2.2.7 Viral Hemorrhagic Septicemia

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A. Name of Disease and Etiological Agent

Viral hemorrhagic septicemia (VHS) is one of the most important viral diseases of finfish worldwide. In the past, VHS was thought to affect mainly rainbow trout *Oncorhynchus mykiss* reared at freshwater facilities in Western Europe where it was known by various names including Egtved disease and infectious kidney swelling and liver degeneration (Wolf 1988). Today, VHS is known as an important source of mortality for cultured and wild fish in freshwater and marine environments in several regions of the northern hemisphere (Dixon 1999; Gagné et al. 2007; Kim and Faisal 2011; Lumsden et al. 2007; Marty et al. 1998, 2003; Meyers and Winton 1995; Skall et al. 2005b; Smail 1999; Takano et al. 2001). Viral hemorrhagic septicemia is caused by the fish rhabdovirus, viral hemorrhagic septicemia virus (VHSV), a member of the genus *Novirhabdovirus* of the family *Rhabdoviridae*.

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Viral hemorrhagic septicemia is endemic among marine and freshwater fish in Western Europe, North America and Eastern Asia. Countries or regions where VHSV has been isolated from natural infections of fish using cell culture methods and confirmed by serological or molecular assays are listed in Table 1. Isolates of VHSV from different geographic areas tend to group into distinct genotypes (Benmansour et al. 1997; Einer-Jensen et al. 2004, 2005 a, b; Elsayed et al. 2006; Kim et al. 2003; Nishizawa et al. 2002; Snow et al. 1999, 2004; Stone et al. 1997; Thiéry et al. 2002; Thompson et al. 2011).

2. Host Species

Over 60 species of freshwater and marine fish are currently known to be natural hosts of VHSV (Table 1). Other species have been shown to be experimentally susceptible (Wolf 1988). In addition, invertebrates including a leech and an amphipod have been found to harbor the virus (Table 1). While large outbreaks of disease associated with high mortality have occurred in aquaculture facilities and in some populations of wild fish, VHSV has also been isolated from fish that appeared normal.

C. Epizootiology

VHSV is readily transmissible to susceptible fish of all ages. The main portal of entry is believed to be the epithelial tissues of the gills or skin, especially at the base of the fins (Harmache et al. 2006). Disease outbreaks are typically seen at water temperatures from 3-12°C and are most severe at lower

temperatures. As temperature increases, mortality and the proportion of virus carriers decreases. At temperatures above 15°C, mortality from VHS is typically low (Goodwin and Merry 2011). Infected fish mount a strong interferon response, which may play a role in transiently mitigating the effects of VHS at higher temperature. The antibody response in survivors of epizootics and in fish with inapparent infections is variable. In captive fish, the disease can recur as a consequence of immunosuppression due to culture conditions and stress.

Table 1. Natural Host and Geographic Range of Viral Hemorrhagic Septicemia Virus.

