterinarians, should coordinate with their State Animal Health Official and Area Veterinarian in Charge (AVIC) upon suspicion or confirmation of NLRAD listed diseases. Confirmed cases of a NLRAD disease should be reported in accordance with NLRAD Standards. Reporting under NLRAD does not supersede State requirements or notification processes for foreign animal emerging disease incidents or other regulated/high-priority endemic disease reporting requirements (Appendix, Table 1). EHN is a WOAH listed notifiable disease.[22, 23] Disease notification requirements and requirements for self-declaration of freedom of EHNV infection for Member nations are found in the WOAH Aquatic Animal Health Code, Chapter 2.3.1 (Appendix, Table 1).[24, 16, 4, 19] EHN is listed as an exotic disease by the European Union health directive and is a reportable disease in Canada.[25, 26] WOAH import/export guidelines specific to EHNV are found in WOAH Aquatic Animal Health Code [19] (Appendix, WOAH Import/Export Recommendations for EHV).

In the United States, there are no Federal regulations specific to the import of live fish, eggs, or gametes and EHNV. Pre-import testing for EHNV is not specifically required for USFWS import health certification. However, EHNV is cultivable in the cell lines that are utilized in for other USFWS import fish health certifications and would likely be detected. The USFWS does require disinfection of salmonid eggs prior to import; however, there are no validated disinfection protocols for fish eggs relative to EHNV.[16] Limited information describing State, Territorial, Tribal, or other local regulation of fish, eggs, or gametes relative to EHNV is found in Appendix, Table 1.

Susceptible Fish Species

Fish species identified by WOAH as susceptible to EHNV are summarized in Table 1.[1, 27] In the United States, rainbow trout are the farmed salmonid species of greatest economic concern relative to infection with EHNV.

Table 1. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to epizootic haematopoietic necrosis virus (EHNV).[1, 27]

Genus species	Common Name
Ameiurus melas	Black bullhead
Bidyanus bidyanus	Silver perch
Esox lucius	Northern pike
Galaxias olidus	Mountain galaxias
Gambusia affinis	Mosquito fish
Gambusia holbrooki	Eastern mosquito fish
Macquaria australasica	Macquarie perch
Melanotaenia fluviatilis	Crimson spotted rainbow fish
Oncorhynchus mykiss	Rainbow trout
Perca fluviatilis	European (redfin) perch

Sander lucioperca	Pike-perch

Geographic Distribution

EHN is endemic only to Australia (Appendix, Table 3).[22, 24, 23, 28, 27] In 1986, the disease emerged in wild redfin perch in New South Wales and subsequently spread to other wild redfin perch populations in Australian Capital Territory, Victoria, and South Australia.[24, 29, 16] Outbreaks of EHN have also occurred in farmed rainbow trout in New South Wales.[22] EHN has remained contained within these regions, causing discontinuous, discrete outbreaks followed by long lapses in occurrence.[23] A search of the WOAH World Animal Health Information System (WAHIS, Appendix, Table 1) database for years that data were available (2005 to 2021) identified reports of EHNV presence in Australia and in Kuwait in 2009 to 2012 (this occurrence could not be verified via a search of scientific literature).[27]

According to the European Union Reference Laboratory for Fish and Crustacean Diseases, EHNV has never been detected in Europe.[23, 26, 27] Additionally, EHNV has never been detected in North America, including the United States.[25, 26, 27] Review of the literature did identify manuscripts describing detection of fish iridioviruses in North America (e.g., white sturgeon iridovirus[30] and Santee-Cooper ranavirus[31, 32]). Santee-Cooper ranavirus includes three virus strains (doctor fish virus, DFV; guppy virus 6, GV-6; and largemouth bass virus, LMBV) which were originally described as viruses similar to EHNV and ECV.[30, 33, 34, 35, 31, 36, 37, 38] These viruses are genetically distinct from EHNV.

Public Health

EHNV is not a zoonotic pathogen. There are no threats to human health.[22, 39]

Epidemiology

In this section, the epidemiology of EHN in the natural host species (e.g., redfin perch, rainbow trout) is summarized. In general, many environmental, pathogen, and host factors of EHNV susceptibility among fish species are poorly described or understood.

Host Characteristics

Redfin perch

Redfin perch are highly susceptible to EHNV infection. The disease is highly fatal in juvenile fish compared to adults.[40, 16] Infection of eggs and early life stages (larvae and fry up to approximately 5 g in weight) are not reported in published literature or the WOAH Manual of Diagnostic Tests for Aquatic Animals.[5] Initial emergence of EHN in this species lead to collapse of the recreational redfin perch fishery and caused severe economic losses when outbreaks occurred in redfin perch aquaculture.[41, 22] Experimental challenge studies have demonstrated high rates of susceptibility following a low virus dose challenge of 0.08 $TCID_{50}$ mL^{-1} (50 percent tissue culture infective dose per milliliter) via immersion bath or intraperitoneal inoculation.[42, 24] Disease outbreaks in wild populations are often followed by years of disease absence.[43] The epidemiology of this pattern of disease occurrence is not fully described but appears related to the dynamic relationship between host population density and environmental conditions optimal for host-virus interaction.[44, 45, 28] High mortality (95 percent) outbreaks have been observed during the summer in fingerling and juvenile perch, while adult fish are largely unaffected.[46, 41, 22, 23] Factors contributing to the high mortality in young perch may include the inability of young fish to mount adequate immune responses to EHNV and behavioral differences between young and adult fish. Young perch tend to reside and feed in shallow warm waters which may increase susceptibility to EHNV infection. Adult fish typically reside and feed in deeper, cooler waters.[44, 41, 24, 16, 39]

