Australian Government
Department of Agriculture

Australian Aquatic Veterinary Emergency Plan

AQUAVETPLAN

Disease Strategy

Viral haemorrhagic septicaemia

Version 2.0, 2014

AQUAVETPLAN is a series of manuals that outline Australia's approach to national disease preparedness and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency.

National Biosecurity Committee

This disease strategy forms part of:

AQUAVETPLAN

This strategy will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to: AQUAVETPLAN Coordinator, Aquatic Pest and Health Policy, Animal Health Policy, Australian Government Department of Agriculture, GPO Box 858, Canberra ACT 2601

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It is the responsibility of the users of this publication to identify and ensure they have complied with all legislative or regulatory requirements of the relevant Australian state or territory and the Commonwealth prior to undertaking any of the response options set out within this publication.

Being a guide only, outbreaks or suspected outbreaks must be assessed on a case by case basis and expert advice should be obtained to determine the most appropriate management plan in response to the risk.

IMPORTANT NOTE: Important regulatory information is contained in the OIE *Aquatic Animal Health Code*, which is updated annually and is available at:

http://www.oie.int/en/international-standard-setting/aquatic-code/access-online

DISEASE WATCH HOTLINE

1800 675 888

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency animal disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance.

Preface

This disease strategy for the control and eradication of viral haemorrhagic septicaemia (VHS) is an integral part of the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN).

AQUAVETPLAN disease strategy manuals are response manuals and do not include information about preventing the introduction of disease.

The Department of Agriculture provides quarantine inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture ICON website (biosecurity import icon). Specific risk management measures for VHS in imported fish are discussed in *Import risk analysis on non-viable salmonids and non-salmonid marine finfish* (http://www.agriculture.gov.au/ba/ira/final-animal/salmon).

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of VHS in Australia. The strategy was scientifically reviewed by the Sub-Committee on Aquatic Animal Health before being endorsed by the Animal Health Committee of the Primary Industries Standing Committee in July 2013.

VHS is listed by the World Organisation for Animal Health (OIE) in the *Aquatic Animal Health Code* (OIE 2013) (http://www.oie.int/en/international-standard-setting/aquatic-code/access-online).

VHS is listed on Australia's *National List of Reportable Diseases of Aquatic Animals* (http://www.agriculture.gov.au/animal-plant-health/aquatic/reporting).

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the enterprise manual. Table 1 lists available AQUAVETPLAN manuals.

AQUAVETPLAN manuals

Disease strategy manuals	**Operational procedures manuals**
Crayfish plague	Disposal
Furunculosis	Destruction
Infectious salmon anaemia	Decontamination
Piscirickettsiosis	
Viral encephalopathy and retinopathy	Enterprise manual
Viral haemorrhagic septicaemia	Includes sections on:
Whirling disease	– open systems
White spot disease	– semi-open systems
Withering syndrome of abalone	– semi-closed systems – closed systems
Management manual	
Control centres management	

The second edition of the VHS disease strategy manual was prepared by Dr Ben Diggles and Dr Matt Landos and revises the earlier version (version 1.0) developed by Dr Paul Hardy-Smith, with the assistance of Professor Ron Hedrick, Professor Barry Hill, Dr Craig Stephens and Dr Mark Crane, which was published in June 2005. The current authors were responsible for reviewing the first edition of the strategy, in consultation with stakeholders from aquaculture, recreational fishing and government sectors throughout areas of Australia in which there are aquatic species susceptible to VHS. The text was amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not necessarily reflect the views of the members of the writing group. Contributions made by others not mentioned above are also gratefully acknowledged.

The format of this manual was adapted from similar manuals from AUSVETPLAN. The format and content have been kept as similar as possible to these documents, to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and permission to use the original AUSVETPLAN documents are gratefully acknowledged.

The revised manual has been reviewed and approved by the following representatives of government and industry:

Government
Department of Primary Industries, New South Wales
Department of Primary Industry and Fisheries, Northern Territory
Department of Agriculture, Fisheries and Forestry, Queensland
Department of Primary Industries, Parks, Water and Environment, Tasmania
Department of Fisheries, Western Australia
Department of Environment and Primary Industries, Victoria
Department of Primary Industries and Regions, South Australia
Biosecurity, Australian Government Department of Agriculture

Industry
Recfishing Research
Recfish Australia
Chair, National Aquatic Animal Health Industry Reference Group

The complete series of AQUAVETPLAN documents is available at
http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan

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1 Nature of the disease

Viral haemorrhagic septicaemia (VHS) is an infectious disease of freshwater and marine fish caused by the viral haemorrhagic septicaemia virus (VHSV), a rhabdovirus. VHS was first recognised by Schaeperclaus in Germany in 1938, and in 1949 the disease was referred to as Egtved disease after a village in Denmark where the outbreak occurred (Jensen 1965). In 1966, the World Organisation for Animal Health (OIE) recommended that the name be changed to viral haemorrhagic septicaemia (Warren 1983). The virus has caused significant mortality and economic loss, first in the aquaculture of salmonids and marine fish in Europe and North America, and more recently in wild fisheries in marine and freshwater environments in North America.

The list of species recorded to have had the disease in the wild continues to increase and includes marine fish such as pilchards (*Sardinops sagax*), Pacific herring (*Clupea pallasii*), flounder (*Paralichthys olivaceus*) and freshwater species in Europe and North America. Isolates differ markedly in virulence and pathogenicity and clinical signs of disease are often not observed in many fish species infected with VHSV. Outbreaks of disease most often occur in susceptible fish populations in water temperatures of 4–14°C. Outbreaks in low water temperatures often result in an extended disease course with low daily mortality but high accumulated mortality. Three forms of the disease (acute, chronic and nervous) have been identified and clinical signs described. The disease generally takes a short course with a modest accumulated mortality at water temperatures of 15–18°C. Mortality and morbidity have rarely been documented when water temperatures are above 18°C, although VHSV has caused at least one fish kill at 20–22°C and some isolates can replicate in vitro at temperatures of up to 25°C (OIE 2013b).

VHSV has never been reported in Australia, despite ongoing passive surveillance and targetted surveillance in some jurisdictions for trade certification purposes. Australia is considered free of VHSV. This manual is a guide for response in case of detection of VHSV in Australia.

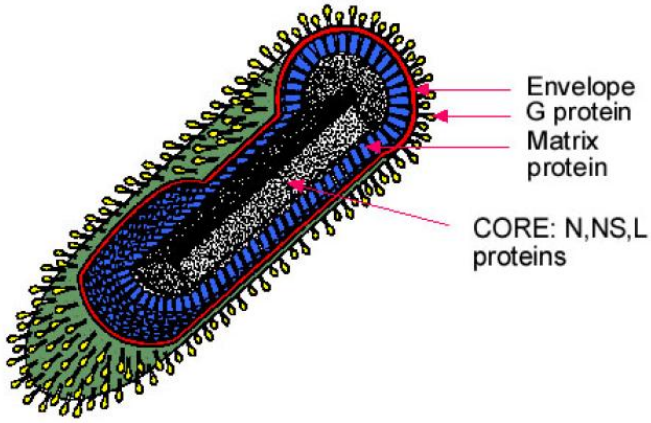
Serotyping, genotyping and challenge trials have confirmed significant differences between VHSV isolates in genome structure and virulence. Separation of isolates into distinct genogroups or types is based primarily on geographic origin. VHSV has never been isolated in Australia.

VHS is listed on Australia's *National List of Reportable Diseases of Aquatic Animals* and is listed by the OIE. Isolation of VHSV, with or without clinical signs of disease, must be reported to the Chief Veterinary Officer (CVO) in the jurisdiction where it was found.

1.1 Aetiology

The causative agent of VHS is a negative sense single-stranded RNA (ssRNA) virus belonging to the genus *Novirhabdovirus* (family *Rhabdoviridae*) (Figure 1) (Tordo et al. 2005).

Figure 1 Schematic representation of a typical rhabdovirus



(courtesy G Traxler)

The virus is bullet shaped, approximately 180 nm in length and 70 nm in diameter. Virions contain a negative-sense ssRNA genome, approximately 11 kb long and encoding six proteins: a nucleoprotein (N), a glycoprotein (G), a phosphoprotein (P), a matrix protein (M), a nonvirion protein (NV) and a polymerase (L) (Snow et al. 2004, Einer-Jensen et al. 2005). The membrane glycoprotein of the envelope of VHSV is the major neutralising surface antigen (Wolf 1988).

The categorisation of isolates is ongoing. Sequence comparisons between isolates have indicated that genetic differences are related to geographic location rather than to host species or year of isolation. Based on sequencing of full-length and/or truncated genes from the G gene (Einer-Jensen et al. 2004, Einer-Jensen et al. 2005), N gene (Snow et al. 1999, Snow et al. 2004, Einer-Jensen et al. 2005) and NV gene (Einer-Jensen et al. 2005), VHSV isolates have been categorised into four distinct genotype groups (designated I–IV). The principal genotypes are:

Type I — Continental Europe freshwater and marine (North and Baltic Sea) group

Genotype I contains isolates considered highly pathogenic for rainbow trout (*Oncorhynchus mykiss*). This genotype has been further divided into five sublineages (Ia–Ie) containing European freshwater isolates, isolates from Kattegat, Skagerrak and the Black Sea, and a group of marine isolates from the Baltic Sea, North Sea and English Channel (Einer-Jensen et al. 2004, Nishizawa et al. 2006, OIE 2013b). Genotype Ia includes isolates of freshwater origin which have caused most outbreaks of VHSV disease in European trout farming (Kahns et al. 2012). Isolates from this group have also been reported to cause disease outbreaks in wild Northern pike (*Esox lucius*), graylings (*Thymallus thymallus*) and white fish (*Coregonus* spp.). Genotype 1b includes isolates from a wide range of wild-caught marine fish species from the North and Baltic Seas. This group has been shown to be of generally low pathogenicity to trout. It is thought that the marine VHSV was introduced into rainbow trout farming in the early days of the industry, when it adapted to virulence and was subsequently propagated through the industry. Genetic evidence suggests this may have occurred on multiple independent occasions (Einer-Jensen et al. 2004).

Type II — European marine group (principally from the Baltic Sea)

Genotype II contains isolates considered less pathogenic for rainbow trout and turbot (*Psetta maxima* [syn. *Scophthalmus maximus*]) (e.g. Snow et al. 2005). Genotype II isolates naturally infect at least eight marine species in the Baltic Sea and North Sea, with an overall prevalence of 4–6%. However, large variations in prevalence can occur between species, sampling locations and sampling periods (Skall et al. 2005a). Wild fish infected with genotype II VHSV isolates appear clinically healthy but are generally more likely to carry external parasitic infections (Skall et al. 2005a).

Type III — North Atlantic marine group

Genotype III comprises isolates from the North Atlantic (from the Flemish Cap to the Norwegian coast, the North Sea, Skagerrak and Kattegat (López-Vázquez et al. 2006, OIE 2013b). A distinct strain from this group was responsible for disease outbreaks in farmed marine rainbow trout from Norway, and for up to 70% mortality in experimental immersion challenges (Dale et al. 2009). Experimental challenges with other strains of this type were far less pathogenic and generally did not cause disease. Genotype III has caused mortality in farmed turbot in the United Kingdom (UK) (Ross et al. 1994, *in* Snow et al. 2005).

Type IV — North American and East Asian group

Type IV genotypes contain two sublineages (IVa and IVb) corresponding to North Western Pacific, Japanese and Korean isolates; and eastern North American isolates, respectively. Genotype IVa emerged in marine fish, particularly Pacific herring *(C. pallasii*) and pilchards *(S. sagax*) in the Pacific Northwest region of North America and subsequently from Japan and Korea (Muroga et al. 2004, Skall et al. 2005a, Kim et al. 2009, Hershberger et al. 2010a, 2010b). In 2003, a novel sublineage of VHSV emerged in the Laurentian Great Lakes basin causing massive fish kills in at least 28 species of freshwater fish (Elsayed et al. 2006, USDA 2006, 2007, Gagné et al. 2007, Groocock et al. 2007, Lumsden et al. 2007, Gustafson 2009, Kim and Faisal 2010). Rainbow trout appear refractory to infection with this genotype (Al-Hussinee et al. 2010). The Great Lakes isolate is distinct from all four previously known genotypes. Since it is phylogenetically closely related to the marine VHSV-IVa isolate, it was designated as VHSV-IVb (Elsayed et al. 2006). The index strain of the Great Lakes VHSV was VHSV IVb-MI03 (GenBank number DQ427105).

The growing number of VHSV isolates and their sequences has necessitated the development of a database (http://www.fishpathogens.eu/vhsv) by the European Union Reference Laboratory for VHSV to keep track of isolates and their sequences (Jonstrup et al. 2009). Phylogenetic analysis suggests that genetic differences appear to be related more to geographic location than to year of isolation or host species (Skall et al. 2005a). One apparent exception is the presence of an isolate belonging to the Type II genotype found in the North Pacific in wild Japanese flounder (*P. olivaceus*) (Takano et al. 2000).

It has been suggested that the European freshwater isolates of VHSV originated from fish in the northern Pacific and Atlantic oceans. The mechanism of transfer was possibly through the feeding of marine feed-fish to cultured freshwater species (Hedrick et al. 2003).

1.2 Susceptible species

Susceptibility and clinical signs of disease can vary between VHSV isolates (Brudeseth et al. 2008, Campbell et al. 2009), fish demographics (e.g. species and age), route of exposure and environmental variables (e.g. water temperature).

Rainbow trout are highly susceptible to isolates of VHSV-Ia (Kahns et al. 2012). Epizootics in rainbow trout have resulted in mortalities of 80–100% in fry weighing 0.3–3 g (Smail 1999). VHSV-II and VHSV-III genotypes cause disease and mortality in turbot, but may cause no or low mortality in rainbow trout exposed by immersion (Skall et al. 2004, Snow et al. 2005). However, significant mortality was reported following intraperitoneal injection of the same VHSV isolates (Skall et al. 2004). One isolate of VHSV-III was identified as the causative agent of an outbreak in farmed marine rainbow trout in Norway, although other type III isolates do not generally cause clinical disease (Dale et al. 2009). Several marine fish species are susceptible to VHSV-IVa, and over 28 freshwater species are susceptible to VHSV-IVb (USDA 2007, Kim and Faisal 2010). Rainbow trout are relatively refractory to VHSV-IVb following intraperitoneal injection (Al-Hussinee et al. 2010).

The range of fish species from which VHSV has been reported continues to increase. The virus has been reported from at least 102 species from marine and freshwater environments (OIE 2013b). Appendix 1 lists fish species from which VHSV has been isolated (with and without clinical signs of disease) and species known to be resistant to challenge by at least one isolate. A further 11 fish species are susceptible to VHSV under experimental conditions.

The susceptibility of Australian native fish species to VHSV has not been investigated, so it is difficult to predict how VHS might manifest in Australia. Recent data show that several species such as red seabream (snapper [*Pagrus auratus*]), Japanese yellowtail (*Seriola quinqueradiata*) and black porgy (*Acanthopagrus schlegelii*) are susceptible to infection through intraperitoneal injection (OIE 2013b). VHSV infection has also been reported from several species of wild marine fish in Korea, including mullet (*Mugil cephalus*) and hairtail (*Trichiurus lepturus*) (Kim et al. 2009). Consequently, Australian fish species such as snapper, mullet, hairtail, bream (*Acanthopagrus* spp.), kingfish, samson fish and amberjack (*Seriola* spp.) may also be susceptible to VHSV infection. Fisheries and industries (including tourism) relying on rainbow trout, Atlantic salmon and species of native freshwater fishes may also be significantly affected.

Genotype has been used as an indicator of pathogenicity due to the association of pathogenicity with genetic origin, but pathogenicity determinants are likely to be based on only a few nucleotides (e.g. Campbell et al. 2009). VHSV is an RNA virus which has repeatedly been shown to become virulent given the opportunity presented in aquaculture situations. Caution should thus be exercised in using genotype as a predictor of virulence.

