

Viral Hemorrhagic Septicemia

*Egtved Disease,
Infectious Nephrotic Swelling and
Liver Degeneration,
Abdominal Ascites of Trout
Infectious Anemia of Trout
Pernicious Anemia of Trout*

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the Center for
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IOWA STATE UNIVERSITY®

College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011
Phone: 515.294.7189
Fax: 515.294.8259
cfsph@iastate.edu
www.cfsph.iastate.edu



INSTITUTE FOR
INTERNATIONAL
COOPERATION IN
ANIMAL BIOLOGICS

Iowa State University
College of Veterinary Medicine
www.cfsph.iastate.edu/IICAB/

Importance

Viral hemorrhagic septicemia (VHS) is a serious systemic disease of fish. The VHS virus (VHSV) is carried by at least 50 species of marine and freshwater fish. The infection is subclinical in some species, but it is associated with severe disease and high mortality rates in others. Clinical infections are economically important in farmed fish, particularly rainbow trout, turbot and Japanese flounder. Outbreaks have also been reported in some wild populations, including Pacific herring and pilchard along the Pacific coast of North America.

Recently, viral hemorrhagic septicemia has become an emerging disease of freshwater fish in the Great Lakes region of North America. The virus was apparently introduced into this region by 2003, and deaths have been reported since 2005. Massive die-offs have occurred in some wild species. Affected fish include several warm-water species previously thought to be resistant to VHS. The epizootic seems to be caused by a new strain of VHSV. The source of this virus is unknown, but it may be a mutated marine virus that became pathogenic for naïve freshwater fish. This isolate causes moderate mortality in salmonid species not affected by other VHSV isolates, including Pacific (chinook) salmon, and could threaten farmed salmonids in the area.

Etiology

Viral hemorrhagic septicemia is caused by the viral hemorrhagic septicemia virus (VHSV or Egtved virus). This virus is a member of the genus *Novirhabdovirus*, family Rhabdoviridae. Currently, the evidence suggests that VHSV contains a single serotype with three subtypes. Both marine and freshwater isolates occur. Marine isolates are indistinguishable from freshwater isolates by routine serology.

Genetic analyses suggest that VHSV strains are closely related to other isolates from the same geographic region, rather than being grouped by host species. Genotype I contains traditional European freshwater isolates and isolates of northern European marine origin. Genotype II consists of marine isolates from the Baltic Sea. Genotype III contains viruses from the North Sea, Skagerrak and Kattegat. The North American isolates belong to Genotype IV. Most Japanese and Korean isolates also belong to the North American genotype, but at least one isolate from Japan is in the European group. The genotypes do not correlate with the sero-grouping system.

VHSV strains differ in their virulence for fish species. European freshwater strains cause severe disease in rainbow trout, while North American or northern European marine isolates are usually low pathogenic or not pathogenic for this species. Some marine isolates are pathogenic for turbot and Atlantic cod. The epizootic in the North American Great Lakes region seems to be caused by a new strain of the North American VHSV genotype. This isolate causes moderate mortality in salmonid species including lake trout, chinook salmon and steelhead trout, as well as warm-water species that were previously thought to be resistant to this disease. Circumstantial evidence suggests that non-virulent VHSV isolates can become virulent.

Species Affected

VHSV has been isolated from at least 50 species of marine and freshwater fish from the Northern Hemisphere, and other species have been infected in the laboratory. Species susceptible to infection include members of the Salmoniformes (salmon and trout), Pleuronectiformes (flounders, soles and other flatfishes), Gadiformes (cod), Esociformes (pike), Clupeiformes (herring and anchovy), Osmeriformes (smelt), Perciformes (perch and drum), Scorpaeniformes (rockfishes and sculpins), Anguilliformes (eels), Cyprinodontiformes (mummichog) and Gasterosteiformes (sticklebacks). Additional species continue to be reported. Many species of marine fish appear to be infected asymptotically, suggesting that VHSV is probably endemic in marine environments.

Clinical disease has been reported in some freshwater and marine species. Species currently known to be affected include rainbow trout, lake trout, steelhead trout, turbot, Japanese flounder, Pacific herring, Pacific hake, Atlantic salmon, Pacific

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salmon (chinook), grayling, whitefish (*Coregonus* spp.) halibut, sea bass, tube snout, Atlantic cod, blackcod, pilchard, ratfish, muskellunge, freshwater drum, round goby, shiner perch, yellow perch, smallmouth bass, white bass, walleye, bluegill, crappie, gizzard shad, redhorse sucker, blunt nose sucker and northern pike. Until recently, most warm-water fish were thought to be resistant to this disease; however, warm-water species such as drum and perch have been affected in recent outbreaks in the Great Lakes.

Geographic Distribution

Viral hemorrhagic septicemia affects farmed rainbow trout and some other freshwater species in continental Europe and Japan. VHSV has also been isolated from a variety of wild marine fish in North Atlantic, the Baltic Sea and the North American part of the Pacific and Atlantic Oceans. This virus has also been reported from Korea.