There is lack of sufficient data to fully describe the potential transmission capability, duration of subclinical infection, or carrier status in wild perch.[47, 48, 23, 28, 16] Virus has been infrequently isolated from wild juvenile and adult perch following natural disease outbreaks.[48, 23, 28, 39] In experimental studies, both resistance to reinfection and lack of isolation of EHNV from individual fish after experimental challenge have been reported.[47, 48, 28, 39] If wild perch are capable of functioning as subclinical carriers, this could contribute to the spread of EHNV and the irregular occurrences of EHN outbreaks in natural water systems.[23] Differences in susceptibility between Australian and European redfin perch stocks following experimental challenge have been described in the literature.[49, 50, 48, 23, 16] It is unknown if this reflects differences in the fish stocks or factors related to the design of the studies (e.g., the challenge strain and dose of EHNV used).[49, 23, 28, 16]

Rainbow Trout

The epidemiology of EHN in rainbow trout is not fully described.[45, 39] Infection can occur at all ages; however, in general rainbow trout appear to be relatively resistant to EHNV infection and the resulting disease is less severe that than observed in redfin perch.[22, 5] Infection of eggs and early life stages (larvae and fry up to approximately 5 g body weight) are not reported.[19] In experimental studies, the immersion bath and intraperitoneal challenge dose required to infect rainbow trout ($1 \times 10^{2.2}$ $TCID_{50}$ mL^{-1}) was greater than that required to infect redfin perch.[42, 24] Typically, only a small proportion of individuals in a population become infected or develop clinical disease.[44, 45, 40] In farmed trout, disease surveillance strategies that incorporate routine moribund sampling would improve detection of EHNV due to the low number of fish exhibiting clinical signs of illness and the low level of mortality observed in this species.[24, 29] Clinical disease is most commonly observed in young fingerlings (up to 125 mm fork length),[22, 16] and is rarely observed in grower and broodstock fish.[45] Rates of EHNV detection via virus isolation during outbreaks in farmed trout range from 60 percent to 80 percent in moribund and dead fish, and 0 percent to 4 percent in clinically normal appearing fish.[44, 45, 24, 16] Post-outbreak, surviving fish appear to develop long-lasting immunity and virus is rarely detected.[44, 45, 16] There is a lack of consensus among researchers regarding the presence of a carrier state in naturally infected rainbow trout.[51, 52, 53, 16] Anti-EHNV antibodies have been detected at low prevalence (0.2 percent to 3.7 percent), which some authors suggest indicate the capability of surviving fish to function as carriers.[45, 16, 43] A literature search did not identify any reports or transmission studies verifying that trout surviving EHNV infection were capable of infecting other fish.

There is currently no evidence that an amphibian reservoir exists.[23] It is currently unknown if other reservoir hosts (other aquatic animals, vectors) may maintain EHNV presence in aquatic environments.[23, 28]

Environmental Characteristics

Environmental factors that appear related to outbreaks of EHN in endemic areas include seasonal variation in water temperature and quality, as well as other factors. Outbreaks in wild redfin perch tend to occur at intervals lasting two to three weeks during summer months, and appear associated with the above described environmental factors and food availability which affect behavior.[44, 16, 43] In farmed rainbow trout, EHN occurrence is associated with environmental factors such as high stocking rates, low water quality and exchange rates, sudden changes in water temperature (low to high), water temperatures ranging from 11–20 °C/51.8–68 °F, and concomitant presence of parasitic, protozoal, fungal, or systemic bacterial infections.[44, 45, 22, 24, 43]

The incubation periods for EHNV are inversely proportional to water temperature in fish challenged by intraperitoneal injection.[23] The incubation period for redfin perch ranged from 10–28 days at 12–18 °C/53.6–64.4 °F and 10–11 days at 19–21 °C /66.2–69.8 °F.[42, 22, 48, 23, 16] In rainbow trout, the incubation period ranged from 14–32 days at 8–10 °C/46.4–50 °F and 3–10 days at 19–21 °C/66.2–69.8 °F.[42, 22, 24, 23, 16] Viral replication in infected fish is also temperature dependent. Peak viral replication was documented in experimentally infected northern pike (*Esox lucius*) at 3 and 7 days post-experimental challenge via bath exposure at 22 °C/71.5 °F and 12 °C/53.6 °F, respectively.[54, 23]

Pathogen Characteristics

Environmental persistence of EHNV appears to be a key factor in the epidemiology of disease occurrence.[41] Under natural conditions, EHNV appears to be highly resistant to drying. According to WOAH, for this reason it should be presumed that EHNV is capable of persisting for months to years on fish farms in water and sediment, and plausibly on plants, equipment and other fomites.[51, 22, 24, 19] Experimentally, the virus remains environmentally stable in distilled water for 97 days. [51, 22, 24] Infectivity persists for approximately 97 and 300 days in water stored at 15 °C/59 °F and 4 °C/39 °F, respectively, 110 days in dried fish tissues, 113 days in dried tissue culture spots stored at 15 °C/59 °F, and for over 300 days in cell cultures stored at 4 °C/39 °F. [51, 22, 24] Viability has been documented for two years in fish tissues frozen at -20 °C/-4 °F and for approximately one year in frozen fish carcasses.[22, 16, 5]