Common name	Scientific name	Geographic location	Reference
Rainbow trout	*Oncorhynchus mykiss*	Europe (most countries) Northeastern US	Wolf 1988; Skall et al. 2005b Thompson et al. 2011
Brown trout	*Salmo trutta*	Western Europe Atlantic Coast - North America	deKinkelin and Le Berre 1977; Gagné et al. 2005
Northern pike	*Esox lucius*	Western Europe Great Lakes – North America	Meier & Jorgensen 1979; Thompson et al. 2011
Grayling	*Thymallus thymallus*	Switzerland	Meier & Wahli 1988
Whitefish	*Coregonus* sp.	Switzerland	Meier et al. 1994
European eel	*Anguilla anguilla*	France	Castric et al. 1992
Largemouth bass	*Micropterus salmoides*	France Great Lakes – North America	deKinkelin et al. 1999; Thompson et al. 2011
Turbot	*Scophthalmus maximus* *Psetta maxima*	Germany, Gigha, Ireland, Black Sea (Turkey)	Schlotfeldt et al. 1991; Ross et al. 1994; Nishizawa et al. 2006
Atlantic cod	*Gadus morhua*	Baltic Sea, North Sea, North Atlantic	Jensen et al. 1979; Mortensen et al. 1999; Smail 2000; King et al. 2001
Atlantic herring	*Clupea harengus*	Baltic Sea; English Channel; Kattegat; Skagerrak, North Sea	Mortensen et al. 1999; Dixon et al. 1997; King et al. 2001
European sprat	*Sprattus sprattus*	Baltic Sea	Mortensen et al. 1999
Common dab	*Limanda limanda*	Kattegat; Baltic Sea	Skall et al. 2005a
European plaice	*Pleuronectes platessa*	Skagerrak; Kattegat	Skall et al. 2005a
European flounder	*Platichthys flesus*	Baltic Sea	Skall et al. 2005a
Sand goby	*Pomatoschistus minutus*	Baltic Sea	Skall et al. 2005a
Sand eel	*Ammodytes* sp.	Baltic Sea	Skall et al. 2005a
Four-bearded rockling	*Rhinonemus cimbrius*	Baltic Sea	Mortensen et al. 1999
Haddock	*Melanogrammus aeglefinus*	North Sea	Smail 2000
Norway pout	*Trisopterus esmarkii*	North Sea; North Atlantic; Skagerrak	Mortensen et al. 1999; King et al. 2001
Poor cod	*Trisopterus minutus*	North Atlantic	King et al. 2001
Whiting	*Merlangius merlangus*	North Sea	Mortensen et al. 1999; King et al. 2001
Lesser argentine	*Argentina sphyraena*	North Sea	Mortensen et al. 1999
Blue whiting	*Micromesistius poutassou*	North Sea	Mortensen et al. 1999
Greenland halibut	*Reinhardtius hippoglossoides*	Flemish Cap - North Atlantic	Dopazo et al. 2002
Chinook salmon	*Oncorhynchus tshawytscha*	Pacific Coast - North America; Great Lakes – North America	Winton et al. 1991; Thompson et al. 2011
Coho salmon	*Oncorhynchus kisutch*	Pacific Coast - North America	Winton et al. 1991
Steelhead	*Oncorhynchus mykiss*	Pacific Coast - North America	Winton et al. 1991
Atlantic salmon	*Salmo salar*	Pacific Coast - North America Spain	Traxler et al. 1995; Jimenez de la Fuente et al. 1988
Pacific cod	*Gadus macrocephalus*	Pacific Coast - North America	Meyers et al. 1992
Pacific herring	*Clupea pallasi*	Pacific Coast - North America	Meyers et al. 1994; Kocan et al.