Generally, viruses in nature co-evolve with their hosts within their natural range to ensure their long-term survival. Translocation of viruses exposes previously naïve hosts to new viruses and brings associated risk (e.g. the 2003 Laurentian Great Lakes basin fish deaths).

1.3 World distribution and occurrence in Australia

The geographic distribution of VHSV encompasses marine and freshwater habitats throughout the Northern Hemisphere, incorporating Europe, America and Asia (Skall et al. 2005a). Specifically, the VHS virus:

- has never been reported in Australia
- has been reported in freshwater fish species from continental eastern and western Europe and is considered endemic in those regions
- has been isolated from marine fish in the northeast Pacific Ocean (from Alaska to California), the North Atlantic, the Baltic Sea, the Mediterranean and Aegean Seas and from flounder in Japan and Korea
- has been reported in at least 28 freshwater fish in the Great Lakes area of North America (thought to have been introduced via ship ballast water)
- is not commonly reported in fish from areas where water temperatures are above 18°C, though VHSV-IVb has been associated with a fish kill at water temperatures of 20–22°C.

1.4 Diagnostic criteria

Detailed reviews of the clinical signs and pathological changes of VHS are provided in Smail (1999) and Skall et al. (2005a). Clinical and pathological signs of VHS are referred to in terms of acute, chronic and latent stages, which relate to degrees of severity of infection rather than progressive stages of the disease.

1.4.1 Clinical signs

Clinical signs of infection vary between species and with severity of infection. Acute, chronic and nervous forms of the disease have been identified in rainbow trout. A carrier state occurs in surviving fish, and the virus can be isolated from persistently infected tissues, such as kidney and brain (Ghittino 1965). Acute forms of the disease have also been observed in other species of fish, including sea bass (*Dicentrarchus labrax*), turbot (*P. maxima*) (Castric and de Kinkelin 1984, Schlotfeldt et al. 1991), freshwater drum (*Aplodinotus grunniens*) and round gobies (*Neogobius melanostomus*) (Lumsden et al. 2007, Groocock et al. 2007). Virus multiplication in endothelial cells of blood capillaries, leukocytes, haematopoietic tissues and nephron cells underlies clinical signs of disease.

Infection is often lethal in susceptible species, since infection results in impairment of the osmotic balance of the fish. This occurs within the clinical context of oedema and haemorrhage. However, these clinical signs are not pathognomonic for VHS.

General signs

VHS may present in an acute, chronic or nervous form depending on the fish species. The following are general clinical signs that may be observed in fish infected with VHSV, irrespective of the form.

External signs

External signs of VHS include:

- loss of appetite
- haemorrhage at the base of fins and in the skin and eyes (Figure 2)

- exophthalmia ('pop eye') due to subretinal haemorrhaging (Figure 3)
- pale gills (anaemia)
- ascites (distended abdomen caused by abnormal accumulation of fluids, which may include blood)
- ataxia (uncoordinated swimming).

Internal signs

Internal signs of VHS include:

- swollen, pale liver
- swollen kidneys, which appear darker red in the early stages of disease (especially in the anterior kidney)
- the head and midsection of the kidney may be totally necrotic (inconsistently reported in the literature)
- bloody ascitic fluid surrounding abdominal organs
- oedema in muscles
- absence of food in the gastrointestinal tract.

Acute form

In its acute form, VHS can result in rapid death. In rainbow trout, the acute stage occurs 2–30 days after experimental infection at 8–12°C. Clinical signs of acute VHS include:

- pale gills with or without petechiae
- ataxia
- lethargy
- darker than normal colour
- crowding of fish to edges of ponds or cages.

Chronic form

Clinical signs might not be evident in the chronic form of VHS but the virus can be isolated from all major internal organs including the kidney, heart, spleen and muscle.

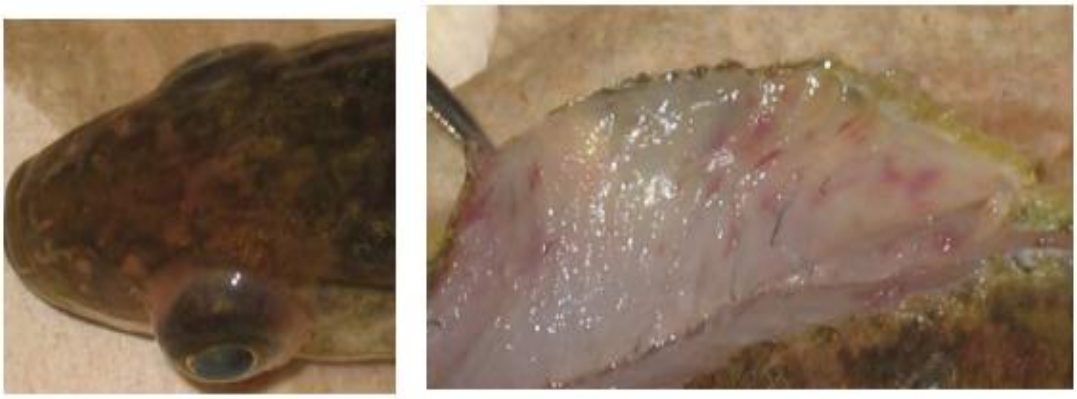
Nervous form

In the nervous stage, there is marked ataxia caused by the effect of the virus on the brain. This is a feature of virulent freshwater strains of VHSV–I that has also been observed in seawater-reared rainbow trout infected with VHSV-III (Dale et al. 2009).

VHSV has been isolated from the brain after experimental infection in marine species, including cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*) and turbot (*P. maxima*) (Snow and Smail 1999). A nervous form of the disease has not been reported in these species.

Figure 2 Haemorrhage on skin and around head of gizzard shad in North America

Figure 3 Exophthalmia (left) and haemorrhage in muscle of gizzard shad



(Photos Credit: Dr. Mohamed Faisal, Michigan State University).

1.4.2 Pathology, histopathology and haematology

The OIE *Manual of Diagnostic Tests for Aquatic Animals* (Chapter 2.3.9) (see Appendix 2) outlines virus identification and isolation techniques for VHS (OIE 2013b). A prominent feature of VHS is widespread haemorrhaging in the internal and external organs, including around the eyes and in the muscle. This has been observed in a number of species, including rainbow trout (*O. mykiss*), Japanese flounder (*P. olivaceus*), turbot (*P. maxima*) and Pacific herring (*C. pallasii*) (Munro 1996, Kocan et al. 1997, Smail 1999, Isshiki et al. 2001), and more recently from freshwater drum (*A. grunniens*), muskellunge (*Esox masquinongy*), yellow perch (*Perca flavescens*) and several other recreationally and ecologically important freshwater species in the United States (US) (Elsayed et al. 2006, USDA 2006, Lumsden et al. 2007, Gagne et al. 2007). Petechial and ecchymotic haemorrhaging has been observed on the swim bladder and in the peritoneum, adipose tissue, gonads, surface tissue of the liver and within muscle. Haemorrhages have also been observed in the epidural area in pike (*E. lucius*).

A notable gross pathological feature observed in turbot was gross body swelling due to fluid retention (Munro 1996). Differentiation has been made on the basis of clinical signs and the different forms of the disease (acute, chronic and nervous). No such differentiation has been made on the basis of histopathology.

Histopathology

Haemorrhage is a prominent feature of VHS. Degenerative changes and necrosis are common, particularly in tissues of endothelial origin (Wolf 1988, Lumsden et al. 2007, Groocock et al. 2007). The principal tissue affected is the kidney, often with necrosis and/or degeneration of haematopoietic tissue. Lymphoid tissue necrosis leads to leukopenia. In acute infections, liver sinusoids are engorged with blood, and hepatocytes exhibit extensive focal changes, including cytoplasmic vacuoles, pyknosis, karyolysis, lymphocytic invasion and occasionally intracytoplasmic and intranuclear inclusions (Stoskopf 1993, Smail 1999).

Extravasation of blood may be found in skeletal muscle; however, muscle fibres and bundles may not show signs of damage. Lumsden et al. (2007) reported perivascular infiltrates in the meninges, kidney and intestine of freshwater drum. Sporadic necrotising and leukocyte-rich lesions that were associated with blood vessels, extending into the surrounding parenchyma, have been reported in the gills, hepatopancreas, swim bladder, dermis, skeletal muscle, stomach and oesophagus (Lumsden et al. 2007). Infections in turbot and freshwater drum have resulted in necrosis and collapse of cardiac muscle (Ross et al. 1994, Lumsden et al. 2007). Likewise, the most prominent pathological changes were observed in the heart tissues of Japanese flounder. In this species, many muscle fibres in the inner layer of the myocardium were necrotic (Isshiki et al. 2001). Lumsden et al. (2007) and Dale et al. (2009) also reported significant meningoencephalitis in drum and rainbow trout, respectively.

Less destructive changes occur in pancreatic tissues. This is in contrast to infectious pancreatic necrosis and infectious haematopoietic necrosis, two other significant differential diagnoses to VHS. Damaged pancreatic islet tissue has been observed in northern pike (*E. lucius*).

Long-term studies of Pacific herring indicate that VHSV is associated with chronic lesions, including mineralisation of the myocardium, hepatocellular necrosis, submucosal gastritis, meningoencephalitis and skin ulcerations (Marty et al. 1998). Inclusion bodies have also been observed in necrotic myocardial cells of infected Japanese flounder (Isshiki et al. 2001).

Haematology

Extensive damage to haematopoietic tissue results in anaemia, leukopenia and thrombocytopenia. There is an increase in damaged erythrocytes and granulocytes, and a marked increase in immature erythrocytes, particularly late in infection (Wolf 1988).

1.4.3 Laboratory tests

The standard diagnostic procedure for VHS is the isolation of VHSV in a cell culture, followed by identification using molecular methods (e.g. reverse-transcriptase [RT] polymerase chain reaction [PCR]) (OIE 2013b). The virus replicates in several piscine cell lines, and the BF-2 cell line is recommended. Alternatively, the EPC or FHM cell lines may be used, and although they are generally less susceptible to infection by genotypes I, II and III, EPC cells are susceptible to genotype IV isolates (Olesen and Vestergård 1992, Lorenzen et al. 1999, OIE 2013b). Cell susceptibility is ranked in the order (most susceptible to least susceptible) BF-2, FHM, RTG-2 and EPC, but other fish cell lines, such as CHSE-214 and SSN-1, are also susceptible. The susceptibility of a cell line to infection depends on a range of parameters including cell-line

lineage and viral strain. For example, the EPC cell line may be more susceptible to VHSV genotype IV isolates than to type I to III isolates (OIE 2013b).

Detection of the virus through a viral cytopathic effect (CPE) in the cell culture is followed by virus identification using either antibody-based assays (e.g. neutralisation, immunofluorescence, enzyme-linked immunosorbent assay [ELISA] and immunoperoxidase staining) or nucleic acid-based assays, including RT-PCR and quantitative RT-PCR (qRT-PCR) (OIE 2013b). The advantage of qRT-PCR over traditional RT-PCR-based procedures is that qRT-PCR is generally more sensitive and viral loads can be quantified. The time to confirmation of results is also reduced when using qRT-PCR, since additional techniques such as electrophoresis, Southern blot hybridisation and/or nested PCR are not required (Chico et al. 2006, Matejusova et al. 2008, Cutrin et al. 2009, OIE 2013b). Immunofluorescence assays, ELISA, immunohistochemistry, RT-PCR and qRT-PCR are rapid diagnostic techniques suitable for obtaining presumptive evidence of VHSV in infected tissues or homogenates. The virus is most abundant in the kidney, spleen and heart. Confirmatory testing for VHSV in Australia must be done by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Animal Health Laboratory (AAHL) in Geelong. The detection and identification of VHSV at the AAHL and procedures for the correct submission of specimens are provided in Appendix 3.

The presence of VHSV can usually be confirmed in a submitted sample within days, depending on the original virus titre in the sample. The genotype to which the isolate belongs can also be determined within days. Genotyping can help to more quickly determine where the isolate may have come from and can significantly help an epidemiological investigation. Subclinical carriers of VHSV can be difficult to detect and are problematic for the control of this disease (OIE 2013b).

Detection of VHSV in the environment

VHSV can be cultured from marine and fresh water, although isolation can be difficult due to large dilution factors. Water samples can be concentrated to increase the probability of detecting VHSV (Watanabe et al. 1988), but the development of RT-PCR and qRT-PCR assays have increased the likelihood of detection of VHSV in environmental samples (Chico et al. 2006, Matejusova et al. 2008, Cutrin et al. 2009, Bain et al. 2010).

Transport of specimens

Suspect fish specimens should initially be sent to the state or territory diagnostic laboratory. After obtaining the necessary clearance from the CVO of the state or territory of the disease outbreak and informing the CVO of Victoria, specimens should then be forwarded to the AAHL for emergency disease testing.

1.4.4 Differential diagnosis

There are several differential diagnoses for VHS (Table 1). Infection with VHSV can only be confirmed by laboratory testing and should be considered when mortality of fish (freshwater or marine species) is observed in conjunction with haemorrhaging of tissues including the liver, muscle, brain and heart. Observation of neurological signs, such as spiralling, should reinforce the urgency for laboratory testing of specimens.

1.5 Resistance and immunity

Innate fish defence mechanisms against VHS include:

- physical barriers (scales, skin and associated mucous layers)
- bioactive molecules (lysozyme and other bacteriolytic enzymes, often found within mucous layers)
- nonspecific cytotoxic cells capable of destroying virus-infected cells
- interferon production.

1.5.1 Innate (nonspecific) immunity

Antiviral cytotoxic cells have been shown in fish (Ellis 2001) and are capable of destroying infected cells before viral replication. The production of interferon peaked three days after VHSV infection in rainbow trout (Dorson et al. 1994). The rapid innate response helps to provide some protection until the active (acquired) immune defences respond to the infection.

1.5.2 Adaptive (specific) immunity

Resistance to reinfection has been shown in survivors of VHS (Hershberger et al. 2007, OIE 2013b). Temperature has a profound effect on the development of active immunity. Fish exposed to the virus at 15°C can usually recover if water temperatures are raised to 20°C or 25°C (Goodwin and Merry 2010). Infection often results in the development of protective immunity in fish populations living in VHSV-endemic areas, particularly where the disease is present in populations of young, VHSV-naïve fish (OIE 2013b). It has been suggested that VHSV antibodies are both neutralising (i.e. reacting with a few epitopes on the glycoprotein of the virus) and non-neutralising (i.e. directed against virus protein), and that non-neutralising antibodies persist longer in fish than neutralising antibodies (Olesen et al. 1991). Neutralising antibodies have been shown in recovering trout. The time for this antibody response to develop can vary substantially (e.g. in 130 g trout, the response time was approximately 4–10 weeks) (Olesen et al. 1986, 1991).

Table 1 Differential diagnoses for viral haemorrhagic septicaemia

Disorder	Pathogen	In Australia	Species affected	Clinical signs	Diagnostic tests
Epizootic haematopoietic necrosis	Epizootic haematopoietic necrosis virus	Yes	Redfin perch, salmonids, Macquarie perch, Murray cod and others	Haemorrhage, necrosis, epizootics in redfin perch	Cell culture, immunodiagnostics, histopathology, PCR
Infectious haematopoietic necrosis	Infectious haematopoietic necrosis virus	No	Salmonids	Haemorrhage, necrosis	Cell culture, immunodiagnostics, histopathology, PCR
Infectious pancreatic necrosis	Infectious pancreatic necrosis virus	No*	Salmonids, flatfish	Extended abdomen, ascites, spiralling, high mortality	Cell culture, immunodiagnostics, histopathology, PCR
Bacterial septicaemia	Various types of bacteria	Yes	All species	Haemorrhage, reddening, extended abdomen, ascites ulcers, abscesses	Bacterial isolation with clinical signs, histopathology, PCR
Infection with rickettsia-like organisms	Rickettsia-like organisms	Yes	Many species, particularly salmonids	Congestion, haemorrhage, anaemia, ascites	Clinical signs, histopathology, PCR

Disorder	Pathogen	In Australia	Species affected	Clinical signs	Diagnostic tests
Whirling disease	*Myxobolus cerebralis*	No	Salmonids (mainly rainbow trout)	Discolouration, whirling, deformities	Clinical signs, histopathology, PCR
Epizootic ulcerative syndrome	*Aphanomyces invadans*	Yes	Many species in freshwater and estuarine areas	External haemorrhages	Clinical signs, histopathology, fungal isolation, PCR
Osmotic stress	NA	Yes	All species	Bloody ascitic fluid	History (e.g. recent transfer in salmon smolts), absence of pathogenic organisms

* Acute IPN has not been recorded in Australia, but a related aquatic birnavirus is endemic to parts of Tasmania (Crane et al. 2000). PCR = polymerase chain reaction. NA = not applicable.