Transmission

Factors associated with transmission are not fully understood. According to the literature, EHNV enters the water column from carcasses and the tissues of infected fish.[23, 28, 16, 55] Movement of EHNV suspended in water and via movement of infected redfin perch are thought to be methods of local and regional spread in rivers, lakes, and ponds.[41, 56, 11, 23, 12, 16] Under natural conditions, EHN outbreaks have been documented in rainbow trout farms using influent water sourced from areas where infected redfin perch were present.[44, 45, 57, 23] Movement of virus suspended in water and the presence of subclinically infected individual fish are thought to be the primary methods of disease spread in farmed rainbow trout.[41]

Horizontal transmission has been documented experimentally via immersion bath.[51, 24, 48, 16] Potential modes of horizontal transmission include oral ingestion of virus present in water and the tissues of infected or dead fish, and contact exposure via the gills or skin lesions.[42, 22, 24, 23] Anthropogenic transmission of EHNV via translocation of subclinically infected fish for aquaculture purposes and the accompanying transport water has been described as a route of introduction into trout farms.[40, 57, 23, 16, 39] Activities of recreational fishermen (use of raw or frozen/thawed fish bait, movement of live fish and transport water) have been implicated in introduction of EHNV to susceptible wild fish populations.[41, 45, 28] Other potential transmission routes include mechanical transmission via fomites (e.g., boats, fishing gear, farm equipment, clothing) and wildlife (e.g., piscivorous birds).[41, 24, 39] Vertical transmission has not been verified in the field or under experimental conditions. Langdon et al. (1987) was unable to isolate EHNV from wild redfin perch eggs or yolk-sac fry,[47] and EHNV has not been detected in rainbow trout ovarian tissues or broodstock.[22, 24, 16] According to the WOAH Manual of Diagnostic Tests for Aquatic Animals, infections in eggs and early life stages (larvae and fry up to approximately 5 g body weight) are not reported.[5]

Clinical Signs and Pathogenicity

Clinical signs are non-specific and may include abnormal swimming behavior at the water surface, abdominal distension, anorexia, darkened skin color, gill hemorrhages, lethargy, loss of equilibrium, opercula flaring, petechial (pinpoint) hemorrhages, reddening at the base of fins, and skin ulcers.[51, 42, 22, 23, 17] Most clinically affected fish die within a few weeks.[28] All ages are susceptible to infection; however, clinical signs are typically most apparent in fingerlings and juvenile fish.[22, 16] Adult fish are most likely to develop clinical signs when the disease is first introduced into a naïve population.[17]

Clinical signs of EHN are often inapparent or observed at low frequency in naturally infected farmed rainbow trout.[44, 45] Development of disease in this species is often associated with poor husbandry (e.g., high stocking density, poor water quality) and the presence of concurrent disease (e.g., external parasites, focal and systemic bacterial infections).[44, 45, 24] Differential diagnosis for EHN include toxicities and other bacterial, fungal, parasitic, or viral pathogens that cause non-specific signs of illness.

Morbidity and Mortality

In naïve redfin perch, rates of infection, morbidity, and mortality in natural outbreaks can approach 95 percent.[46, 47, 41, 22, 27] In populations where the disease is recurrent, the highest rates of morbidity and mortality are observed in fingerling and juvenile fish, while adult fish are rarely affected.[41, 24] In rainbow trout, under natural farm conditions, the infection rate is low. When individual fish do become infected, they typically die from the disease. Infection is often present on a farm but goes unnoticed because the low infection rate leads to low daily observed morbidity and mortality rates that are within expected standard loss rates (0.2 percent daily mortality, up to 4 percent total mortality).[45, 22, 24, 23, 16] Mortalities are most common in fingerlings (up to 125 mm fork length and 500 g body weight) and can reach 90 percent in this age group.[58, 51, 44, 45, 23] Infection has not been confirmed in broodstock.[44, 45, 27]

Treatment

There is no treatment or vaccine.[16] There have been no formal EHNV resistance breeding programs for redfin perch or rainbow trout.[23, 16]

Diagnostic Testing

EHNV infects a wide range of cell types, including the hematopoietic cells, hepatocytes, and the endothelial cells of organs. Target organs include kidney, liver, and spleen. Capability of EHNV to infect reproductive tissues (i.e., gonadal tissues, milt, ovarian fluid) and the suitability of these tissues for surveillance of EHNV in broodstock is unknown.[16]

Gross lesions may not be present in some affected fish. When present, lesions are more often observed in redfin perch[22] and may include gastrointestinal ulceration; pale focal necrosis in the liver; petechial hemorrhages or redness at the base of the fins; petechial hemorrhages on the viscera; serosanguineous peritoneal effusion; and swelling of the kidney, spleen, and/or liver.[22, 23, 17] The spleen may also appear small and pale.[22] Histological lesions include basophilic intra-cytoplasmic inclusion bodies in hepatocytes adjacent to necrotic foci in the liver; fibrinous exudate; hemorrhage, hyperplasia, multifocal necrosis, and thrombosis in gill tissues; focal to extensive necrosis in the hematopoietic kidney, liver, spleen, heart, lamina propria of the intestine, and pancreas; and degenerate vascular endothelial cells and necrotic hematopoietic cells in organs and blood vessels.[15, 57, 23, 28, 39]