			2001
Tube-snout	*Aulorhynchus flavidus*	Pacific Coast - North America	Traxler et al. 1995
Walleye pollock	*Theragra chalcogramma*	Pacific Coast - North America	Meyers et al. 1999
Pacific hake	*Merluccius productus*	Pacific Coast - North America	Meyers et al. 1999
Pacific tomcod	*Microgadus proximus*	Pacific Coast - North America	Meyers et al. 1999
English sole	*Parophrys vetula*	Pacific Coast - North America	Hershberger et al. 1999
Pacific sand lance	*Ammodytes hexapterus*	Pacific Coast - North America	Kocan et al. 2001
Pacific sardine (Pilchard)	*Sardinops sagax*	Pacific Coast - North America	Hedrick et al. 2003
Sablefish (Black cod)	*Anoplopoma fimbria*	Pacific Coast - North America	Hedrick et al. 2003
Eulachon	*Thaleichthys pacificus*	Pacific Coast - North America	Hedrick et al. 2003
Surf smelt	*Hypomesus pretiosus*	Pacific Coast - North America	Hedrick et al. 2003
Shiner perch	*Cymatogaster aggregata*	Pacific Coast - North America	Hedrick et al. 2003
Pacific mackerel	*Scomber japonicus*	Pacific Coast - North America	Hedrick et al. 2003
Japanese (Olive) flounder	*Paralichthys olivaceus*	Japan, Korea	Takano et al. 2000, 2001
Japanese sandlance	*Ammodytes personatus*	Japan	Watanabe et al. 2002
Mebaru (Black rockfish)	*Sebastes inermis*	Japan	Isshiki et al. 2003
3-spine stickleback	*Gasterosteus aculeatus*	Pacific Coast - North America Atlantic Coast - North America	Kent et al. 1998; Olivier 2002
Mummichog	*Fundulus heteroclitus*	Atlantic Coast - North America	Olivier 2002
Striped bass	*Morone saxatilis*	Atlantic Coast - North America	Gagne et al. 2007
Freshwater drum	*Aplodinotus grunniens*	Great Lakes - North America	Lumsden et al. 2007
Muskellunge	*Esox masquinongy*	Great Lakes - North America	Elsayed et al. 2006
Round goby	*Neogobius melanostomus*	Great Lakes - North America	Groocock et al. 2007
Channel catfish	*Ictalurus punctatus*	Great Lakes - North America	Thompson et al. 2011
Common carp	*Cyprinus carpio*	Inland Lakes - North America	Thompson et al. 2011
Lake trout	*Salvelinus namaycush*	Inland Lakes - North America	Thompson et al. 2011
Pumpkinseed	*Lepomis gibbosus*	Great Lakes - North America	Thompson et al. 2011
White perch	*Morone americana*	Great Lakes - North America	Thompson et al. 2011
Sea lamprey	*Petromyzon marinus*	Great Lakes - North America	Thompson et al. 2011
Yellow perch	*Perca flavescens*	Great Lakes - North America	Kane-Sutton et al. 2009
Smallmouth bass	*Micropterus dolomieu*	Great Lakes - North America	Thompson et al. 2011
White bass	*Morone chrysops*	Great Lakes - North America	Thompson et al. 2011
Walleye	*Sander vitreus*	Great Lakes - North America	Thompson et al. 2011
Burbot	*Lota lota*	Great Lakes - North America	Thompson et al. 2011
Trout-perch	*Percopsis omiscomaycus*	Great Lakes - North America	Thompson et al. 2011
Emerald shiner	*Notropis atherinoides*	Great Lakes - North America	Thompson et al. 2011
Gizzard shad	*Dorosoma cepedianum*	Great Lakes - North America	Thompson et al. 2011
Bluntnose minnow	*Pimephales notatus*	Great Lakes - North America	USDA-APHIS 2008
Bluegill	*Lepomis macrochirus*	Great Lakes - North America	Thompson et al. 2011
Brown bullhead	*Ameiurus nebulosis*	Great Lakes - North America	Thompson et al. 2011
Lake whitefish	*Coregonus clupeaformis*	Great Lakes - North America	Thompson et al. 2011
Shorthead redhorse sucker	*Moxostoma macrolepidotum*	Great Lakes - North America	Thompson et al. 2011
Silver redhorse sucker	*Moxostoma anisurum*	Great Lakes - North America	Kim and Faisal 2011
Rock bass	*Ambloplites rupestris*	Great Lakes - North America	Thompson et al. 2011
Black crappie	*Pomoxis nigromaculatus*	Great Lakes - North America	Thompson et al. 2011
Spottail shiner	*Notropis hudsonius*	Great Lakes - North America	Kim and Faisal 2011
Lake herring	*Coregonus artedi*	Great Lakes – North America	Thompson et al. 2011
Piscicolid leech	*Myzobdella lugubris*	Great Lakes – North America	Faisal and Schulz 2009
Amphipods	*Diporeia* sp.	Great Lakes – North America	Thompson et al. 2011

D. Disease Signs

A variety of clinical signs and histopathological changes can be apparent in infected fish (Wolf 1988; Smail 1999; Lumsden et al. 2007; Kim and Faisal 2010). Some fish can show profound clinical manifestation whereas others appear to be nearly normal. Historically, clinical and histological signs of VHS have been categorized into acute, chronic, and latent forms or stages. Such descriptions represent degrees of severity rather than progressive forms or stages of the disease.

External clinical signs of disease can include exophthalmia, abdominal distention, darkened coloration, anemia, lethargy, hyperactivity, and hemorrhages in the eyes, skin, gills, and at the base of fins. Internally, visceral mesenteries can show diffuse hemorrhage, the kidneys and liver can be hyperemic, swollen, and discolored, and hemorrhages can occur in skeletal muscle.