1.5.3 Vaccination

At the time of publication, there are no commercially available VHS vaccines, but DNA-based vaccine technology has been proven to be highly effective in stimulating specific and nonspecific immunity under experimental conditions (Ortega-Villaizan et al. 2009, Chico et al. 2009), although protection may be influenced by variables such as water temperature (Lorenzen et al. 2009). There have been several attempts to develop vaccines. Despite their ability to induce efficient protection under experimental conditions, live vaccines are, so far, unsafe for field use, and inactivated vaccines require high doses. Different recombinant subunit vaccines based on the VHSV membrane glycoprotein have been less successful, but DNA vaccines encoding the same viral glycoproteins have been developed and can provide protection when used in small doses (as little as 10 ng in trout fry) as early as 4–8 days, for up to 2 years after vaccination (Lorenzen et al. 1993, Lecocq-Xhonneux et al. 1994, Anderson et al. 1996, Lorenzen et al. 1998, Kim et al. 2000, Lorenzen et al. 2000, Sommerset et al. 2003). RNA interference (RNAi) holds promise for reducing future impacts of VHSV (Ruiz et al. 2009).

1.6 Epidemiology

1.6.1 Transmission and incubation period

Transmission occurs horizontally through water, with excretion of virus in the urine. There is no indication or evidence of true vertical transmission of VHSV (OIE 2013b). The virus gains entry through the gills of the fish (Neukirch 1985, Brudeseth et al. 2008), through wounds, and possibly through the skin (Yamamoto et al. 1992). Oral exposure through predation on infected fish is also a route of transmission (CFSPH 2007, Schönherz et al. 2012), as is exposure to blood-feeding vectors such as leeches (Faisal and Schulz 2009). Viral multiplication may take place at the site of entry, or the virus may pass through without primary multiplication, which subsequently occurs in the endothelial cells of the vascular system (primarily the kidney, spleen and brain). The virus is shed primarily in the urine and reproductive fluids (ovarian fluids and milt). Virus shed with sex products is via surface contamination of the eggs and is thought to be readily dissipated. This virus has also been reported in the faeces, but shedding is low. Reservoirs of infection include clinically ill fish and subclinical carriers. Virus carriage seems to be long-term, but shedding appears to be intermittent in carriers.

Pathological changes in cells lining the circulatory system 48 hours after infection have been described (Smail 1999). Necrosis of liver hepatocytes occurs by day 4 after infection (Evensen et al. 1994). The incubation period for European freshwater VHSV-I isolates is 1–2 weeks at warmer temperatures (12°C), and 3–4 weeks at cooler temperatures (1°C). Mortalities in Pacific herring began 4 days after experimental infection (by immersion) with a marine VHSV-IVa isolate (Kocan et al. 1997). Mortalities in small, medium and large Japanese flounder at 15°C began 7, 11 and 20 days, respectively, after injection with a VHS-IVa isolate (Isshiki et al. 2001). Fathead minnows injected with a VHS-IVb isolate showed lesions characteristic of VHS after 9 days at 12°C (Al-Hussinee et al. 2010).

1.6.2 Virus shedding from infected host

Virus shedding from infected fish occurs rapidly. With Pacific herring, detectable levels of virus in the water were first noted 48 hours after exposure, peaking at days 4–5 (Kocan et al. 1997). At that time, each infected herring was shedding virus at an average rate of more than $10^{6.5}$ plaque forming unit (PFU)/hour (Kocan et al. 1997). In experimentally infected Pacific herring held at 8.5°C, VHSV shedding rates reached 1.8–5 × 10^8 PFU/fish/day. The onset of viral shedding was dose-dependent and preceded initial mortality by 2 days (Hershberger et al. 2010a). Although it has been proposed that most viral shedding occurs via urine (Neukirch 1985), some shedding can occur in mucus and from other tissues (e.g. skin, gills and skin ulcers) (Smail 2000).

1.6.3 Persistence of virus

The European freshwater isolates of VHSV are heat-labile and acid-labile (at pH 3). These isolates are stable at pH 5–10, and stable through several freeze–thaw cycles (Wolf 1988). There may be some variation in susceptibility to freezing and thawing depending on the strain of VHSV. For example, North American marine strains (VHSV-IVa) are more sensitive to freeze–thaw cycles than European freshwater strains (Arkush et al. 2006).

The length of time VHSV can survive in the environment will depend on:

- temperature
- salinity
- solar radiation
- presence of chemical pollutants
- bacterial antagonism
- water hardness
- suspended solids.

VHSV can survive in freshwater and marine environments. Hawley and Garver (2008) examined the stability of three VHSV-IV isolates and one VHSV-I isolate taken from marine, freshwater or estuarine hosts, from raw and filtered fresh water and seawater, at temperatures of 4–30°C. All four isolates were substantially more stable in fresh water and at lower water temperatures. The average time required for 99.9% inactivation of VHSV in raw fresh water at 15°C was 13 days, and in raw seawater, VHSV was inactivated within an average of 4 days (Hawley and Garver 2008). The virus has been documented to persist in fresh water for 28–35 days at 4°C (Parry and Dixon 1997) and to be infective for over 1 year at 4°C in filtered fresh water (Hawley and Garver

2008). Freezing at –20°C maintains infectivity of VHSV-I isolates for many years (Wolf 1988). However, normal commercial freezing practices resulted in a significant (up to 99%) reduction in titres of VHSV-IVa on thawing, with infectious virus remaining in thawed fish tissue at a concentration of 5.5×10^3 PFU/g (Arkush et al. 2006).

Significant reductions in VHSV titre have been reported in untreated seawater compared to sterilised or filtered seawater, suggesting that there may be considerable inactivation due to the action of bacteria or other microorganisms (Mori et al. 2002, Hawley and Garver 2008). In contrast, Kocan et al. (2001) reported that the addition of crude oil at 10 parts per billion had no effect on virus survival. In fresh water, VHSV survival appears to decrease as water hardness increases (Hawley and Garver 2008).

Disinfection with an iodophore will rapidly inactivate VHSV on the surface of fish eggs (Bovo et al. 2005, OIE 2013b).

Birds may spread the virus from farm to farm by physically carrying infected fish or by eating infected fish at one farm and regurgitating it at another. VHSV will not survive passage through the gut of the bird, due to the high acidity in the anterior digestive tract and the high internal body temperature of birds. The virus remains infectious after passage through the gut of leeches (Faisal and Schulz 2009).

1.6.4 Sources of VHSV

VHSV is distributed throughout the Northern Hemisphere from wild and cultured fish in marine and freshwater habitats where temperatures are 4–20°C (OIE 2013b). Prevalence of the virus may vary through the year, and tends to be higher in winter and early spring, which can be attributed to the reduced water temperatures at those times (Altuntas and Ogut 2010).

1.6.5 Factors influencing transmission

Age and size

Fish age influences susceptibility to and severity of VHS in rainbow trout. Fish weighing 0.3–3 g are most susceptible. Mortality at 9–12°C in fish of this weight with virulent isolates of VHSV is 80–100%. In fingerlings and growers, mortality is significantly lower, given the same conditions (Smail 1999). Larval and juvenile Pacific herring were also particularly susceptible to VHS compared to adult fish (Kocan et al. 1997, Hershberger et al. 2007). In general, older fish that experience high VHS mortality have never been in contact with VHSV (OIE 2013b).

Temperature

Water temperature is an important factor in the propagation and spread of VHSV. Transmission of the virus occurs readily over a temperature range of 1–15°C (OIE 2013b). At temperatures above 15°C, virus shedding from infected fish and survivability of the virus outside the fish host are significantly reduced. However, Castric and de Kinkelin (1984) found an upper temperature threshold of 18–20°C for in vivo infections in marine fish. Furthermore, VHSV-IVb caused mortalities in freshwater fishes at water temperatures up to 18°C in the Great Lakes in the US (Goodwin and Merry 2010). At water temperatures of 15–18°C, the disease generally takes a short course with a modest accumulated mortality, but transmission can occur in temperatures up to 20°C (OIE 2013b). Although VHSV-IVb infection has resulted in mortalities at 20–22°C (OIE 2011),

VHSV-related mortalities at temperatures above 18°C may be due to damage sustained during cooler temperatures before discovery of the outbreak (Goodwin and Merry 2010). Different isolates of VHSV in different fish species may show variance in temperature tolerance.

Serial passages of VHSV in cell culture at increasing temperatures of 14–25°C resulted in a temperature-resistant variant able to replicate efficiently at 25°C (de Kinkelin et al. 1980). The resulting variant had a reduced virulence for rainbow trout when tested at 8–12°C (de Kinkelin et al. 1980).

Infectious dose

Units of measurement for infectious dose vary in the literature (e.g. $10^{3.5–4.5}$ PFU/mL, 10^5 tissue culture infective dose [TCID]50/mL) (Kocan et al. 1997, King et al. 2001). The infectious dose varies considerably between host species and can be influenced by temperature, route of exposure (see below) and, more importantly, the age and size of the host. For example, outbreaks of acute VHS in juvenile Pacific herring followed waterborne exposure to VHSV concentrations as low as 27 PFU/mL (Hershberger et al. 2010a). Similarly, the minimum dose of VHSV required to initiate disease in juvenile Pacific herring by waterborne exposure for 1 hour was $10^{1.5–2.5}$ PFU/mL (Kocan et al. 1997).

Due to the high levels of shedding of the virus from individual fish, VHSV can spread very quickly in schooling species (e.g. sardines and herring), resulting in clinical VHS outbreaks (Kocan et al. 1997, Hershberger et al. 2010a).

Route of exposure

The most common routes of infection are downstream by water flow, upstream by migrating fish, across water catchments by fish-predating birds (herons, cormorants, crows and gulls) and human activities and trade (Bovo et al. 2005). Exposure of farmed fish to naturally occurring isolates of VHSV has caused mortalities in farmed turbot and trout (e.g. Snow et al. 2005). Infection of fish through eating other infected fish has also been shown (Schönherz et al. 2012). True vertical transmission has not been demonstrated (Bovo et al. 2005).

Many species have been infected experimentally but results of infection vary with method. 'Less natural' methods, such as intraperitoneal (IP) injection, may produce clinical disease, but 'more natural' methods, such as immersion, may not (e.g. Skall et al. 2004). For some fish species–virus strain combinations, disease is evident only when the virus is injected, and the fish remain healthy and/or refractory to infection when exposed to the virus by immersion (de Kinkelin and Castric 1982, Skall et al. 2005b, Dale et al. 2009). Examples include fathead minnows that were infected with VHSV-IVb via immersion and IP injection, and exhibited earlier mortalities, higher mortality rates and required a smaller infectious dose when IP injection alone was used (Al-Hussinee et al. 2010). Similar results have been reported for other fish species (Skall et al. 2004, 2005a, Dale et al. 2009).

Species

VHSV has been isolated from at least 101 species of marine and freshwater fish (Appendix 1) but all temperate marine species may be susceptible to infection with VHSV under suitable conditions (Stone et al. 1997). Many species of freshwater fish are susceptible to infection with marine and freshwater strains of VHSV. For example, VHSV-III, a marine genotype not initially considered to be pathogenic for rainbow trout was the causative agent of a VHS outbreak in

seawater-reared rainbow trout in Norway (Dale et al. 2009). Consequently, caution should be exercised before assuming a species is resistant to infection. The highest prevalence of natural infections in the marine environment has a tendency to occur in schooling fish such as herring and sprat (Skall et al. 2005a).

A broad range of Australian temperate marine and freshwater fish species are likely to be susceptible should VHSV be introduced into Australia (Section 1.2).

1.6.6 Inactivation

VHSV is rapidly inactivated by chlorine and iodophore disinfectants (Bovo et al. 2005). Dose rates and time until inactivation depend on the type of disinfectant (Table 2).

Table 2 Dose rates and time required until VHSV inactivation using physical and chemical agents

Agent	Treatment required for inactivation*
Physical agents	
Heat	45°C for 60 minutes, or 60°C for 15 minutes, or 70°C for 1 minute
Ultraviolet light	1–3 × 103 µW s/cm2
Chemical agents	
Chlorine	100 mg/L for 10 minutes, or 515 mg/L for 2 minutes
Sodium hypochlorite	100 mg/L for 5 minutes
Iodine	100 mg/L for 4 minutes
Sodium hydroxide	10 g/L for 5 minutes, or pH > 12 for 7 hours
Formic acid	pH < 4 for 24 hours, pH 3 for 3 hours
Quaternary ammonia	10 mg/L for 5 minutes
Ozone	8 mg/L/minute for 3 minutes (redox potential 600–750 mV)
Virkon S	1% solution
Formaldehyde	3% for 5 minutes, 1% for 16 hours
Ethanol	40% in seawater for 2 minutes
Propanol	20% in seawater for 2 minutes, or 30% in seawater for 30 seconds

* Affected by factors such as amount of suspended solids, salinity and organic load (see AQUAVETPLAN Operational procedures manual: decontamination, at http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan/operational_procedures_manual_-_decontamination)

(Bovo et al. 2005 and Kebus 2007)

2 Principles of control and eradication

2.1 Introduction

VHS has caused significant mortality and economic loss in cultured and wild fish species overseas. Some fish species in Australia are known to be susceptible to this disease (Section 1.2), but the susceptibility of many other species remains uncertain.

Possible scenarios for the isolation of VHSV in Australia include virus from:

Atlantic salmon or rainbow trout in salt water or fresh water as part of routine surveillance, with no clinical signs of disease

- Atlantic salmon or rainbow trout in salt water or fresh water, accompanied by haemorrhaging, increasing morbidity and mortality
- wild fish around southern bluefin tuna, yellowfin tuna or yellowtail kingfish cages
- southern bluefin tuna, yellowfin tuna or yellowtail kingfish showing no clinical signs of disease, or showing morbidity and mortality
- pilchards or herrings during mortality events
- wild marine fish other than pilchards or herring, with or without clinical signs of disease
- wild freshwater fish, with or without clinical signs of disease.

Each scenario may require a different control strategy, the choice of which will be influenced by the circumstances at the time. The above list is not exhaustive and takes no account of the different strains of VHSV.

2.2 Methods to prevent spread and eliminate pathogens

There are essentially three disease control strategies that could be adopted if VHSV is detected in Australia, including:

- eradication: the scale may be national (i.e. from Australia), local (e.g. from a local trout farm) or intermediate (e.g. from a sea cage farm, region or state)
- containment, control and zoning: excluding VHSV from defined geographic areas and unaffected populations (e.g. by quarantine) and containing the virus to areas with enzootic infection
- control and mitigation: managing the frequency and severity of disease episodes in infected populations and keeping them within acceptable levels.

The basic principles of eradication and the other control responses are described in the AQUAVETPLAN *Enterprise Manual* (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan) and *Control Centres Management Manual* (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan), which detail state and territory legislation relating to disease control and eradication.