Clinical signs and gross pathological lesions caused by EHNV infection are not pathognomonic. Definitive diagnosis must be confirmed using laboratory diagnostic assays that utilize genomic sequencing to differentiate this EHN from disease caused by other closely related iridoviruses.[16] Direct diagnostic test methods include histological examination of fixed tissues, polymerase chain reaction (PCR) and genetic sequencing, and virus isolation (VI) in cell culture.[5] In the United States, Title 50 diagnostic testing methods include use of virus isolation/culture methods using cell lines sensitive to EHNV infection.

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.[16, 5, 19] Briefly, because infection can go unnoticed due to low daily and total mortality rates, surveillance sampling should be focused on moribund fish and fresh mortalities.[19] This testing strategy is also encouraged by USDA APHIS Comprehensive Aquaculture Health Program Standards (CAHPS) (Appendix, Table 1). In the United States, confirmatory testing at the USDA National Veterinary Services Laboratory (NVSL) is required following first detections (Appendix, Table 1). All suspected EHNV detections or outbreaks of ENV are reportable to USDA–APHIS–VS as the Federal competent authority for animal health. Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL.[59]

Prevention and Control

Biosecurity measures are the most important control measures available to prevent the introduction and spread of EHNV through infected fish, including apparently healthy carriers and survivors of disease outbreaks.

Recommended import biosecurity measures include pre-import certification of live fish, eggs, and gametes, and their source for EHNV freedom.[23] Under some circumstances, countries may implement more stringent measures. For example, Australia enforces quarantine restrictions on the importation of some fish species because of the risk of EHNV introduction.[23, 39] Suggested farm biosecurity measures include sourcing fish stocks only from sources demonstrated to be free from EHNV.[23] On farm quarantine should be a standard practice for all incoming fish.[23]

Water is one of the most common introductory pathways of aquatic pathogens into aquaculture establishments.[56, 11, 12] Therefore, disinfection of influent water is recommended to prevent exposure of farmed fish to pathogenic agents.[23] Treatment of effluent water prior to release is recommended to prevent downstream exposure of susceptible aquatic animals (including downstream farmed fish) to pathogenic agents. Additional disease control measures include maintaining good husbandry practices and optimal environmental conditions (water quality, flow, and temperature) and minimizing physiological stressors (bacterial and fungal pathogens, external parasites, high stocking densities, inadequate nutrition).[44, 45, 24, 16, 60]

EHNV is highly resistant to drying, desiccation, and many of the physical conditions and chemical agents used to inactivate other aquatic viruses.[51, 22, 24] Equipment should be thoroughly cleaned to remove biofilms and debris prior to disinfection.[22] The virus is temperature tolerant across a wide thermal range, but is inactivated when heated to 40 °C/104 °F for 24 hours or 60 °C/140 °F for 15 minutes.[51, 23] On dry surfaces, the EHNV is resistant to sodium hypochlorite, but can be inactivated by application of 70 percent ethanol for 120 minutes wet contact time.[22, 16] On wet surfaces or in liquid suspensions, sodium hypochlorite (200mg/L) and other disinfectants in the 4.0 to 12.0 pH range (chlorhexidine 150 mg/mL for 1 minute; potassium peroxymonosulfate (Virkon®) 200 mg/L) are effective.[22, 24, 23] Ultraviolet sterilization units may have some inactivation efficacy.[39] According to the literature, lime may be used to disinfect earthen ponds or raceways.[22] Disinfection protocols for eggs and larvae have not been validated.[16]

Prevention and control measures should include development of risk-based surveillance strategies for susceptible farmed and wild fish populations, and contingency plans for EHNV containment and eradication if introduction occurs. Whittington et al. (2009) recommends sampling of unexpected mortalities in redfin perch and “routine” mortalities of rainbow trout, instead of random samples of live fish.[29, 23] According to Whittington, given the low prevalence of pathogen prevalence in apparently healthy subclinically infected fish, certification practices based on random sampling of apparently healthy fish may lead to misclassification of the population as EHNV-free.[29] WOAH recommends that Members consider use of passive surveillance strategies to identify zones free from infection to facilitate the trade of live fish.[23, 19]

In the United States, EHN is a reportable foreign animal disease. Reporting of EHNV detection is required under USDA APHIS NLRAD and WOAH notifiable disease reporting requirements.[1] If

EHNV 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.[1] Control measures utilized by 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.[22, 16, 19] Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of EHN introduction. A summary of WOAH import/export guidelines specific to EHN, U.S. regulations, and other regulatory information related to aquaculture in the United States is summarized in the Appendix, WOAH Pathogen Specific Import Export Recommendations.

Summary

EHNV is a WOAH-listed viral disease affecting wild and farmed redfin perch and farmed rainbow trout. The economic impact to redfin perch aquaculture can be high given the high rates of susceptibility and mortality in this species. The economic impact to the rainbow trout industry is relatively low, due to the low infection rate and level of morbidity that occurs in this species.[27] In Australia, where EHNV is endemic, control of disease occurrence is difficult in wild fish populations (redfin perch) once introduction has occurred. Farmed rainbow trout are less susceptible to EHNV infection, which may go undetected due to low associated rates of mortality. The impact that EHN may have on wild rainbow trout populations is not known.