Transmission

VHSV is shed primarily in the urine and reproductive fluids (ovarian fluids, sperm). This virus has also been reported in the feces, but shedding is low. Reservoirs include clinically ill fish and asymptomatic carriers. Virus carriage seems to be lifelong, but shedding appears to be intermittent in carriers.

Transmission can occur through the water or by contact. VHSV is thought to enter the body through the gills or possibly through wounds. Predation on infected fish is also thought to be a route of transmission. Fish-eating birds can introduce VHSV into areas by acting as mechanical vectors.

Virus survival outside the host appears to vary with the strain. North American marine strains seem to be more sensitive to freeze-thaw cycles than European freshwater strains. Virus survival is inversely correlated with temperature, and is shorter at 20°C (68°F) than 4°C (40°F). Temperatures above 20°C (68°F) are particularly detrimental. Proteins such as ovarian fluids or serum prolong virus survival. One marine VHSV isolate remained infectious for more than 10 months in salt water with 1% serum, maintained at 4°C (40°F).

Incubation Period

The incubation period varies with water temperature. Between 1°C (34°F) and 12°C (54°F), the incubation period for European freshwater VHSV isolates is 1 to 2 weeks at warmer temperatures and 3 to 4 weeks at colder temperatures. Pacific herring infected experimentally with a marine isolate (by immersion) began to die after 4 to 6 days.

Clinical Signs

Affected rainbow trout are usually anorexic and may be either lethargic or hyperactive. Swimming behavior can also be abnormal. The coloring is usually darker than

normal but the gills are pale due to anemia and may have petechial hemorrhages. Hemorrhages can also be seen in the eyes and at the base of the fins, and sometimes on the body surface. Bilateral or unilateral exophthalmia and ascites may be present. A neurologic form characterized only by abnormal swimming behavior, such as constant flashing and/or spiraling, can also occur in this species. Chronic carriers may be asymptomatic.

Limited information is available on the symptoms in other species. In species such as turbot, Japanese flounder and sea bass, the clinical signs resemble those seen in rainbow trout. In other species, the classical syndrome may not be seen. After intraperitoneal injection of VHSV, limited exophthalmia and ascites were the major symptoms in juvenile Atlantic cod. In Pacific herring, the symptoms included petechial hemorrhages on the lower jaw, isthmus and eyes. A marine strain caused ascites and darkening of the skin in halibut.

Post Mortem Lesions [Click to view images](#)

Scattered hemorrhages may be seen in the skeletal muscles, perivisceral adipose tissue in the abdomen, swim (air) bladder, intestines and other organs. The spleen is usually enlarged and darker red than normal. The liver is also dark red early in the infection, but may later be a pale, chalky gray color. It can contain petechiae or mottling. The kidneys are dark red in the early stage of disease, but can be severely necrotic in moribund fish. The body cavity may be filled with ascitic fluid, and the gastrointestinal tract is usually empty of food. Fish with the nervous form may have no significant gross lesions.

Histopathologic lesions typically include extensive focal necrosis and degeneration in the kidney, liver and spleen. Evidence of hemorrhages may be seen in the muscles.

Morbidity and Mortality

VHSV infections appear to be particularly common in marine species. These infections are often subclinical. In parts of the Baltic Sea, the prevalence of this virus is 0-17% in herring and 6-8% in sprat. In coastal Oregon and California waters, one study reported a prevalence of 4-8% in apparently healthy sardines, mackerel, and smelt.

Clinical disease has been reported in freshwater fish and occasionally in marine species. In rainbow trout, most epizootics occur on freshwater farms, but outbreaks have also been reported when these fish are cultured in brackish water or seawater. Mass mortalities have been reported in wild North American populations, including marine fish along the Pacific coast and freshwater fish from the Great Lakes region. Clinical disease can occur at any age, but younger fish appear to be most susceptible. Stress is a predisposing factor, and outbreaks can occur in subclinical carriers after a stressful event. Water temperature also influences the likelihood of infection. The optimal temperature for active infection is 9-12°C (48-54°F); most

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outbreaks occur when water temperatures are less than 15°C (59°F). Viral hemorrhagic septicemia has not been reported when water temperature is above 18°C (64°F). Outbreaks often occur in the spring, when the temperature of the water is either rising or fluctuating.

The morbidity and mortality rates vary with the environmental conditions as well as the species of fish, strain of virus and route of infection. The mortality rate can be as high as 80-100% in rainbow trout fry. In older rainbow trout, it is usually 10-70%. Cumulative mortality rates of 0% to 96% have been reported in turbot. In VHSV-infected wild Atlantic salmon, cumulative mortality of 10% was reported in one population, and 2% mortality per week in another. By injection, mortality rates as high as 78% have been reported in this species. Some North American VHSV isolates are highly pathogenic to Pacific herring by immersion, with a mortality rate approaching 100%. In Japanese flounder, these isolates result in mortality rates as high as 50–70% during outbreaks, and up to 100% after experimental infection. In halibut, a marine strain caused mortality rates of 2-20% when the fish were infected by immersion and 28-80% after intraperitoneal injection.