Response measures may involve any or all of the following:

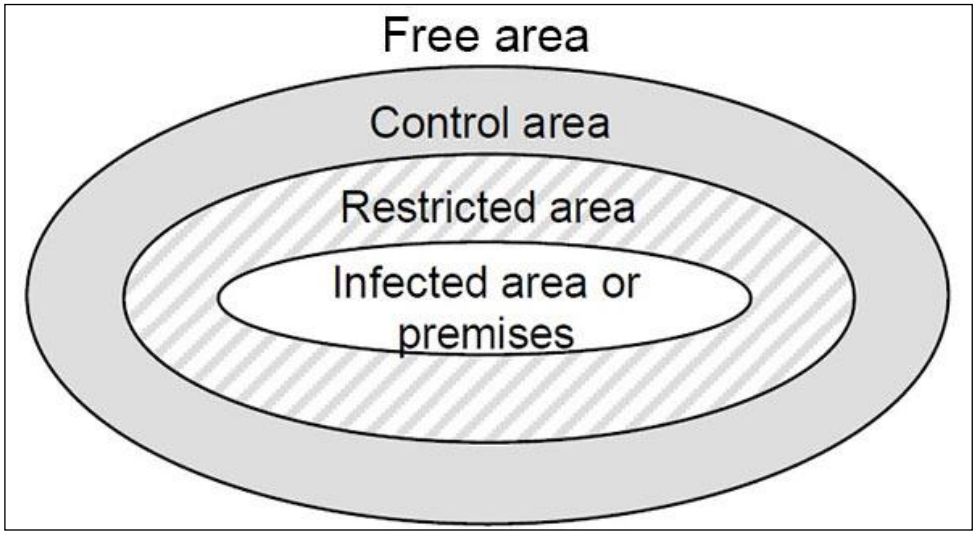
- early detection and identification of VHSV and any associated clinical signs of disease
- rapid definition of the nature and extent of the problem, including delineation of the geographic area of the outbreak
- testing of wild fish species to assess whether virus is present in wild fish populations and, if so, its prevalence and geographic extent
- seizure, quarantine or destruction of infected fish (which may not always be possible or warranted)
- tracing, seizure and quarantine or destruction of potentially infected fish (which may not always be possible or warranted)
- movement controls over fish and fish products
- movement controls over water (where possible) and/or disinfection of water to ensure inactivation of virus
- movement controls over people, vehicles, fish transporters, equipment and other means of mechanical spread of the virus
- good communication between all relevant government and industry stakeholders
- media liaison and development of extension materials.

2.2.1 Quarantine and movement controls

If quarantine and movement controls are to be implemented, the basic principles to be followed are:

- establishment of specified areas (Figure 4):
  - declared area (includes restricted area and control area)
  - restricted area (an area around an infected premises or area)
  - control area (a buffer between the restricted area and free areas)
  - free area (noninfected area not considered a declared area and may include large areas of Australia in which the presence or absence of VHSV remains unassessed)
- bans on the movement of live fish and fish products from restricted areas into areas where VHSV is considered absent
- restrictions or bans on releasing fish into river or freshwater lake systems or marine zones in designated areas
- restrictions or bans on the movement of fish and fish products between river systems and between marine zones in designated areas
- restrictions or bans on the use and movement of vehicles (e.g. cars and boats) and equipment (e.g. nets, buckets, footwear and fishing gear) within and between marine and freshwater areas
- controls on access of predators, such as birds, to potentially infective material (e.g. fish carcasses and hatchery tanks).

Figure 4 Establishment of specified areas to control viral haemorrhagic septicaemia



Practices that would be affected by the implementation of quarantine and movement controls include:

- transportation of live fish or fish eggs between and within freshwater operations (including broodstock)
- live fish transportation between freshwater and saltwater operations
- translocation of fish and fish products as bait
- fish harvesting (wild and farmed) and transportation to processing plants
- discharge of processing plant effluent
- transportation of consumer-ready products
- disposal of dead fish
- movements of boats and other vehicles.

The imposition of restrictions can significantly reduce spread of the pathogen in the early stages of control of a disease outbreak. Imposing restrictions may also 'buy time' while the true extent of the problem is assessed but, as demonstrated in the outbreaks of VHSV-IVb in the Great Lakes of North America, even if restrictions are implemented, the virus can still spread through natural movements of water, fish and other vectors (Appendix 4) (Gustafson 2009, Bain et al. 2010, Gustafson et al. 2010, VHSV Expert Panel and Working Group 2010).

If VHSV is detected, it may be difficult to determine the size of the specified areas. For example, during an outbreak of VHS on a turbot farm in Scotland, all farms within 20 km of the infected premises were deemed suspect and placed under movement controls (Munro 1996). The 20 km radius was the distance at which virus concentration fell below 1 infectious virion/m^3 of seawater (with the assumption that natural factors did not inactivate the virus). In another VHS outbreak on a rainbow trout farm in the UK, control orders were placed on all fish farms within five river catchments that drained into the Humber estuary, and all fish at the affected farm were slaughtered on site (CEFAS 2007). Furthermore, the risk of infectious salmon anaemia (ISA) virus infection

increased by a factor of eight if the site was closer than 5 km to another ISA-positive site (Jarp and Karlsen 1997). For the outbreaks of VHSV-IVb in the Great Lakes, an expert panel considered that the cut-off for high-risk linear distance from a known VHSV-positive watershed was around 100 km, based on the distance a piscivorous bird might travel from feeding grounds to nest (VHSV Expert Panel and Working Group 2010). The same panel estimated that the cut-off for low-risk linear distance was around 500 km, based on a day's travel by car (VHSV Expert Panel and Working Group 2010).

The extent of restrictions should take into account movements of fish and water, and the possibility that the virus is widespread in the region in which it is first isolated. Rapid determination of the nature and extent of the problem is important for the decision-making process.

Semi-open systems

In semi-open fish production systems, there is virtually no control over the aquatic environment. Fish are contained in cages moored in estuaries or sheltered positions. Cages and nets can become damaged, allowing fish to escape into the wild. There is often significant interaction between wild and farmed fish, including wild fish entering sea cages holding farmed fish. With such close interaction, the only way to prevent release of virus from infected fish into the surrounding environment in a semi-open system is to remove infected captive fish from the water. If wild fish have already become infected, this may not eliminate the risk of disease propagation.

Semi-closed systems

Semi-closed fish production systems have more control over water than semi-open systems. However, there are differences between farms in the extent to which input and output water can be contained. Semi-closed systems are not designed to be self-contained, so preventing the inflow or outflow of water may have adverse effects on the capacity of a farm to sustain adequate water quality for support of its captive fish population.

Output water control and treatment to control VHSV are possible, but are usually not economically viable. The virus was successfully eradicated in Scotland from a pump-ashore tank farm in Scotland through disinfection of effluent water before release (Munro 1996).

Fish input and output may be controlled. Fish inputs into freshwater farms may be from onsite hatcheries or from freshwater or marine farms (e.g. broodstock). Fish are also able to enter farm waterways, and possibly ponds, via intake water from the water source. Movement restrictions would significantly interrupt some farm management practices and production.

VHSV was successfully eliminated from fish hatcheries in Denmark (Jørgensen 1980). Eradication protocols included draining, disinfection and drying, for at least 1 month, of all VHSV-infected trout farms using water from the same stream, starting with the farm at the top of the stream and working progressively downstream. The farms were then restocked with VHSV-free fish. There was no destruction of wild fish. VHSV has also been eradicated from fish farms in Scotland (Munro 1996) and England (CEFAS 2007) through slaughter of fish followed by disinfection and fallowing.

Closed systems

It is possible to isolate a closed fish production system, such as an aquarium or recirculation system. Therefore, it is possible to prevent the spread of VHSV from a closed system.

Zoning

Principles of zoning for infected and noninfected zones in Australia are outlined in the AQUAPLAN *Zoning Policy Guidelines* (Zoning Policy Guidelines). Zoning may be possible if VHSV is isolated from a single culture facility or if the virus has been carried into a freshwater environment from a marine source (e.g. through feeding of marine trash fish to freshwater cultured fish), even if the virus is known to be present in wild marine species.

If VHSV becomes enzootic in specific regions of Australia, a zoning policy that is specific for VHSV may be necessary to protect noninfected areas and to prevent further spread of infection. A corresponding surveillance and monitoring program for VHSV will also be required to support and validate a zoning policy.

2.2.2 Tracing

The tracing of fish, fish products, people and equipment may be difficult, depending on the areas from which VHSV was isolated. Some facilities culturing fish are involved in restocking programs where there is extensive movement of live fish. Other facilities move fish products daily to distant markets. Often, very little is known of wild fish movements in water bodies receiving discharge from fish farms.

A thorough and comprehensive epidemiological investigation, such as the one that occurred in Yorkshire in 2007 (CEFAS 2007), requires trained personnel with adequate time and resources.

Immediate tracing steps to aid in the epidemiological investigation include:

- trace-back of all movements of infected fish to help establish the origin of the outbreak (were the infected fish exposed to VHSV at their current location? Did they carry the virus to that location?)
- trace-forward of all contacts with infected fish, premises and sites to establish the current and potential spread of infection (where did fish, water and equipment from the infected facility go, within the period of infection or exposure?).

Tracing should include:

- fish (broodstock, juveniles, fish used for restocking and harvested wild fish)
- fish products (fish for consumption, used as bait or berley or as aquaculture feed, and effluent and waste products from slaughter and processing)
- water (input and output)
- equipment, vehicles and personnel (bearing in mind that VHSV is not a robust virus and that these items are readily disinfected).

Diagnostic tools such as RT-PCR (Section 1.4.3) may also be useful in identifying the strain of VHSV. This information can be used to determine the most likely route of entry of the virus.

Neighbouring fish populations

For wild marine fish, 'neighbouring populations' are numerous and extensive. Detection of VHSV in wild marine fish does not necessarily indicate that the virus is a recent introduction. Wild fish must always be considered potential carriers of the virus.

If VHSV is isolated from a freshwater facility, it may be possible to quarantine the facility to prevent the spread of the virus to neighbouring farm sites and to wild fish populations (CEFAS 2007). Quarantine and movement controls (Section 2.2.1) need to be considered.

2.2.3 Surveillance

Surveillance is critical in any control strategy. Surveillance can be costly and may require:

- field personnel
- laboratory personnel
- administrative assistance
- equipment and instruments
- validated tests in accredited laboratories
- diagnostic reagents.

In Australia, VHSV must be confirmed at the AAHL, Geelong. In the development of a surveillance and monitoring program, the capacity of the AAHL to handle large numbers of samples must be considered. Note that surveillance may be carried out by other competent laboratories if RT-PCR-positive controls are noninfectious (i.e. positive RNA).

The OIE *Manual of Diagnostic Tests for Aquatic Animals* (aquatic manual) provides specific, up-to-date information on the tests required (two real-time RT-PCR assays have been found to be suitable for identification of VHSV of all genotypes) and other information useful for designing a targeted surveillance program. Any surveillance program would need to be epidemiologically designed on a fit-for-purpose basis, depending on the specific situation.

2.2.4 Treatment of infected fish

There are currently no treatments for VHS, but experimental vaccines (Section 2.2.8) have been developed to reduce or prevent the impacts of disease. RNAi technology holds promise for reducing the impacts of VHSV (Ruiz et al. 2009).

2.2.5 Destruction and disposal of fish

Destruction of infected fish will eliminate a major potential source of virus, and reduce the virus load in the surrounding environment and consequently the risk of infection of other wild or farmed fish. Measures to minimise the spread of virus while fish are destroyed should be implemented where possible. Destruction of wild fish is rarely feasible or practical.

In North America, large numbers of Pacific salmon growing in enhancement hatcheries were destroyed after VHSV was detected (Meyers and Winton 1995). Subsequently, the isolated strain was shown to be different from the classical European strain, enzootic in the region, and avirulent for salmonids.

Destruction of large quantities of fish requires considerable resources and logistical planning to handle the carcasses. For example, boats or trucks

capable of safely containing potentially infective material and composting or rendering facilities or burial sites are required.

For more details on destruction and disposal of fish, see the AQUAVETPLAN operational procedures manuals *Destruction* (Destruction) and *Disposal* (Disposal).

2.2.6 Treatment of fish products and by-products

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered when determining the processing methods and the final use of fish products and by-products.

VHSV is not a resilient virus, but survives reasonably well at low temperatures. Freezing will not completely inactivate the virus, but freeze–thaw cycles significantly reduce the overall infectious virus titre (Arkush et al. 2006). Freezing at –20°C maintains infectivity of VHSV-I isolates for many years (Wolf 1988).

The virus may be very difficult to detect in carrier fish, particularly in those in the very early stages of infection or in convalescent fish. However, the titre of virus may be substantial in susceptible species in the preclinical phase of infection. The titre of virus in the tissues of Pacific herring experiencing mortality was 10–10,000 times as high during the acute phase of an epizootic than during the recovery phase (Hershberger et al. 2010b). Selecting fish that do not have the acute disease, and through the use of appropriate controls, it may be feasible to harvest and safely process fish without signs of disease. During the control of an outbreak of VHSV in Scotland, market-sized fish were eviscerated on the farm site, the viscera were destroyed by burning, and the remainder of the carcasses were sent to market. Hence, organs that were most likely to contain the highest titre of virus were removed and destroyed (Munro 1996). The eradication strategy on this farm was successful.

2.2.7 Decontamination

Successful disinfection requires effective cleaning prior to the disinfection process. Drying and sunlight will effectively destroy VHSV and the virus can be readily inactivated using several disinfectants (Table 2 and Section 1.6.6). Processing plants handling infected or potentially infected fish are potential sources of spread of the pathogen (CEFAS 2007). If an emergency harvest is performed and some of the fish show clinical signs, there may be high virus titres in processing plant effluent. For example, titres of infectious haematopoietic necrosis virus (family Rhabdoviridae) have been shown to be approximately 1.3–4.3 × 10^3 PFU/mL in processing water when the fish being processed were from an infected site. This is well above levels known to initiate infection of several VHSV isolates. Hence, water would require disinfection before release into the aquatic environment to reduce risk of infection.

Disinfection protocols for freshwater facilities need to be determined on a case-by-case basis, due to differences between farming enterprises. This will involve the farm manager, the state or territory CVO and/or the director of fisheries. The disinfection protocol should take into consideration the epidemiological factors (Section 1.6), including the following:

- source and location of infection

- type of enterprise (e.g. facilities using well water versus those using water from a river, lake or stream, or a sea-cage culture enterprise)
- design of the site and its proximity to other waterways
- environmental impact of the protocol
- availability of approved, appropriate and effective disinfectants.

The AQUAVETPLAN operational procedures manual *Decontamination* (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan) provides further information.

Environmental considerations

Large numbers of dead fish can be the source of unpleasant odours and can be unsightly if not covered. Decontamination operations must comply with all legislation and regulations concerning the disposal or discharge of chemicals and cleaning agents into the environment.

2.2.8 Vaccination

There are currently no commercial vaccines available for VHS, but DNA-based vaccine technology has been proven to be highly effective at stimulating both specific and nonspecific immune systems under experimental conditions (Ortega-Villaizan et al. 2009, Chico et al. 2009), although protection may be influenced by variables such as temperature (Lorenzen et al. 2009).

2.2.9 Vector control

While VHSV cannot survive passage through the acidic intestinal environment of a bird or fish, birds can carry infected fish and drop them in an uninfected region. Predator fish and mammals can also move infected material from sea cages. Effective precautions should be taken to prevent birds and mammalian predators or scavengers (e.g. dogs) from accessing infected fish, including from disposal sites. Wild fish which live around sea cages are also potential carriers that can spread the disease agent to new areas, but it is extremely difficult to control their movements. VHSV has also been detected in parasitic leeches by PCR (prevalence 72.5%) and cell culture (prevalence 62.6%) (Faisal and Schulz 2009), suggesting that these (and other ectoparasites) could play a significant role in transmission of VHSV as vectors and/or reservoirs of infection.

2.2.10 Restocking

VHSV can infect many species, but strains of VHSV differ significantly in their virulence for different species. For example, rainbow trout are highly susceptible to the VHSV-I genotype, and Pacific herring are highly susceptible to the VHSV-IVa genotype.