In the United States, there are no Federal regulations specific to the import of live fish, eggs, or gametes and EHNV. Pre-import testing for EHNV is not specifically required for USFWS import health certification. However, EHNV is cultivable in the cell lines that are utilized in other required USFWS import health certifications and would likely be detected. The USFWS does require disinfection of salmonid eggs prior to import; however, there are no validated disinfection protocols for fish eggs relative to EHNV.[16] Information describing State and Tribal regulation of live salmonid fish, eggs, or gametes relative to EHNV that guide local aquatic animal health and import requirements is found in Appendix, Table 1. Suggested best practices to prevent introduction of EHN into rainbow trout aquaculture in the United States include development of policies that would ensure live fish, eggs, and gametes are imported from EHN-free sources and that importation complies with the guidelines described in the WOAH Aquatic Animal Health Code.[19]

The potential impact that EHNV introduction may have on the U.S. rainbow trout industry is unknown due to lack of susceptibility testing of native farmed rainbow trout in the United States, as well as other knowledge gaps and data deficiencies. The impact that EHNV introduction may have on wild or stocked rainbow trout populations is likewise unknown. The susceptibility of other farmed and wild fish species in the United States is also unknown. EHNV is a WOAH-listed reportable disease. Therefore, detection of EHNV in cultured or wild fish stocks in the United States would likely result in significant trade impacts.

Appendix

Tables

Table 1. Links to manuals, websites, and other resources relevant to the ENV and other resource materials associated with aquaculture and aquatic animal diseases.

Topic	Link
Guide to State and Tribal aquaculture regulations	USDA APHIS | Interactive Maps
National Veterinary Service Laboratory National Animal Health Laboratories	USDA APHIS | Diagnostic Testing at the NVSL USDA APHIS | General NVSL Information USDA APHIS | Laboratory Information and Services USDA APHIS | Laboratories
USDA APHIS Comprehensive Aquaculture Health Program Standards (CAHPS)	USDA APHIS | Comprehensive Aquaculture Health Program Standards
USDA APHIS National Animal Health Reporting System (NAHRS)	USDA APHIS | National Animal Health Reporting System (NAHRS)
USDA APHIS National Aquaculture Health Plan & Standards (NAHP&S): 2021–2023	USDA APHIS | National Aquaculture Health Plan & Standards (NAHP&S): 2021–2023
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) 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, 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 Aquaculture Overview - National Agricultural Law Center (nationalaglawcenter.org)
FAO Aquaculture Regulatory Frameworks	AQUA-CULTURE REGULATORY FRAMEWORKS (fao.org)
United States Fish and Wildlife National Fish Health Survey Mapper	National Wild Fish Health Survey Mapper | U.S. Fish & Wildlife Service (fws.gov)
United States Fish and Wildlife Importation Guidelines	Steps for Importing Salmonids into the United States of America | U.S. Fish & Wildlife Service (fws.gov) Information for Importers & Exporters | U.S. Fish & Wildlife Service (fws.gov) CFR-2016-title50-vol1.pdf (govinfo.gov)

	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) Import/Export Requirements for Aquaculture Products (fdacs.gov)

Table 2. Countries for which APHIS has a negotiated export health certificate that can used to ship live salmonids (fish or eggs) that require testing for some of the pathogens described in this assessment.

Country	**Epizootic Haematopoietic Necrosis**
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	Yes
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	Yes
Slovakia	Yes
Slovenia	Yes
South Africa	Yes
Spain	Yes
Sweden	Yes
Switzerland	Yes
Taiwan	Yes
Turkey	Yes
Turks and Caicos Islands	Yes
Ukraine	No
United Arab Emirates	Yes
United Kingdom	Yes

Table 3. Countries in which presence of the six World Organisation for Animal Health (WOAH)-listed pathogens have been reported historically (wild and or farmed fish species) Note. This table presents summary data from 2010 through 2022. The WOAH WAHIS database (Appendix, Table 1), should be consulted for information regarding current country status.[27, 19]

Country	**Epizootic haematopoietic necrosis virus**
Australia	Yes
Austria	–
Belgium	–
Canada	–
Chile	–
China	
Costa Rica	–
Croatia	–
Czech Republic	–
Denmark	–
Estonia	–
Faroe Islands	–
Finland	–
France	–
Georgia	–

Germany	–
Iceland	–
Iran	–
Ireland	–
Italy	–
Japan	–
Latvia	–
Netherlands	–
North Macedonia	–
Norway	–
Poland	–
Romania	–
Russia	–
Slovakia	–
Slovenia	–
South Korea	–
Spain	–
Sweden	–
Switzerland	–
Turkey	–
Ukraine	–
United Kingdom (England, Scotland)	–
United Kingdom (Scotland)	–
United States	–
Vietnam	–

WOAH Import/Export Recommendations For EHV

WOAH import/export guidelines specific EHV are found in WOAH Aquatic Animal Health Code.[19] Briefly,