Histopathological changes are generally confined to the liver, kidney, spleen, and skeletal musculature. In the liver, kidney and spleen, focal to extensive necrotic changes can occur, i.e., vacuolation, pyknosis, karyolysis and lymphocytic infiltration. The hematopoietic areas of the kidney and spleen are the principal sites of viral replication.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Clinical signs and histopathological changes associated with VHS are variable and cannot be used for definitive diagnosis or to distinguish VHS from the other fish viral diseases. The absence of clinical signs does not indicate that the fish are free from VHSV. Consequently, virological examination is required for diagnosis of VHS. Virological examination consists of assaying tissue and reproductive fluid samples in cell culture or by a validated serological or molecular assay. For cell culture assays, the *Epithelioma papulosum cyprini* (EPC) and fathead minnow (FHM) cell lines are considered to be the most sensitive, although VHSV will replicate in the rainbow trout gonad (RTG-2), bluegill fry (BF-2) and to a lesser degree in Chinook salmon embryo (CHSE-214) cell lines. During incubation, it is critically important that the pH of the medium remain within the range of 7.4 - 7.8 because the glycoprotein of VHSV undergoes a pH-dependent conformational change that can prevent development of cytopathic effect (CPE) in acidic cultures (Gaudin et al. 1995). This is especially problematic for cell lines derived from coolwater species that continue to metabolize efficiently at the incubation temperatures of the assay. In recent years, polymerase chain reaction (PCR) assays have become widely used for presumptive diagnosis of the disease (Winton and Einer-Jensen 2002).

2. Confirmatory Diagnosis

Confirmatory diagnosis of VHS and identification of VHSV can be performed using a serum neutralization assay, immunoblot assay (McAllister and Owens 1987; McAllister and Schill 1986), enzyme-linked immunosorbent assay (ELISA; Mourton et al. 1992; Olesen and Jorgensen 1991; Way and Dixon 1988), fluorescent antibody test (FAT; Lorenzen et al. 1988), DNA probe (Batts et al. 1993), PCR (Winton and Einer-Jensen 2002) and sequence analysis (Snow et al. 2004). For the serum neutralization assay, the cell cultures and the conditions of incubation and pH control must be maintained as indicated above. Antiserum specific to each serotype must be used in the serum neutralization assay because three serological types of VHSV can be distinguished by certain neutralizing antisera. For the ELISA and FAT, polyclonal or monoclonal antibodies are available that react with all VHSV serotypes. Primer sets for RT-PCR and quantitative RT-PCR assays that react with all genotypes of VHSV can be used for confirmatory diagnosis of the disease as well as for

identification of VHSV that has been isolated in cell culture (Winton and Einer-Jensen 2002; Garver et al. 2011). Confirmed isolates of VHSV from a new host species or a new geographic area should be sent to a reference laboratory for additional molecular or serological analysis and the finding reported to state or national fisheries agencies and, in the United States, to USDA-APHIS.

F. Procedures for Detecting Subclinical Infections

Subclinical infections can be detected by cell culture assay and by PCR. In some instances, VHSV has only been detected by examination of certain tissues or organs such as the brain.

G. Procedures for Determining Prior Exposure to the Etiological Agent

The specific immune response among survivors of VHS epizootics and unapparent virus carriers varies with both the fish and season of the year. Nevertheless, detection of VHSV-specific antibody can be useful as part of a VHSV surveillance program for evidence of past infections and for determining the status of the protective immune response following vaccination (Bernard et al. 1983; Olesen and Jorgensen 1986).

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

Samples should be sent on ice, preferably below 4°C, but not frozen. Tissue samples may be moistened with buffered physiological saline (pH 7.4 - 7.8) or cell culture medium (pH 7.4 - 7.8). Samples should be processed within 48hrs. Samples should not be frozen nor should glycerol be used to preserve specimens because VHS virus has been shown to be inactivated by these methods.

I. Procedures for Enumeration of VHSV

The EPC cell line is recommended for enumeration of VHSV infectious units via a plaque assay. Virus adsorption to EPC cells can be enhanced by pretreating the cells with a 7% solution (final concentration) of polyethylene glycol (PEG: 20,000 MW (Sigma Aldrich Catalog # P-2263); Batts and Winton 1989) or by adding DEAE dextran (Campbell and Wolf 1969; 50 μg/mL final concentration. Quantitative RT-PCR assays have been developed that can be used to estimate virus loads for some (Chico et al. 2006; Hope et al. 2010) or all strains of VHSV (Garver et al. 2011).

J. Procedures for Determination of Disease-free Status

Inspection procedures for determination of disease-free status rely upon virological assays using cell culture. This is because all strains of VHSV can be grown in cells and because any isolates obtained can be examined using one or more of the confirmatory tests discussed above. In addition, cell culture assays have a long track record of use and, while potentially less sensitive than some newer PCR-based methods, are considered the gold standard. In the future, it is expected that some of the newer molecular assays will be sufficiently validated against this gold standard to become accepted methods for determination of disease-free status.

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