If a highly virulent strain of VHSV is isolated in Australia and is accompanied by signs of disease, restocking with a different species of fish could be considered, ideally after determining the identity of refractory species through controlled infection trials. Restocking would only be considered after the initial outbreak has been effectively dealt with and sufficient site fallowing has been undertaken.

2.2.11 Public awareness

A public awareness campaign emphasising education, surveillance and cooperation from industry and the community is essential. The public should be informed that:

- VHSV is not infective for humans
- eating fish that may have been exposed to VHSV is not considered a health risk.

A media kit should be immediately distributed to ensure that the media can help reduce any potential public fear or perception of risk. Examples of the types of extension materials that can be used to raise public awareness of issues that would become relevant during an outbreak of VHS are contained in Appendix 4.

During significant mortality events of either marine or freshwater fish species, VHSV may be isolated from sick or moribund fish even though the virus may not be identified as the causative agent of the mortalities. Although the virus may not be identified as the causative agent of the mortality, it nevertheless is likely to be associated with it. Moribund fish may wash up on beaches or river banks and be of concern to the public. It is important that the public has the confidence that appropriate people or authorities are taking responsibility for the investigation and that the problem is being addressed.

2.3 Feasibility of specific options for control in Australia

Feasibility for control of VHS in Australia will depend on the circumstances of the detection. There is little that can be done to control wild fish that have been exposed or potentially exposed to VHSV, although containment remains a high priority in that situation (CEFAS 2007, Gustafson 2009, Gustafson et al. 2010, VHSV Expert Panel and Working Group 2010).

2.3.1 Eradication

Eradication is not a feasible option if epidemiological investigations determine that:

- the infection is widespread
- the outbreak has no point source and cannot be contained
- the infection is present or potentially present in wild fish species in freshwater or marine environments.

This is due to:

- the ability of VHSV to spread and establish reservoirs of infection in wild fish populations
- the ability of VHSV to infect many different species of fish in fresh water and in salt water
- the ability of VHSV to infect fish but remain undetectable
- the lack of a full understanding of how VHSV survives in the aquatic environment
- the ability of infected wild fish to transmit and establish VHSV infection in rivers and the sea
- the close contact between, and relative lack of control over, some farmed and most wild fish populations and water in Australian salmonid, kingfish and tuna farming operations
- previous experience that an aquatic pathogen cannot be eradicated once reservoirs of infection become established in wild fish populations and the natural environment.

Eradication may be feasible if the initial isolation of the virus is from a freshwater facility or from a closed aquaculture system (such as a semi-closed system or aquarium) (Munro 1996, CEFAS 2007).

If eradication is considered feasible, fish must be dealt with as follows:

Unexposed fish

Unexposed fish may be destroyed as a precaution in order to remove a potential source of virus before exposure and infection occur, but if exposure can be prevented in the first place, there will be no further propagation of virus and healthy fish will not need to be destroyed. Therefore, rather than destruction of unexposed fish being the first choice, the principle should be to determine first if exposure can be prevented and destruction avoided.

Exposed or potentially exposed, clinically normal fish

If there is doubt about whether fish have been exposed to VHSV, they should be treated as if exposed. Immediate destruction of exposed fish prevents further virus propagation by reducing the infectious load at a site and minimising the spread of infection. A normal or controlled grow-out (to market size) is only an option if there is no possibility that during the grow-out period the pathogen will spread beyond the declared area. Depending on the number of fish involved, emergency harvesting can depopulate an area as quickly as destruction and disposal.

Clinically diseased fish

Immediate removal, destruction and disposal of all clinically diseased and moribund fish are essential for effective eradication. Clinically diseased fish, along with infectious waste, are the main source of VHSV in the environment. In a given population of infected fish, it is likely that some will show clinical signs of disease while others show none. In these circumstances, all fish in the population should be treated as diseased.

2.3.2 Containment, control and zoning

The detection of VHSV in wild marine fish in Australia would make control and containment of the virus very difficult. The geographic and biological distribution of VHSV would need to be investigated to determine if zoning is feasible. Environmental conditions in many parts of Australia are not suitable for the establishment of VHSV, which may make zoning easier.

If VHSV is isolated from fish in a freshwater establishment, there is the possibility of the virus becoming established in wild freshwater fish. In Denmark, control of VHS in trout farms has been practised for many years without measures being taken to remove wild fish populations (Jørgensen 1974).

Unexposed fish

Containment and control options for unexposed fish are the same as those outlined for eradication (Section 2.3.1). A zoning program and associated control measures to maintain uninfected zones will be necessary.

Exposed or potentially exposed, clinically normal fish

A successful zoning program for farmed fish relies on movement restrictions on exposed or potentially exposed fish to prevent infection spreading to uninfected zones. The feasibility of implementing a zoning program depends on farm management practices, the extent to which infection has already

spread and the location of reservoirs of infection. Feasibility can only be assessed at the time of the outbreak, taking into account such factors as movement restrictions required for fish, people, vehicles and boats, and market access for the fish products and by-products.

In a declared area, normal or controlled grow-out and slaughter may be feasible without further spread of infection. Harvested fish must be processed to the degree required for the designated market. For infected fish, evisceration will remove the organs likely to have the highest titre of virus, potentially allowing their sale for direct consumption within the affected area (CEFAS 2007). Freezing and thawing fish products will further reduce, but not eliminate virus in the product.

Clinically diseased fish

Diseased fish, along with infectious wastes, are the most likely means of spreading the virus to uninfected zones. There are currently no treatments for VHS, so fish that survive an outbreak can potentially become carriers and a source of infection. Destruction and adequate disposal of diseased fish is therefore the best option if the disease is to be contained within a zone.

2.3.3 Control and mitigation of disease

In a control and mitigation program, the aim may simply be to reduce the existing disease to biologically and/or economically acceptable levels. Critically, there may be a level of disease in the population below which the cost of further expenditure on control would be greater than the benefit. VHSV is an ssRNA virus, and RNA viruses tend to have higher rates of mutation than DNA viruses (Steinhauer and Holland 1987, Dale et al. 2009). If VHSV is not associated with clinical disease, the potential for the strain to adapt to a new host or to be virulent in alternative host species should be considered (Dale et al. 2009).

If farmed fish infected with VHSV are allowed to grow out for harvest, there should be a fallowing period between emptying the farm and restocking, as this will help break the VHSV cycle in the facility, depending on the presence of reservoir hosts. After an outbreak of VHS in a rainbow trout farm in the UK, the farm was disinfected and completely dried out (no water supply to the farm) for a minimum of 4 months (CEFAS 2007).

2.3.4 Trade and industry considerations

Trade regulations, market requirements and food safety standards must be considered as part of a control strategy. Permits may be required from the relevant authorities to allow products derived from disease control programs to be released and sold for human consumption.

Export and domestic markets

VHSV is listed by the OIE and is enzootic in North America, Japan and throughout much of Europe. Isolating VHSV in Australia would not necessarily mean that trade in fish products to these regions would be seriously affected.

Many overseas countries require imports such as fertilised fish eggs (embryos) to be certified as being free from VHSV. This export trade might still be possible even if some parts of Australia were considered infected with the virus, especially if such products came from a VHSV-free zone or farm.

Generally, evisceration of fish before export will satisfy international trade requirements for fish harvested from a VHSV-positive region. The Department

of Agriculture should be contacted for current information on export market requirements.

A cautious approach is required for the salvage of VHSV-exposed or potentially exposed product for the domestic market. Decisions about the release of fish or fish products will depend on the control strategy implemented. Evisceration will remove the organs most likely to contain the highest titre of virus in infected fish. If areas of Australia remain free of VHSV, restrictions on the release of fish product to the domestic market may help maintain freedom in those areas. If VHSV becomes enzootic in Australia, conditions associated with the import of products from VHSV infected areas would be reviewed by the Department of Agriculture.

3 Policy and rationale

3.1 Overall policy

Viral haemorrhagic septicaemia (VHS) is reportable in Australia and is an OIE-listed disease (OIE 2013a).

The policy for response to an outbreak of VHS in Australia depends on the nature of the outbreak and on the disease management strategy to be adopted. The response option will be decided by the Chief Veterinary Officer (CVO) and/or the Director of Fisheries of the state or territory in which the outbreak occurs. Epidemiological investigation will be used to assist with this decision.

There are three possible response options:

- *eradication*, with the aim of returning Australia to freedom from VHS
- *containment, control and zoning* to confine the VHS virus to enzootic areas, prevent further spread and protect uninfected areas, or
- *control and mitigation* of the disease through management practices that decrease the incidence and severity of the disease.

Epidemiological information on which to base a decision may initially be limited. The policy initially implemented may change as more information becomes available. For example, eradication may eventually be chosen as a long-term policy even when the containment, control and zoning response option was initially implemented.

Strategies that may be used under these control options include:

- *quarantine and movement controls* on fish, fish products, boats, cars and other fomites in declared areas to prevent spread of infection
- *prevention of access* by predators and/or scavengers (e.g. birds) to infected fish
- *destruction and disposal* of clinically diseased and dead fish to prevent further virus release into the environment
- *decontamination* of facilities to inactivate the virus
- *surveillance* to determine the extent of possible fish hosts that are infected, and to provide proof of freedom from the virus
- *zoning* to define infected and VHS-free zones and to maintain VHS-free zones
- *restocking* with older, less susceptible fish or less susceptible species unlikely to develop clinical disease
- *a public awareness campaign* to assist cooperation from industry and the community.

If VHSV, with or without disease, is confirmed in Australia, the director of fisheries and/or the CVO of the state or territory from which VHSV is detected will be responsible for implementing disease control measures in accordance with relevant legislation. The Aquatic Consultative Committee on Emergency Animal Diseases (comprising representatives of the state or territory and Australian governments and representatives of the affected industries) will be convened to discuss options for responses to the incident, and the agreed

management strategy will then be implemented by the state or territory of the outbreak. Detailed control measures will be determined using the principles of control and eradication and epidemiological information about the incident. In some instances, changes to legislation may be required to facilitate a more effective response (e.g. Appendix 4).

For information on the responsibilities of the state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN *Control Centres Management* manual (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan).

3.2 Overview of response options

The control option to be adopted will be decided after or during the initial response to the outbreak of VHS and/or isolation of VHSV. This decision may need to be made with only very limited epidemiological information. While it is important that the initial choice is decisive, it is also important that the decision is dynamic. As more information becomes available, the decision may be modified.

Below are some key criteria for the adoption of a policy option. These criteria are not exhaustive and are given only as a guide. The flow chart in Figure 5 provides possible scenarios and is designed to aid the initial decision-making process. For full details of measures to be taken under each control option, see Section 2.2.

3.2.1 Option 1: Eradication

Eradication may be feasible and chosen as the preferred control option when:

- epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the virus (e.g. in a closed system such as an aquarium or in a fully recirculating system)
- there is no possibility of virus being in wild fish stocks (unless such stocks are in a landlocked system where their complete destruction is possible).

3.2.2 Option 2: Containment, control and zoning

Containment, control and zoning may be the preferred control option when:

- VHSV is isolated from wild or farmed fish confined to a specific geographic area (the determination of which may require a comprehensive monitoring and surveillance program) and virus containment is possible
- there is clinical disease associated with the outbreak
- eradication is not considered to be an option.

When containment, control and zoning are chosen as the initial option, the policy may later evolve into one of control and mitigation of disease.

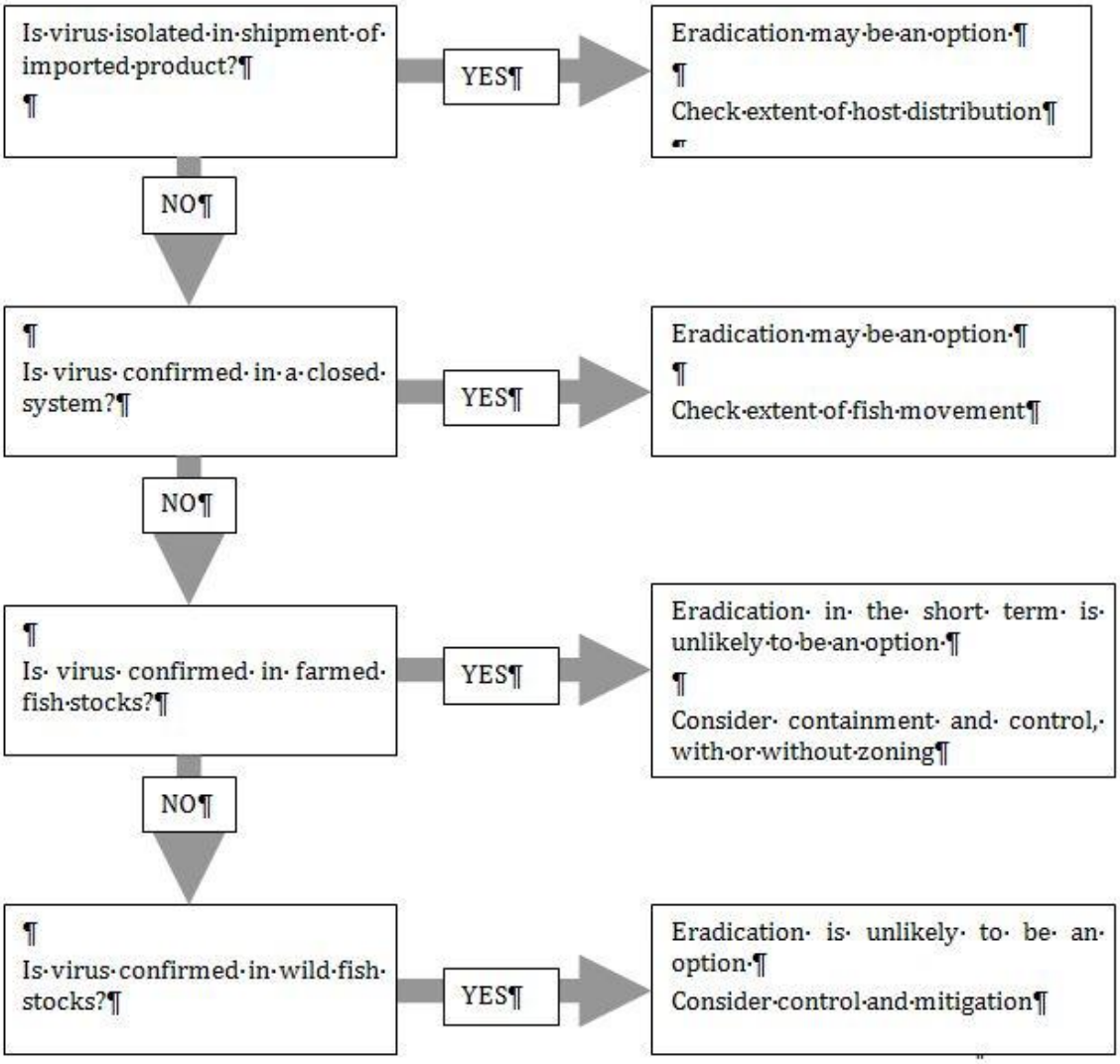
3.2.3 Option 3: Control and mitigation of disease

Control and mitigation of disease is the preferred control option when:

- VHSV is considered to be widespread in wild fish stocks and/or farmed fish stocks and distributed widely in an area or areas where zoning would be difficult

- there is no possibility of limiting the spread of the virus.

Figure 5 Response options for viral haemorrhagic septicaemia disease outbreaks



3.3 Strategies for control and eradication

On suspicion or confirmation of VHSV in Australia, and while the extent of the outbreak or spread of the virus is being determined, the following steps should be taken to minimise or prevent further impact or spread of disease.

3.3.1 Epidemiological investigations

A comprehensive epidemiological investigation, including tracing and surveillance, should be initiated immediately to determine:

- which genogroup or type the isolate is most likely to belong to; this will help identify the possible source of the virus, and possible extent of disease
- how widespread the virus may be, geographically and biologically (i.e. the range of susceptible fish species that may be infected). Until adequate

data become available, potentially exposed fish populations should be assumed to have been exposed to the virus

- how to prevent further spread of the virus from the infected location, if prevention is considered possible.