1. When live aquatic animals or aquatic animal products are imported from a country, zone, or compartment declared free from EHNV infection, the Competent Authority of the importing country should require that the shipment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country.[19] The international aquatic animal health certificate should state that the place of production of the aquatic animal or aquatic animal products is located in a country, zone or compartment declared free from EHNV infection.[19]

2. When importing aquaculture or aquatic animals from a country, zone or compartment that is NOT free from EHNV infection, the Competent Authority of the importing country should assess the risk in accordance with the WOAH Aquatic Animal Health Code, Chapter 2.1, and consider the following risk mitigations:[19]

a) For grow out and harvest of the imported aquatic species, there should be direct delivery and lifelong holding of the imported animals in a quarantine facility from which the animals do not leave unless they are first killed and processed. All transport water, equipment, effluent, and waste materials in this facility must be treated to inactivate EHNV in accordance with WOAH Aquatic Animal Health Code, Chapters 4.4, 4.8, and 5.5.
b) If the intention is establishment of new stock for aquaculture, the exporting country must identify potential source populations, evaluate their aquatic animal health records, test the source population(s) in accordance with the WOAH Aquatic Animal Health Code, Chapter 1.4, and select a founder population (F-0) with a high health status for infection with EHNV. The importing country should import the F-0 population into a quarantine facility and determine the suitability of the population for broodstock by testing for EHNV in accordance with the WOAH Aquatic Animal Health Code, Chapter 1.4. A first generation (F-1) population should be produced, cultured and tested in quarantine to establish/confirm freedom of EHNV as per the WOAH Aquatic Health Code, Chapter 1.4, and the WOAH Manual of Diagnostic Tests for Aquatic Animals (the Aquatic Manual), Chapter 23.1.[16] If EHNV is not detected, the F-1 population may be defined as free from EHNV infection and released from quarantine. If EHNV is detected the aquatic animals remain in quarantine until they can be killed and disposed per the WOAH Aquatic Animal Health Code, Chapter 4.8.

References

1. WOAH. (2022). "World Organisation for Animal Health Terrestrial Animal Health Code. World Organisation for Animal Health." from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=sommaire.htm

2. Bruckner, G., S. MacDiarmid, N. Murray, F. Berthe, C. Muller-Graf, K. Sugiura, C. Zepeda, S. Kahn, and G. Mylrea (2010). "Handbook on import risk analysis for animals and animal products." Office International des Epizooties, Paris.

3. WOAH. (2017). "OIE Report of the Meeting of the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases. World Organisation for Animal Health." from https://www.woah.org/app/uploads/2021/10/a-ahg-susceptibility-of-fish-april-2017.pdf.

4. WOAH (2022). Aquatic Animal Health Code, Chapter 10.4, Infection with Infectious Salmon Anemia Virus, World Organisation for Animal Health.

5. WOAH. (2022). "OIE Manual of Diagnostic Tests for Aquatic Animals 2022. World Organisation for Animal Health." from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/

6. Hedrick, R. (1998). "Relationships of the host, pathogen, and environment: implications for diseases of cultured and wild fish populations." Journal of Aquatic Animal Health 10(2): 107-111.

7. Jiménez-Valverde, A., A. T. Peterson, J. Soberón, J. Overton, P. Aragón, and J. M. Lobo (2011). "Use of niche models in invasive species risk assessments." Biological invasions 13(12): 2785-2797.

8. Engering, A., L. Hogerwerf and J. Slingenbergh (2013). "Pathogen–host–environment interplay and disease emergence." Emerging microbes & infections 2(1): 1-7.

9. James, T. Y., L. F. Toledo, D. Rödder, D. da Silva Leite, A. M. Belasen, C. M. Betancourt-Román, T. S. Jenkinson, C. Soto-Azat, C. Lambertini and A. V. Longo (2015). "Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research." Ecology and Evolution 5(18): 4079-4097.

11. Oidtmann, B. C., E. J. Peeler, M. A. Thrush, A. R. Cameron, R. A. Reese, F. M. Pearce, P. Dunn, T. M. Lyngstad, S. Tavornpanich and E. Brun (2014). "Expert consultation on risk factors for introduction of infectious pathogens into fish farms." Preventive Veterinary Medicine 115(3-4): 238-254.

12. Sitjà-Bobadilla, A. and B. Oidtmann (2017). Integrated pathogen management strategies in fish farming. Fish diseases, Elsevier**:** 119-144.

13. Oidtmann, B., P. Dixon, K. Way, C. Joiner and A. E. Bayley (2018). "Risk of waterborne virus spread–review of survival of relevant fish and crustacean viruses in the aquatic environment and implications for control measures." Reviews in aquaculture **10**(3): 641-669.

14. Romero, J. F., I. A. Gardner, L. Hammell, D. Groman, D. Whelan, N. O'Brien, L. J. Hawkins, H. Burnley and K. Thakur (2022). "Descriptive epidemiology of variants of infectious salmon anaemia virus in four Atlantic salmon farms in Newfoundland and Labrador, Canada." Journal of Fish Diseases **45**(6): 919-930.

15. Crane, M., and A. Hyatt (2011). "Viruses of fish: an overview of significant pathogens." Viruses 3(11): 2025-2046.

16. WOAH. (2019). "Manual of Diagnostic Tests for Animals, Chapter 2.3.1 Infection with Epizootic Haematopoietic Necrosis Virus. World Organisation for Animal Health." from https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_ehn.pdf.