Diagnosis

Clinical

Viral hemorrhagic septicemia should be suspected in rainbow trout, turbot, Japanese flounder and other susceptible species with hemorrhages, exophthalmia, nervous signs or other symptoms consistent with this disease. Water temperatures are expected to be in the 1-18°C (34-64°F) range; disease has not been reported in temperatures above this range.

The differential diagnosis includes infectious hematopoietic necrosis, enteric red mouth disease and furunculosis.

Laboratory tests

Viral hemorrhagic septicemia can be diagnosed by virus isolation in cell cultures; appropriate cell lines include BF-2 (Bluegill fry) and RTG-2 (Rainbow trout gonad) cells. EPC (Epithelioma papulosum cyprini) and FHM (fathead minnow) cells can also be used, but are less susceptible to infection by freshwater European strains. EPC cells are the preferred cell line in North America. Virus identity is confirmed by virus neutralization, immunofluorescence (IFA), an enzyme-linked immunosorbent assay or a polymerase chain reaction (PCR)-based assay.

Viral antigens can also be identified directly in tissues, particularly the kidney and spleen, by immunofluorescence, immunohistochemistry or ELISA. PCR can also be used.

Serology by virus neutralization or ELISA may be effective in detecting carriers, but has not yet been validated for routine diagnosis.

Samples to collect

VHSV is most abundant in the kidney, spleen, encephalon and heart. The samples to collect depend on the size of the fish. Small fish (alevin and yolk sac fry less than or equal to 4 cm) should be sent whole, but the yolk sac should be removed if it is present. The viscera including the kidney should be collected from fish that are 4 to 6 cm long. The kidney, spleen, heart and encephalon should be sent from larger fish. Samples of ovarian fluid should also be collected from broodfish at spawning. Samples should be taken from ten diseased fish and combined to form pools with approximately 1.5 g of material (no more than five fish per pool).

The pools of organs or ovarian fluids should be placed in sterile vials. The samples may also be sent in cell culture medium or Hanks' balanced salt solution with antibiotics. They should be kept cold [4°C (39°F)] but not frozen. If the shipping time is expected to be longer than 12 hours, serum or albumen (5-10%) may be added to stabilize the virus. Ideally, virus isolation should be done within 24 hours after fish sampling.

Recommended actions if viral hemorrhagic septicemia is suspected

Notification of authorities

Viral hemorrhagic septicemia should be reported to state or federal authorities immediately upon diagnosis or suspicion of the disease.

Federal: Area Veterinarians in Charge (AVIC):

www.aphis.usda.gov/animal_health/area_offices/

State Veterinarians:

www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf

Control

Viral hemorrhagic septicemia is a highly contagious disease; quarantines are necessary to control outbreaks. There is evidence that VHSV is transferred from wild fish to farmed fish and vice versa. Current control methods include fish health surveillance programs and measures such as eradication and fallowing. These procedures have eliminated viral hemorrhagic septicemia from parts of Europe. VHSV can survive for long periods in the bottom of farm ponds if the ponds are not dried and disinfected.

VHSV is sensitive to many common disinfectants including formalin, iodophor disinfectants, sodium hydroxide and sodium hypochlorite. The virucidal activity of disinfectants is reduced when they are diluted in seawater. VHSV is very sensitive to UVC (280-200 nm wavelength) irradiation, which can be used to treat inflow water for hatcheries, or treat water in recirculation systems. It is also highly thermolabile. In addition, this virus is inactivated by drying and pH 2.5 or 12.2. The effectiveness of lime disinfection is suspect.

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Methods to decrease the impact of viral hemorrhagic septicemia in endemic areas include hatchery disinfection, the use of specific-pathogen free (SPF) stock and spring or bore water, and management methods that decrease physiological stressors. Co-cultivation of flatfish and salmonids (particularly rainbow trout) should be avoided, as VHSV can be transmitted between species and non-virulent isolates could become virulent. There are no effective antiviral agents for the control of this disease, and no commercial vaccines exist.

Public Health

There is no indication that this disease is a threat to human health.

Internet Resources

- USDA APHIS Viral Hemorrhagic Septicemia
http://www.aphis.usda.gov/newsroom/hot_issues/vhs/vhs.shtml
- World Organization for Animal Health (OIE)
<http://www.oie.int>
- OIE Manual of Diagnostic Tests for Aquatic Animals
<http://www.oie.int/international-standard-setting/aquatic-manual/access-online/>
- OIE Aquatic Animal Health Code
<http://www.oie.int/international-standard-setting/aquatic-code/access-online/>

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