It can be difficult to isolate the virus from carrier fish. This must be taken into consideration when designing and performing any epidemiological investigation and assessment of the results. Fish from waters cooler than 18°C should be the primary focus.

3.3.2 Quarantine and movement controls

Quarantine and movement controls (Section 2.2.1) must be imposed on anything capable of transmitting the virus. Control areas will be established if the virus or the disease has been found in fish in an area conducive to control. Only limited epidemiological information may be available on which to make a decision. Control area boundaries can be refined as more information becomes available.

3.3.3 Treatment of fish

There are no treatments currently available for VHS.

3.3.4 Vaccination

There are currently no commercially available vaccines for VHSV, but DNA-based vaccine technology has been shown to be effective under experimental conditions (Ortega-Villaizan et al. 2009, Chico et al. 2009, Lorenzen et al. 2009). RNAi technology holds some promise for reducing future impacts from VHSV (Ruiz et al. 2009). Vaccines and RNAi are unlikely to be suitable for emergency control of disease outbreaks, but can be used for controlling spread of VHSV to unexposed populations in long-term control programs.

3.3.5 Destruction of fish

The decision to humanely destroy fish must be made based on the circumstances of the outbreak. Virus shedding from clinically diseased fish is likely to be high. An outbreak associated with significant clinical disease may warrant destruction of fish to limit contamination of the environment and reduce the risk of further spread of the virus into neighbouring fish populations.

If the outbreak occurs in natural waterways, it is possible that by the time a control strategy is implemented the virus will have established in wild fish populations. In these circumstances, the humane destruction of cultured fish will assist in minimising the virus load in the area, but it will not eradicate the virus from the infected area. See the AQUAVETPLAN operational procedures manual *Destruction* (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan) for details of destruction operations.

3.3.6 Treatment of fish products and by-products

The treatment of fish products and by-products must consider trade regulations, market requirements, food safety standards and potential spread of the pathogen via products.

Harvested fish should be eviscerated as a minimum, and preferably filleted, to remove organs likely to contain the highest titre of virus. Harvested fish can safely be frozen until infection is definitively diagnosed or discounted. The freeze–thaw cycle will reduce virus titre.

A decision on the use of fish products and by-products will depend on the control option selected (Section 2.2.6).

Any harvesting or processing equipment used must be treated as contaminated and disinfected accordingly (see the AQUAVETPLAN operational procedures manual *Disposal* (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan).

3.3.7 Vector control

Effective vector control is essential in the initial response, to prevent predators and scavengers (e.g. birds and rodents) eating infected carcasses or carrying them away from the infected premises.

3.3.8 Public awareness

In the early stages of an outbreak investigation, education and public relations, especially with the media, is critical. The use of trained communications managers as media contact points is essential to ensure effective communication with stakeholders and the public. A vital aspect of the response program will be to address the concerns of the public (especially groups such as fishers) by conveying the fact that the authorities are taking all necessary measures to control the situation. It must also be clearly stated that VHS poses no health risk for humans. It is also important to inform and educate the public and key stakeholders on critical issues, especially when their support and cooperation is required. This can be done using extension materials such as the poster and brochure in Appendix 4. The use and monitoring of social media are necessary to provide correct information and dispel incorrect rumours early.

3.4 Social and economic effects

Australia's aquatic animal health status for VHS will change if the VHS virus is isolated from Australian fish. This change may only be temporary if the virus is successfully eradicated.

3.4.1 Export markets

If VHSV is isolated from Australian fish species, its occurrence must be reported to the OIE. In this event, industries exporting fish products (e.g. fertilised eggs) will need to confirm the requirements of countries importing Australian products. Fish exported from temperate areas of Australia are usually eviscerated, so it is not expected that there would be substantial impacts on Australian exports.

Increased monitoring and surveillance, with comprehensive sampling of fish populations in affected industries, may be required to satisfy the requirements for proof of freedom from VHSV for importing countries. Fish egg exporters already have a monitoring and surveillance program in place.

Permits may be required from the relevant authorities to allow products from within disease control programs to be released and sold for human consumption.

The Department of Agriculture website should be used for the most current information about export market requirements (fish export requirements).

3.4.2 Domestic markets

Decisions about the release of fish or fish products to the domestic market will depend on the control strategy implemented (Section 2.3.4). If VHSV is isolated

in Australia and becomes enzootic, the entry of fish products from other parts of the world where VHS is endemic into domestic markets might be expected.

3.5 Criteria for proof of freedom

Proof of freedom from VHSV may be important for trade. Proof of freedom can be demonstrated at the aquaculture establishment, zone and country levels. Criteria for proof of freedom at each level are given in the OIE *Aquatic Animal Health Code* (OIE 2013a) (see Appendix 2).

3.6 Funding and compensation

There are currently no national cost-sharing agreements in place for emergency responses to VHS. It is the responsibility of the users of this publication to seek advice in relation to any relevant funding or compensation arrangements within the relevant jurisdiction.

Appendix A: Species susceptibility to viral haemorrhagic septicaemia

Main sources: the OIE *Manual of Diagnostic Tests for Aquatic Animals*, chapter 2.3.9 (updates from OIE notifications include up to February 2014), and the European Food Safety Authority Panel on Animal Health and Welfare Scientific Opinion: Aquatic species susceptible to diseases listed in Directive 2006/88/EC (http://www.efsa.europa.eu/en/efsajournal/pub/808.htm).

Table A1 Species susceptibility to viral haemorrhagic septicaemia

Susceptible Species	Susceptible Species
Alaska pollock (*Theragra chalcogramma*)	Armoured weaselfish (*Hoplobrotula armata*)
Atlantic cod (*Gadus morhua*)	Atlantic halibut (*Hippoglossus hippoglossus*)
Atlantic herring (*Clupea harengus*)	Atlantic salmon (*Salmo salar*)
Black crappie (*Pomoxis nigromaculatus*)	Black porgy (*Acanthopagrus schlegelii*)
Blue whiting (*Micromesistius poutassou*)	Bluegill (*Lepomis macrochirus*)
Bluntnose minnow (*Pimephales notatus*)	Brook trout (*Salvelinus fontinalis*)
Brown bullhead (*Ictalurus nebulosus*)	Brown trout (*Salmo trutta*)
Burbot (*Lota lota*)	Channel catfish (*Ictalurus punctatus*)
Chinook salmon (*Oncorhynchus tshawytscha*)	Chum salmon (*Oncorhynchus keta*)
Coho Salmon (*Oncorhynchus kisutch*)	Cuckoo wrasse (*Labrus mixtus*)
Dab (*Limanda limanda*)	Emerald shiner (*Notropis atherinoides*)
English sole (*Parophrys vetulus*)	Eulachon (*Thaleichthys pacificus*) (smelt)
European eels (*Anguilla anguilla*)	European river lamprey (*Lampetra fluviatilis*)
European sprat (*Sprattus sprattus*)	Flounder (*Platichthys flesus*)
Fourbeard rockling (*Enchelyopus cimbrius*)	Freshwater drum (*Apliodinotus grunniens*)
Gilthead seabream (*Sparus aurata*)	Gizzard shad (*Dorosoma cepedianum*)
Golden trout (*Salmo aquabonita*)	Grayling (*Thymallus thymallus*)
Greater amberjack (*Seriola dumerii*)	Greenland halibut (*Reinhardtius hippoglossoides*)
Haddock (*Gadus aeglefinus*)	Hairtail (*Trichiurus lepturus*)
Hong Kong grouper (*Epinephelus akaara*)	Hybrid (rainbow trout × coho salmon) (*Oncorhynchus mykiss × O. kisutch*)
Iberian nase (*Pseudochondrostoma polylepis*)	Japanese amberjack (*Seriola purpurascens*)
Japanese flounder (*Paralichthys olivaceus*) (hirame)	Japanese yellowtail (*Seriola quinqueradiata*)
Korean flounder (*Glyptocephalus stelleri*)	Lake trout (*Salvelinus namaycush*)
Lake whitefish (*Coregonus clupeaformis*)	Largemouth bass (*Micropterus salmoides*)
Lesser argentine (*Argentina sphyraena*)	Marbled flounder (*Pleuronectes yokohamae*)
Mullet (*Mugil cephalus*)	Mummichog (*Fundulus heteroclitus*)
Muskellunge (*Esox masquinongy*)	Norway pout (*Trisopterus esmarki*)
Olive flounder (*Paralichthys olivaceus*)	Pacific cod (*Gadus macrocephalus*)
Pacific hake (*Merluccius productus*)	Pacific herring (*Clupea pallasii*)
Pacific mackerel (*Scomber japonicus*)	Pacific salmon (*Oncorhynchus* spp.)
Pacific sand eel (*Ammodytes personatus*)	Pacific sand lance (*Ammodytes hexapterus*)
Pacific sardine (*Sardinops sagax*)	Pacific tomcod (*Microgadus proximus*)
Pike (*Esox lucius*)	Plaice (*Pleuronectes platessa*)
Poor cod (*Trisopterus minutus*)	Pumpkinseed (*Lepomis gibbosus*)

Susceptible Species	Susceptible Species
Rainbow trout (*Oncorhynchus mykiss*)	Red seabream (*Pagrus auratus*)
Rock bass (*Ambloplites rupestris*)	Rockfish (*Sebastes* spp.)
Rockling (*Rhinonemus cimbrius*)	Round goby (*Neogobius melanostomus*)
Sablefish (*Anoplopoma fimbria*)	Sand goby (*Pomatoschistus minutus*)
Sandeel (*Ammodytes* spp.)	Sea bass (*Dicentrarchus labrax*)
Senegalese sole (*Solea senegalensis*)	Shiner perch (*Cymatogaster aggregata*)
Shorthead redhorse (*Moxostoma macrolepidotum*)	Silver pomfret (*Pampus argenteus*)
Silver redhorse (*Moxostoma anisurum*)	Smallmouth bass (*Micropterus dolomieui*)
Sockeye salmon (*Oncorhynchus nerka*)	Splake (lake trout × brook trout) (*Salvelinus namaycush × S. fontinalis*)
Spottail shiner (*Notropis hudsonius*)	Striped bass (*Morone saxatilis*)
Surf smelt (*Hypomesus pretiosus*)	Three-spine stickleback (*Gasterosteus aculeatus*)
Trout-perch (*Percopsis omiscomaycus*)	Tube snout (*Aulorhynchus flavidus*)
Turbot (*Psetta maxima*)	Walleye (*Sander vitreus*)
Walleye pollock (*Theragra chalcogramma*)	White bass (*Morone chrysops*)
Whitefish (*Coregonus* spp.)	White perch (*Morone americanus*)
Whiting (*Merlangius merlangus*)	Yellow perch (*Perca flavescens*)
Yellowback seabream (*Dentex tumifrons*)	

Fish species (freshwater and marine) from which VHSV has been isolated

Main sources: the OIE *Manual of Diagnostic Tests for Aquatic Animals*, chapter 2.3.9 (updates from OIE notifications include up to February 2014), and the European Food Safety Authority Panel on Animal Health and Welfare Scientific Opinion: Aquatic species susceptible to diseases listed in Directive 2006/88/EC (http://www.efsa.europa.eu/en/efsajournal/pub/808.htm).

Table A2 Species from which VHSV has been isolated where clinical signs of disease have been observed

Ballan wrasse (*Labrus bergylta*)*	Burbot (*Lota lota*)
Corkwing wrasse (*Symphodus melops*)*	Cuckoo wrasse (*Labrus mixtus*)
Eulachon (*Thaleichthys pacificus*) (smelt)	Freshwater drum (*Apliodinotus grunniens*)
Goldsinny wrasse (*Ctenolabrus rupestris*)*	Greater amberjack (*Seriola dumerii*)
Japanese amberjack (*Seriola purpurascens*)	Japanese flounder (*Paralichthys olivaceus*) (hirame)
Mummichog (*Fundulus heteroclitus*)	Muskellunge (*Esox masquinongy*)
Olive flounder (*Paralichthys olivaceus*)	Pacific hake (*Merluccius productus*)
Pacific herring (*Clupea pallasii*)	Pacific sardine (*Sardinops sagax*)
Pike (*Esox lucius*)	Rainbow trout (*Oncorhynchus mykiss*)
Rockcook (*Centrolabrus exoletus*)*	Round goby (*Neogobius melanostomus*)
Sablefish (*Anoplopoma fimbria*)	Smallmouth bass (*Micropterus dolomieui*)
Surf smelt (*Hypomesus pretiosus*)	Turbot (*Psetta maxima*)
Walleye pollock (*Theragra chalcogramma*)	Yellow perch (*Perca flavescens*)

Table A3 Species challenged with at least one VHSV isolate (generally Type I) and found not to be susceptible*

Chinook salmon (*Oncorhynchus tshawytscha*)	Coho salmon (*Oncorhynchus kisutch*)
Common carp (*Cyprinus carpio*)	Eurasian perch (*Perca fluviatilis*)
Goldfish (*Carassius auratus*)	Roach (*Leuciscus rutilus*)
Tench (*Tinca tinca*)	

* This does not mean that these species are not susceptible to other VHSV isolates.

Appendix B OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals

OIE Aquatic Animal Health Code

The objective of the *Aquatic Animal Health Code* is to prevent the spread of aquatic animal diseases while facilitating international trade in fish and fish products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade.

The latest edition of the OIE Aquatic Code is available at http://www.oie.int/international-standard-setting/aquatic-code/access-online. Chapter 10.9, Viral haemorrhagic septicaemia is relevant to this manual.

OIE Manual of Diagnostic Tests for Aquatic Animals

The purpose of the *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2013b) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use internationally.

The current edition of the OIE Aquatic Manual is available at http://www.oie.int/international-standard-setting/aquatic-manual/access-online. Chapter 2.3.9, Viral haemorrhagic septicaemia is relevant to this manual.

Further information

Further information about the OIE Aquatic *Code* and *Manual* is available on the OIE website (http://www.oie.int/en). An internet search for 'OIE aquatic code' or 'OIE aquatic manual' will help find the web pages.

Appendix C Detection and identification of viral haemorrhagic septicaemia

The following methods are used for the detection and identification of viral haemorrhagic septicaemia virus (VHSV) at the Australian Animal Health Laboratory (AAHL) Fish Diseases Laboratory (AFDL) at CSIRO Livestock Industries, Geelong.

Examination and culture of specimens

Sampling

Suspected fish specimens should initially be sent to the state or territory diagnostic laboratory. After obtaining the necessary clearance from the chief veterinary officer (CVO) of the state or territory of the disease outbreak and informing the CVO of Victoria of transport of specimens to Geelong, specimens will be forwarded to the AAHL for exotic disease testing. Tissue samples should be collected according to the Australian and New Zealand Standard Diagnostic Procedure *Collection and submission of samples for investigation of diseases of fin fish* (finfish sampling). Tissues or fluids from affected fish may be pooled in one container with transportation medium at a ratio of 1 part tissue (weighing a minimum of 0.5 g) to 5 parts medium, representing one pooled sample. During transportation to the AAHL, pooled tissues may be stored (on ice but not frozen) in transportation medium. The recipe for transportation medium is as follows:

Reagents: 400 mL Hank's balance salt solution (HBSS), 200 IU penicillin/200 μg streptomycin (pen–strep)/mL (final dilution), and 2% (v/v) foetal bovine serum (FBS).

Method: using an aseptic technique, add 8 mL FBS to 400 mL HBSS. Thaw out antibiotic solution. Aseptically add antibiotic solution to the HBSS–FBS solution.

Storage and transport: samples should not be frozen before processing but should be maintained at 4–10°C (shipped on wet ice in a styrofoam shipping container). To maximise sensitivity, samples should be processed and assayed within 24 hours of sampling but, when this is not possible, they must be processed within 72 hours of sampling, during which time storage must be at 4°C. Samples to be assayed after 72 hours after collection should be frozen at –70°C to –80°C.