17. CEFAS. (2022). "International Database on Aquatic Animal Diseases: Disease Data: Epizootic Haematopoietic Necrosis. Centre for Environment, Fisheries and Aquaculture Science." from https://www.cefas.co.uk/international-database-on-aquatic-animal-diseases/disease-data/?id=44

18. EURL. (2022). "Diagnostic Manuals. European Union Reference Laboratory for Fish and Crustacean Diseases." from https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals.

19. WOAH. (2022). "Aquatic Animal Health Code. World Organisation for Animal Health ", from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/

20. USDA-APHIS. (2022). "Voluntary 2022 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections and Infestations List. United States Department of Agriculture (USDA), Animal Plant and Health Inspection Service (APHIS) National List of Reportable Animal Diseases (NLRAD)." from https://www.aphis.usda.gov/animal_health/nahrs/downloads/nlrad-nahrs-disease-list.pdf.

21. USDA-APHIS. (2022). "National Animal Health Reporting System (NAHRS). United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) ", from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/sa_disease_reporting/ct_usda_aphis_animal_health.

22. Spickler (2007). Epizootic Hematopoietic Necrosis. Center for Food Security and Public Health, Iowa State University.

23. Hick, P., E. Ariel, and R. Whittington (2017). "Epizootic hematopoietic necrosis and European catfish virus." Fish Viruses and Bacteria: Pathobiology and Protection; PTK Woo, RC, Ed: 38-50.

24. Peeler, E., A. Afonso, F. Berthe, E. Brun, C. Rodgers, A. Roque, R. Whittington, and M. Thrush (2009). "Epizootic haematopoietic necrosis virus—an assessment of the likelihood of introduction and establishment in England and Wales." Preventive Veterinary Medicine 91(2-4): 241-253.

25. CFIA. (2022). "Epizootic Hematopoietic Necrosis. Canadian Food Inspection Agency.", from https://inspection.canada.ca/animal-health/aquatic-animals/diseases/reportable-diseases/epizootic-haematopoietic-necrosis-disease/fact-sheet/eng/1337100098837/1337100190147

26. EURL. (2022). "Epizootic Haematopoietic Necrosis (EHN). European Union Reference Laboratory for Fish and Crustacean Diseases." from https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals/ehn.

27. WOAH. (2022). "World Animal Health Information System (WAHIS) database. World Organisation for Animal Health." from https://wahis.woah.org/#/home.

28. Becker, J. A., D. Gilligan, M. Asmus, A. Tweedie and R. J. Whittington (2019). "Geographic Distribution of Epizootic haematopoietic necrosis virus (EHNV) in Freshwater Fish in South Eastern Australia: Lost Opportunity for a Notifiable Pathogen to Expand Its Geographic Range." Viruses 11(4): 315.

29. Whittington, R., R. Dixon, L. Li, T. Nguyen, A. Hyatt, and I. Marsh (2009). "Epizootic haematopoietic necrosis virus: epidemiology and uncertainty." Asian Fisheries Science 22(4): 1235-1255.

30. Hedrick, R., T. McDowell, J. Groff, S. Yun, and W. Wingfield (1992). "Isolation and some properties of an iridovirus-like agent from white sturgeon Acipenser transmontanus." Diseases of aquatic organisms 12(2): 75-81.

31. Gray, M. J., and V. G. Chinchar (2015). Ranaviruses: lethal pathogens of ectothermic vertebrates, Springer Nature.

32. Jancovich, J. K., N. K. Steckler and T. B. Waltzek (2015). Ranavirus taxonomy and phylogeny. Ranaviruses, Springer, Cham: 59-70.

33. Hedrick, R., and T. McDowell (1995). "Properties of iridoviruses from ornamental fish." Veterinary Research 26(5-6): 423-427.

34. Plumb, J. A., J. M. Grizzle, H. E. Young, A. D. Noyes, and S. Lamprecht (1996). "An iridovirus isolated from wild largemouth bass." Journal of Aquatic Animal Health 8(4): 265-270.

35. Mao, J., J. Wang, G. D. Chinchar, and V. G. Chinchar (1999). "Molecular characterization of a ranavirus isolated from largemouth bass Micropterus salmoides." Diseases of Aquatic Organisms 37(2): 107-114.

36. Chinchar, V. G., P. Hick, I. A. Ince, J. K. Jancovich, R. Marschang, Q. Qin, K. Subramaniam, T. B. Waltzek, R. Whittington and T. Williams (2017). "ICTV virus taxonomy profile: Iridoviridae." Journal of General Virology 98(5): 890-891.

37. Fu, X., Q. Lin, L. Liu, H. Liang, Z. Huang, and N. Li (2017). "The pathogenicity and biological features of Santee-Cooper Ranaviruses isolated from Chinese perch and snakehead fish." Microbial pathogenesis 112: 269-273.

38. Zhao, R., Y. Geng, Z. Qin, K. Wang, P. Ouyang, D. Chen, X. Huang, Z. Zuo, C. He and H. Guo (2020). "A new ranavirus of the Santee-Cooper group invades largemouth bass (Micropterus salmoides) culture in southwest China." Aquaculture 526: 735363

39. CABI. (2022). "Epizootic Haematopoietic Necrosis. CABI International Digital Library." from https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.83967#sec-5.

40. Whittington, R., J. Becker, and M. Dennis (2010). "Iridovirus infections in finfish–critical review with emphasis on ranaviruses." Journal of fish diseases 33(2): 95-122.

41. Whittington, R., C. Kearns, A. Hyatt, S. Hengstberger and T. Rutzou (1996). "Spread of epizootic haematopoietic necrosis virus (EHNV) in redfin perch (Perca fluviatilis) in southern Australia." Australian Veterinary Journal 73(3): 112-114.

42. Reddacliff, L. and R. Whittington (1996). "Pathology of epizootic haematopoietic necrosis virus (EHNV) infection in rainbow trout (Oncorhynchus mykiss Walbaum) and redfin perch (Perca fluviatilis L)." Journal of comparative pathology 115(2): 103-115.

43. NACAP. (2022). "Diseases of Finfish: Viral Diseases - Epizootic Haematopoietic Necrosis. Network of Aquaculture Centers in Asia-Pacific." from https://library.enaca.org/Health/FieldGuide/html/fv001ehn.htm.

44. Whittington, R., A. Philbey, G. Reddacliff and A. Macgown (1994). "Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, Oncorhynchus mykiss (Walbaum): findings based on virus isolation, antigen capture ELISA and serology." Journal of Fish Diseases 17(3): 205-218.

45. Whittington, R., L. Reddacliff, I. Marsh, C. Kearns, Z. Zupanovic and R. Callinan (1999). "Further observations on the epidemiology and spread of epizootic haematopoietic necrosis virus (EHNV) in farmed rainbow trout Oncorhynchus mykiss in southeastern Australia and a recommended sampling strategy for surveillance." Diseases of aquatic organisms 35(2): 125-130.

46. Langdon, J., J. Humphrey, L. Williams, A. Hyatt, and H. Westbury (1986). "First virus isolation from Australian fish: an iridovirus-like pathogen from redfin perch, Perca fluviatilis L." Journal of Fish Diseases 9(3): 263-268.

47. Langdon, J., and J. Humphrey (1987). "Epizootic haematopoietic necrosis, a new viral disease in redfin perch, Perca fluviatilis L., in Australia." Journal of Fish Diseases 10(4): 289-297.

48. Becker, J. A., A. Tweedie, D. Gilligan, M. Asmus and R. J. Whittington (2016). "Susceptibility of Australian redfin perch Perca fluviatilis experimentally challenged with epizootic hematopoietic necrosis virus (EHNV)." Journal of Aquatic Animal Health 28(2): 122-130.

49. Ariel, E., and B. B. Jensen (2009). "Challenge studies of European stocks of redfin perch, Perca fluviatilis L., and rainbow trout, Oncorhynchus mykiss (Walbaum), with epizootic haematopoietic necrosis virus." Journal of Fish Diseases 32(12): 1017-1025.

50. Borzym, E. and J. Maj-Paluch (2015). "Experimental infection with epizootic haematopoietic necrosis virus (EHNV) of rainbow trout (Oncorhynchus mykiss Walbaum) and European perch (Perca fluviatilis L.)." Journal of Veterinary Research 59(4): 473-478.

51. Langdon, J. (1989). "Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, Perca fluviatilis L., and 11 other teleosts." Journal of Fish Diseases 12(4): 295-310.

52. Ahne, W., Z. Matasin and G. Bovo (1995). "Antigenic relationship of epizootic haematopoietic necrosis virus (EHNV) and iridovirus-like isolates from European green frogs (Rana esculenta)." Bulletin of the European Association of Fish Pathologists 15(4): 142-144.

53. Monini, M. and F. M. Ruggeri (2002). "Antigenic peptides of the epizootic hematopoietic necrosis virus." Virology 297(1): 8-18.

54. Jensen, B. B., R. Holopainen, H. Tapiovaara and E. Ariel (2011). "Susceptibility of pike-perch Sander lucioperca to a panel of ranavirus isolates." Aquaculture 313(1-4): 24-30.

55. Rajendran, M. a. (2022). Viral Vaccines for Farmed Fish. Fish Immune System and Vaccines. R. Makesh. Singapore, Springer.

56. Oidtmann, B., C. Joiner, D. Stone, M. Dodge, R. Reese and P. Dixon (2011). "Viral load of various tissues of rainbow trout challenged with viral haemorrhagic septicaemia virus at various stages of disease." Diseases of aquatic organisms **93**(2): 93-104.

57. Becker, J. A., A. Tweedie, D. Gilligan, M. Asmus and R. J. Whittington (2013). "Experimental infection of Australian freshwater fish with epizootic haematopoietic necrosis virus (EHNV)." Journal of Aquatic Animal Health **25**(1): 66-76.

58. Langdon, J., J. Humphrey, and L. Williams (1988). "Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, Salmo gairdneri Richardson, in Australia." Journal of Fish Diseases 11(1): 93-96.

59. USDA-APHIS. (2022). "General NVSL Information. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Program (APHIS) ", from

https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/SA_About_NVSL/CT_About_nvsl.

60. Faheem (2021). Some Important Viral Diseases of Farmed Fish. Veterinary Pathobiology & Public Health. K. Abbas. Pakistan, Unique Scientific Publishers.