Tissues: tissues to be examined will depend on the size of fish in the population being tested and the time of year. During spawning, reproductive fluids (preferably ovarian fluid but sometimes milt) should be tested. Tissue samples obtained during nonspawning season will be either whole fry (for the current year class) or selected fish tissues (from older fish of previous year classes), collected aseptically. Samples for testing could include any of the following:

Table C1 Fish tissues collected for VHSV testing

Fish size (length)	Tissues
< 4 cm	Entire fish (remove yolk sac if present)
4–6 cm	Entire viscera including kidney
> 6 cm	Kidney, liver, spleen, encephalon, heart and gill filaments
Sexually mature	Ovarian fluids, kidney, liver, spleen, encephalon, heart and gill filaments

VHSV is very sensitive to enzymatic degradation, therefore sampling tissues with high enzymatic activities such as viscera and liver should be avoided (OIE 2013b).

Culture

It is recognised that some fish cell lines are more susceptible to virus infection and support growth and development of some viruses better than other cell lines. Thus, as part of a disease investigation where involvement of a viral pathogen is suspected, the AFDL will use a range of fish cell lines in an attempt to isolate the virus. Based on international protocols, the AFDL will use two or more of the cell lines BF-2, EPC, RTG-2, CHSE-214 and FHM for isolation of VHSV.

Tissue samples submitted to the AAHL are homogenised using a frozen, sterile mortar and pestle to assist release of a portion of any virus particles present. Diluted aliquots of the supernatants, obtained by centrifuging the prepared tissue homogenates, are inoculated onto cell culture monolayers. These are then incubated at 15°C over a period of several days to allow the development of any viral cytopathic effect, which would be due to the presence of specific viruses (Crane and Williams 2008).

Identification

Immunocytochemistry

Virus identification by various immunoassays has become a standard procedure for viruses where specific antibodies are available. At the AFDL, immunocytochemistry using an immunoperoxidase test is favoured. Virus-infected cell cultures are fixed and incubated with a primary antibody preparation containing either monoclonal or polyclonal antibodies that will bind to specific epitopes if present. Excess primary antibody is removed by washing, and a secondary biotinylated antibody (e.g. biotinylated anti-rabbit Ig if the primary antibody was raised in rabbits) is added. After an incubation period, excess secondary antibody is removed by washing, and streptavidin–peroxidase conjugate is added. After incubation, excess conjugate is removed by washing, a substrate (e.g. with 3 amino-9-ethylcarboxyole) is added and colour is allowed to develop. Finally, after washing in water, cells are counterstained with Mayer's haematoxylin, rinsed in water and blued with Scott's tap water. Any virus that is recognised by the primary antibody will yield a positive colour reaction (Crane et al. 2000).

Similarly, the immunoperoxidase test can be performed on fixed tissues from affected fish (Crane et al. 2000).

Polymerase chain reaction

Tissue samples (homogenised, frozen and thawed, centrifuged and supernatant fluids collected) and tissue culture supernatants are inactivated by adding them to an appropriate commercially prepared buffer (e.g. Qiagen AVL buffer) containing guanidinium isothiocyanate.

Nucleic acid is obtained from cell-free samples using the QIAamp viral RNA extraction kit or from tissues using the RNeasy viral RNA extraction kit. RT-PCR is undertaken using the SuperScript III One-Step RT-PCR with Platinum Taq kit using primers and cycling conditions described in the OIE Aquatic Manual (OIE, 2013a) which are based on Snow et al. (2004).

Table C2 RT-PCR primers to produce a 505bp product from the N gene (OIE 2013b)

Primer	Sequence
VHSV VNF	5'-ATG-GAA-GGA-GGA-ATT-CGT-GAA-GCG-3'
VHSV VNR	5'-GCG-GTG-AAG-TGC-TGC-AGT-TCC-C-3'

Sequencing of PCR products is required for definitive diagnosis, and will help strain identification.

Further reading

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Appendix D Example of a VHSV risk mitigation policy – Michigan DNR, USA

FO-245.09

Fish disease control

By authority conferred on the Department of Natural Resources by sections 41101 through 41105 of 1994 PA 451, MCL 324.41105, it is ordered that effective, December 4, 2008, for a period of five years, the following regulations are established for fish disease control:

The goals of the Department's actions under this Fish Disease Control Order are to protect the aquatic resources of the State, minimize the spread of Pathogens of concern to uninfected waters, and protect the Department's fish hatchery system. The Department will address the control of diseases of fish through the development of regulations for specific Management Areas that are designed to contain or slow the spread of Pathogens of concern.

Definitions

As used in this Fish Disease Control Order, specific terms are defined as follows:

Baitfish –live or dead species of fish, or parts of fish excluding Roe, that are used by anglers to catch fish.

Baitfish and Roe Exclusion Zone- waters designated as critical to hatchery operations where possession and use of baitfish and roe as defined is prohibited.

Certification Process – a process used by the Department to grant or deny applications from individuals who want to conduct certain activities that are regulated by this Fish Disease Control Order.

Inland Waters – all public waters of the State except for the Great Lakes and their connecting waters.

Pathogen – viruses, bacteria, fungi, and parasites that cause disease in living organisms.

Prohibited Fish Species – Baitfish and other species of fish identified by the Department as infected with one or more Pathogens of concern.

Roe – eggs of fish.

Baitfish and roe exclusion zones

The Department has identified locations vital to the protection of hatchery operations in an effort to control or contain pathogen movement and reduce disease risks to these facilities. In these critical areas restrictions on the possession and subsequent use of baitfish and roe must be implemented. Therefore, baitfish and roe shall not be possessed on the following waters of the State:

Benzie County
Brundage Creek and tributaries Kinney Creek and Stanley Creek, from its confluence with the Platte River (T26N, R13W, S7), to their headwaters, including Brundage Spring Pond.

Chippewa County
Pendills Lake (T47N, R4W, S25, 26) including tributary Pendills Creek downstream to its confluence with Lake Superior (T47N, R4W, S28).

Sullivan Creek from its headwaters (T46N, R4W, S32) downstream to its confluence with the North Branch of the Pine River (T45N, R4W, S23).

Viddian Creek from its headwaters (T47N, R4W, S32) downstream to its confluence with Pendills Creek (T47N, R4W, S28).

Marquette County
Cherry Creek from the location of the Cherry Creek Road (T47N, R24W, S18) to the headwaters at County Road 480 (T47N, R25W, S22).

Regulation by management area
The Department uses a classification system to categorize waters based on their disease status. Specific Management Areas identified by the Department under this system include: 1) Named Pathogen Positive Management Area, where the presence of a named Pathogen has been confirmed; 2) Named Pathogen Surveillance Management Area, where a named Pathogen is likely to be found in the near future; and 3) Named Pathogen Free Management Area, where a named Pathogen has not been confirmed to date. Regulatory actions are designed for each Management Area to reduce the risk of spreading a Pathogen from, and in some cases containing a Pathogen within, a Named Pathogen Positive Management Area. Unique regulatory actions will be developed for each new Pathogen that is discovered, and such actions will take into account the likely dispersal routes for a Pathogen.

GENERAL STATEWIDE PROVISIONS [In this order the terms State-licensed baitfish retail and State-licensed baitfish wholesale operations are those that are required to be licensed by the state of Michigan for these activities]

1. The official list of Prohibited Fish Species for each Pathogen of concern as identified by the Department will be available from the Department and will be posted on Fisheries Division's web site (http://www.michigan.gov/dnrfishing). The list of Prohibited Fish Species for each Pathogen of concern as of June 28, 2007 is attached to this Fish Disease Control Order for reference only (Appendix A). Updates to the list of Prohibited Fish Species will be made as necessary. Those changes will be immediately posted on Fisheries Division's web site, and incorporated into this Fish Disease Control Order at least annually.

2. The official list of waters classified by Named Pathogen Management Area for each Pathogen of concern as identified by the Department shall be posted on Fisheries Division's web site (http://www.michigan.gov/dnrfishing). The list of waters classified by Named Pathogen Management Area for each Pathogen of concern as of June 28, 2007 is attached to this Fish Disease Control Order for reference only (Appendix B). Updates to the list of list of waters classified by Named Pathogen Management Area will be made as necessary. Those changes will be immediately posted on Fisheries Division's web site, and incorporated into this Fish Disease Control Order at least annually.

3. It shall be unlawful to import into this state any uncertified baitfish species found on the list of Prohibited Fish Species (Appendix A).

4. The Certification Process includes two Parts: Part A) Status of a Facility and Part B) Status of Fish Health. The *Guide for Certification of Status of a Facility and Status of Fish*

Health will identify the specific steps to be completed for each Pathogen of concern as identified by the Department, as well as when a Certification for Status of a Facility that has been granted, or a Certification for Status of Fish Health that has been granted, shall expire. The *Guide for Certification of Status of a Facility and Status of Fish Health* will be available from the Department and will be posted on Fisheries Division's web site (http://www.michigan.gov/dnrfishing).

Part A) Status of a Facility – Applicants who wish to keep Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, in a facility shall have that facility reviewed by the Department to ensure that there is minimal risk to public waters of the State and that appropriate biological security measures are in effect for the facility. The Department will review the results for each application under Part A) and respond to the applicant in writing with a letter of Certification for Status of a facility that either grants or denies the application and includes the Department's rationale for the decision as well as the actions necessary for achieving certification. This Part A) does not apply to: 1) an owner of an aquaculture facility regulated and permitted by theMichigan Department of Agriculture; 2) an owner of a State-licensed Baitfish retail operation; or 3) an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

Part B) Status of Fish Health – Applicants who wish to offer for sale or sell Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, shall have those Baitfish or that Roe tested for each Pathogen of concern by a certified laboratory that has been approved by the State of Michigan. Applicants shall submit the results of all tests to the Department. The Department will review the results for each application under Part B) and respond to the applicant in writing with a letter of Certification for Status of Fish Health that contains a transaction number, and that either grants or denies the application and includes the Department's rationale for the decision. This Part B) does not apply to an owner of a State-licensed Baitfish retail operation or an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

5. A person shall not stock Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, in holding facilities, ponds, or other waters that discharge into public waters of the State prior to completing Part A) of the Certification Process and receiving a letter of Certification for Status of a Facility from the Department permitting such activity. This Provision 4 does not apply to 1) an owner of an aquaculture facility regulated and permitted by the Michigan Department of Agriculture; 2) an owner of a State-licensed Baitfish retail operation; or 3) an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

6. If an approved Certification for Status of a Facility is issued by the Department under Part A) of the Certification Process, an applicant shall carry that letter of Certification for Status of a Facility with them when transporting and stocking Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, in holding facilities, ponds, or other waters that discharge into public waters of the State. That letter of Certification for Status of a Facility shall be shown upon request. This Provision 5 does not apply to 1) an owner of an aquaculture facility regulated and permitted by the Michigan Department of Agriculture; 2) an owner of a

State-licensed Baitfish retail operation; or 3) an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

7. A person shall not stock Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, in public waters of the State prior to receiving a Fish Stocking Permit from the Department permitting such activity.

8. If an approved Fish Stocking Permit is issued by the Department, an applicant shall carry that Fish Stocking Permit with them when transporting and stocking Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, in public waters of the State. That Fish Stocking Permit shall be shown upon request.

9. Except as otherwise provided for in this Fish Disease Control Order, a person shall not offer for sale or sell any Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, prior to completing Part B) of the Certification Process and receiving a letter of Certification for Status of Fish Health from the Department permitting such activity. This Provision 8 does not apply to an owner of a State-licensed Baitfish retail operation or an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

10. If an approved Certification for Status of Fish Health is issued by the Department under Part B) of the Certification Process, an applicant shall carry that letter of Certification for Status of Fish Health with them when transporting, offering for sale, or selling Baitfish or live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species. That letter of Certification for Status of Fish Health shall be shown upon request. This Provision 9 does not apply to an owner of a State-licensed Baitfish retail operation or an owner of a State-licensed commercial fishing operation specifically for that part of the operation related to the sale for human consumption of Roe taken from fish that are listed as Prohibited Fish Species.

11. An owner of a State-licensed Baitfish wholesale operation shall provide to each wholesale purchaser at the point of sale a copy of the receipt that contains specific information as identified by the Department in the *Guide for Certification of Status of a Facility and Status of Fish Health*, as well as the transaction number from the letter of Certification for Status of Fish Health. An owner of a State licensed Baitfish wholesale operation shall retain the original receipts for at least one year after the date of sale. Receipts shall be shown upon request.

12. A wholesale purchaser shall retain copies of receipts for purchases from a State-licensed Baitfish wholesale operation for at least one year after the date of sale. Receipts shall be shown upon request.

13. An owner of a State-licensed Baitfish retail operation shall provide to each retail customer at the point of sale a receipt that contains specific information as identified by the Department in the *Guide for Certification of Status of a Facility and Status of Fish Health*, as well as the transaction number from the letter of Certification for Status of Fish Health.

14. A retail customer shall retain and show upon request the receipt for purchases of Baitfish or Roe from a State-licensed Baitfish retail operation. A receipt shall be valid for 14 days from the date of sale for all certified baitfish, except frozen baitfish, and 3 days from the date of sale for all uncertified bait. Receipts for certified frozen baitfish shall be good for 6 months from the date of sale and original packaging showing certification status must be kept with the baitfish for verification. Receipts for uncertified frozen baitfish shall be valid for 3 days from the date of sale.

15. All species of live fish and Roe maintained at a location by an owner of a State-licensed commercial fishing operation, an owner of a State-licensed Baitfish wholesale operation, or an owner of a State licensed Baitfish retail operation shall be considered uncertified if live fish that are listed as Prohibited Fish Species, or Roe taken from fish that are listed as Prohibited Fish Species, are also maintained at that location and those live fish or that Roe have not been approved as required under Part B) of the Certification Process.

16. A person, who catches fish in a lake or a Great Lake, shall not release those fish alive in any public waters of the State if those fish are listed as Prohibited Fish Species, except that those fish may be released alive in that lake, or that Great Lake, or in a connecting body of water to that lake, or that Great Lake, so long as those fish can freely move between the original location of capture and the location of release. This Provision 15 does not apply to Baitfish.

17. A person who catches fish in a stream shall not release those fish alive in any public waters of the State if those fish are listed as Prohibited Fish Species, except that those fish may be released alive in any part of that stream, or in a connecting body of water to that stream, so long as those fish can freely move between the original location of capture and the location of release. This Provision 16 does not apply to Baitfish.

18. Except as further restricted in this Fish Disease Control Order, a person shall not use or otherwise release Baitfish that are listed as Prohibited Fish Species, or Roe harvested from fish that are listed as Prohibited Fish Species, in any public waters of the State, unless that person is fishing and those Baitfish or that Roe are attached to a hook.

19. A person who trailers a boat over land shall drain all water from the live well(s) and the bilge of their boat upon leaving any body of water.

SECTION I – Viral Hemorrhagic Septicemia Virus (VHSv)

Viral hemorrhagic septicemia virus (VHSv) is a disease of fish that has caused large-scale mortalities of fish in aquaculture operations in Europe, in certain populations of wild fish along the Pacific Coast of North America, and now in various populations of wild fish in several areas of the Great Lakes Basin. There are four known genetic types of the virus, three in Europe where VHSv originated and one in North America. The genetic type found in the Great Lakes Basin (VHSv IVb) is most similar to the strain of VHSv previously isolated from the Atlantic Coast of Eastern North America. VHSv types I, II, and III have caused significant mortalities, particularly in rainbow trout in European aquaculture facilities, and type IVa has caused large mortalities in Pacific herring in the Puget Sound area. In the Great Lakes Basin, VHSv IVb has caused mortalities in several species of wild fish inhabiting Lake Huron, the St. Clair River, Lake St. Clair, the Detroit River, Lake Erie, the Niagara River, Lake Ontario, and the St. Lawrence River, along with a few inland waters in the basin.

On November 10, 2008, a Federal Interim Rule was issued by the Animal and Plant Health Inspection Service of the US Department of Agriculture in an effort to prevent the spread of VHSv between States and between the U.S. and Canada, thus hopefully protecting economically important sport fisheries and aquaculture operations. This Fish Disease Control Order is consistent with the Federal Interim Rule, and it provides for additional protections to slow the spread of VHSv throughout the Great Lakes and Inland Waters under the jurisdiction of Michigan that are not covered by the federal Emergency Order (as amended).

VHSv is likely to continue spreading in fish throughout the Great Lakes Basin via the natural movements of infected fish. Although the virus may take years to infect fish in Lake Superior, it could be rapidly moved by ships that discharge untreated ballast water, the stocking of infected fish, or the unintended movement of water by boaters and anglers that contains either the virus itself or live fish that are infected with the virus. While containment and eradication of VHSv are likely not possible, the regulations specified in this Fish Disease Control Order are necessary to slow the spread of VHSv, thus providing the time required to develop strategies for managing the Pathogen. The restrictions detailed below will help the Department to 1) protect populations of wild fish in Inland Waters, and in several areas of the Great Lakes, that are not yet infected with the virus; 2) protect wild populations of fish used as broodstock for fisheries management, and 3) prevent the infection of fish being reared in State-owned fish hatcheries.

Management Area Regulations for Viral Hemorrhagic Septicemia virus (VHSv)

VHSv Positive Management Area

On all waters designated within the VHSv Positive Management Area, the following regulations further restrict the transportation, sale, use, and release of Baitfish and fish that are listed as Prohibited Fish Species, and Roe taken from fish that are listed as Prohibited Fish Species, that **have not** been approved as required under Part B) of the Certification Process.

A person who catches Baitfish that are listed as Prohibited Fish Species, or harvests Roe from fish that are listed as Prohibited Fish Species, in a body of water that is included in the VHSv Positive Management Area shall not use or otherwise release those Baitfish or that Roe in any public waters of the State, except that those Baitfish or that Roe may be used in any waters included in the VHSv Positive Management Area subject to Provision 17 under the General Statewide Provisions.

Recreational anglers

1. A person who purchases Baitfish that are listed as Prohibited Fish Species, or purchases Roe from fish that are listed as Prohibited Fish Species, shall not use or otherwise release those Baitfish or that Roe in any public waters of the State, except that those Baitfish or that Roe may be used in any waters included in the VHSv Positive Management Area subject to Provision 17 under the General Statewide Provisions.

2. A person who catches Baitfish that are listed as Prohibited Fish Species, or harvests Roe from fish that are listed as Prohibited Fish Species, in a body of water that is included in the VHSv Positive Management Area shall not use or otherwise release those Baitfish or that Roe in any public waters of the State, except that those Baitfish or that Roe may be used in any waters included in the VHSv Positive Management Area subject to Provision 17 under the General Statewide Provisions

State-licensed Baitfish wholesale operations; State-licensed Baitfish retail operations

1. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation shall not transport Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, if those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Positive Management Area, unless that owner has documentation demonstrating proof that those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Positive Management Area

2. Written documentation demonstrating proof (water body, county, and date) that each species of Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, has been harvested from a body of water that is included in the VHSv Positive Management Area shall accompany all shipments of those Baitfish or that Roe, and that documentation shall be shown upon request.

3. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation shall not offer for sale or sell Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, if those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Positive Management Area, unless that owner has clearly identified at the point of sale that those Baitfish or that Roe may only be used in a body of water that is included in the VHSv Positive Management Area.

VHSv Surveillance Management Area

On all waters designated within the VHSv Surveillance Management Area, the following regulations further restrict the transportation, sale, use, and release of Baitfish and fish that are listed as Prohibited Fish Species, and Roe taken from fish that are listed as Prohibited Fish Species, that **have not** been approved as required under Part B) of the Certification Process.

Recreational anglers

1. A person who catches Baitfish that are listed as Prohibited Fish Species, or harvests Roe from fish that are listed as Prohibited Fish Species in a body of water that is included in the VHSv Surveillance Management Area shall not use or otherwise release those Baitfish or that Roe in any public waters of the State, except that those Baitfish or that Roe may be used in any waters included in either the VHSv Positive Management Area or the VHSv Surveillance Management Area subject to Provision 17 under the General Statewide Provisions.

2. A person who purchases Baitfish that are listed as Prohibited Fish Species, or purchases Roe from fish that are listed as Prohibited Fish Species, shall not use or otherwise release those Baitfish or that Roe in any public waters of the State, except that those Baitfish or that Roe may be used in any waters included in either the VHSv Positive Management Area or the VHSv Surveillance Management Area subject to Provision 17 under the General Statewide Provisions.

State-licensed Baitfish wholesale operations; State-licensed Baitfish retail operations

1. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation shall not transport Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, if those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Surveillance Management Area, unless that owner has documentation demonstrating proof that those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Surveillance Management Area.

2. Written documentation demonstrating proof (water body, county, and date) that each species of Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, has been harvested from a body of water that is included in the VHSv Surveillance Management Area shall accompany all shipments of those Baitfish or that Roe, and that documentation shall be shown upon request.

3. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation shall not offer for sale or sell Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, if those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Surveillance Management Area, unless that owner has clearly identified at the point of sale that those Baitfish or that Roe may only be used in a body of water that is included in either the VHSv Positive Management Area or the VHSv Surveillance Management Area

VHSv Free Management Area

On all waters designated within the VHSv Free Management Area, the following regulations apply to the transportation, sale, use, and release of Baitfish and fish that are listed as Prohibited Fish Species, and Roe taken from fish that are listed as Prohibited Fish Species, that **have not** been approved as required under Part B) of the Certification Process.

Recreational anglers

1. A person who catches Baitfish that are listed as Prohibited Fish Species, or harvests Roe from fish that are listed as Prohibited Fish Species in a body of water that is included in the VHSv Free Management Area may use those Baitfish or that Roe in any public waters of the State, subject to Provision 17 under the General Statewide Provisions.

2. A person who purchases Baitfish that are listed as Prohibited Fish Species, or purchases Roe from fish that are listed as Prohibited Fish Species, that has been harvested from a body of water included in the VHSv Free Management Area may use those Baitfish or that Roe in any public waters of the State, subject to Provision 17 under the General Statewide Provisions.

State-licensed Baitfish wholesale operations; State-licensed Baitfish retail operations

1. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation may transport Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, so long as those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Free Management Area and that owner has documentation demonstrating proof that those Baitfish or that

Roe has been harvested from a body of water that is included in the VHSv Free Management Area.

2. Documentation demonstrating proof that each species of Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, has been harvested from a body of water that is included in the VHSv Free Management Area shall accompany all shipments of those Baitfish or that Roe, and that documentation shall be shown upon request.

3. An owner of a State-licensed Baitfish wholesale operation or a State-licensed Baitfish retail operation may offer for sale or sell Baitfish that are listed as Prohibited Fish Species, or Roe from fish that are listed as Prohibited Fish Species, so long as those Baitfish or that Roe has been harvested from a body of water that is included in the VHSv Free Management Area and that owner has clearly identified at the point of sale that those Baitfish or that Roe may be used in any waters of the State.

This order shall be assigned number FO-245.09, and is entitled "Fish Disease Control." If a discrepancy occurs between this Fish Disease Control Order, FO-245.09, and other orders and laws currently in existence, then the more restrictive regulation shall take precedence.

This order supersedes the order entitled "Fish Disease Control" effective April 1, 2008, and assigned number FO-245.08.

This order shall take effect April 1, 2009, and shall remain effective through March 31, 2014.

Appendix E: Fish disease control order, FO-245

The official list of Prohibited Fish Species for each Pathogen of concern as identified by the Department shall be posted on Fisheries Division's web site (http://www.michigan.gov/dnrfishing). This Appendix E, which contains the list of Prohibited Fish Species for each Pathogen of concern as identified by the Department of Natural Resources as of December 4, 2008, is incorporated by reference into this Fish Disease Control Order, FO- 245.

Table E1 Prohibited Fish Species for each Pathogen

Common name	**Prohibited Fish Species 1 Scientific name**	**Pathogen of concern Section I VHSv**
Black crappie	*Pomoxis nigromaculatus*	X
Bluegill	*Lepomis macrochirus*	X
Bluntnose minnow	*Pimephales notatus*	X
Brown bullhead	*Ictalurus nebulosus*	X
Brown trout	*Salmo trutta*	X
Burbot	*Lota lota*	X
Channel catfish	*Ictalurus punctatus*	X
Chinook salmon	*Oncorhynchus tshawytscha*	X
Coho salmon	*Oncorhynchus kisutch*	X
Emerald shiner	*Notropis atherinoides*	X
Freshwater drum	*Aplodinotus grunniens*	X
Gizzard shad	*Dorosoma cepedianum*	X
Lake whitefish	*Coregonus clupeaformis*	X
Largemouth bass	*Micropterus salmoides*	X
Muskellunge	*Esox masquinongy*	X
Northern pike	*Esox lucius*	X
Pacific herring	*Clupea pallasi*	X
Pink salmon	*Onchorhynchus gorbuscha*	X
Pumpkinseed	*Lepomis gibbosus*	X
Rainbow trout	*Oncorhynchus mykiss*	X
Rock bass	*Ambloplites rupestris*	X
Round goby	*Neogobius melanostomus*	X
Shorthead redhorse	*Moxostoma macrolepidotum*	X
Silver redhorse	*Moxostoma anisurum*	X
Smallmouth bass	*Micropterus dolomieu*	X
Spottail shiner	*Notropis hudsonius*	X
Trout perch	*Percopsis omiscomaycus*	X
Walleye	*Sander vitreus*	X
White bass	*Morone chrysops*	X
White perch	*Morone Americana*	X
White sucker	*Catostomus commersonii*	X
Yellow perch	*Perca flavescens*	X

Note: An "**X**" for a species of fish under each Section/named Pathogen signifies that the species has been identified by the Department as infected with that Pathogen of concern. Such species of fish are therefore subject to the restrictions imposed by this Fish Disease Control Order, FO-245.

Appendix F: Fish disease control order, FO-245

The official list of waters classified by Named Pathogen Management Area for each Pathogen of concern as identified by the Department shall be posted on Fisheries Division's web site (http://www.michigan.gov/dnrfishing). This Appendix B, which contains the list of waters classified by Named Pathogen Management Area for each Pathogen of concern as identified by the Department of Natural Resources as of December 4, 2008, is incorporated by reference into this Fish Disease Control Order, FO-245.

SECTION I – Viral Hemorrhagic Septicemia Virus (VHSv)

VHSv Positive Management Area

Lake Huron including Saginaw Bay, the St. Clair River, Lake St. Clair, the Detroit River, and Lake Erie are classified as a VHSv Positive Management Area. All tributaries to Lake Huron including Saginaw Bay, to the St. Clair River, to Lake St. Clair, to the Detroit River, and to Lake Erie are classified as a VHSv Positive Management Area in their entirety or from their confluence upstream to the first barrier that prevents the upstream passage of fish if such a barrier exists. VHSv has been documented in Budd Lake (Clare County) resulting in its addition to the VHSv Positive Management Area.

VHSv Surveillance Management Area

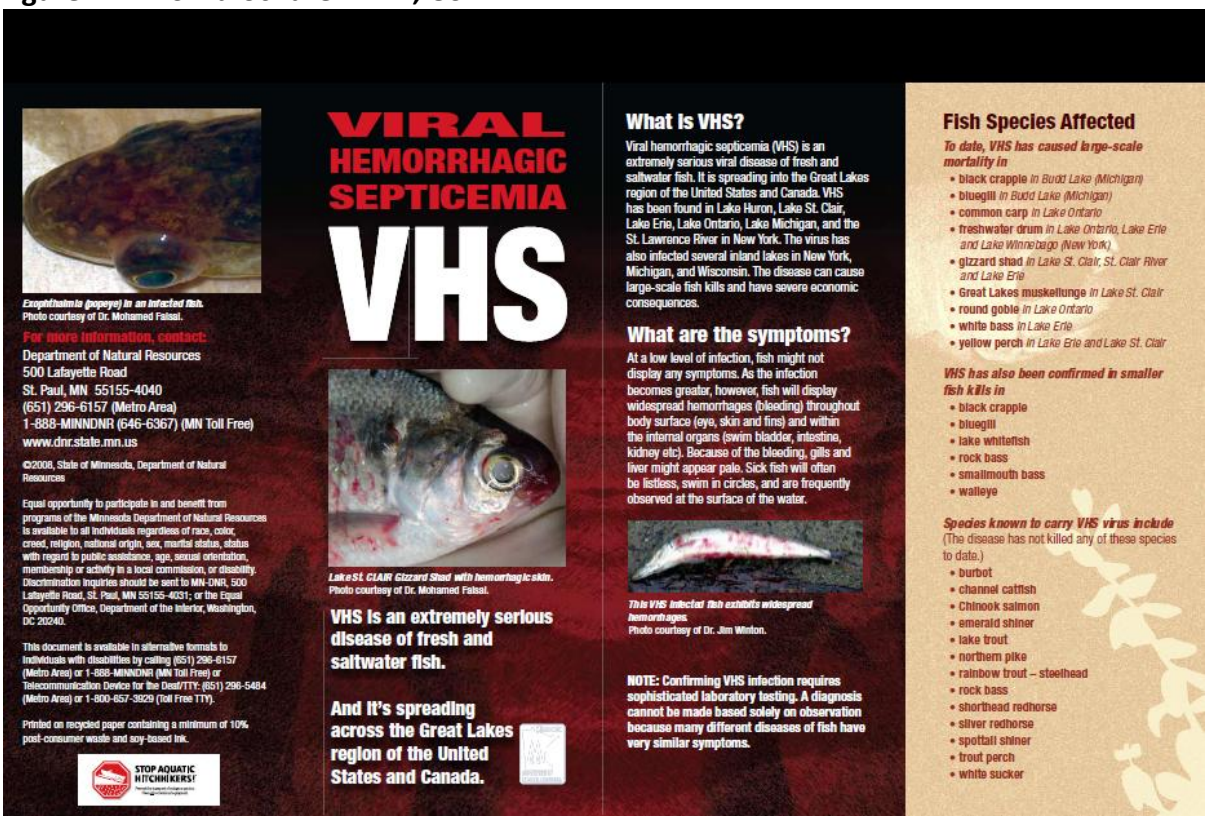
Lake Michigan including Grand Traverse bays and bays de Noc, and the St. Marys River are classified as a VHSv Surveillance Management Area. All tributaries to Lake Michigan including Grand Traverse bays and bays de Noc, and to the St. Marys River are classified as a VHSv Surveillance Management Area in their entirety or from their confluence upstream to the first barrier that prevents the upstream passage of fish if such a barrier exists. All Inland Waters in the watersheds of Lake Huron including Saginaw Bay, of the St. Clair River, of Lake St. Clair, of the Detroit River, and of Lake Erie are classified as a VHSv Surveillance Management Area, except for those tributaries to Lake Huron including Saginaw Bay, to the St. Clair River, to Lake St. Clair, to the Detroit River, and to Lake Erie that are classified as a VHSv Positive Management Area.

VHSv Free Management Area

Lake Superior and all Inland Waters in the watersheds of Lake Superior are classified as a VHSv Free Management Area. All Inland Waters in the watersheds of Lake Michigan including Grand Traverse bays and bays de Noc, and of the St. Marys River are classified as a VHSv Free Management Area, except for those tributaries to Lake Michigan including Grand Traverse bays and bays de Noc, and to the St. Marys River that are classified as a VHSv Surveillance Management Area.

Figure F1 VHSV poster – Michigan DNR, USA

Figure F2 VHSV brochure – MN, USA



Glossary

ACVO	Australian Chief Veterinary Officer. The nominated senior veterinarian in the Australian Government Department of Agriculture who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. *See also* CVO.
Anaemia	A decrease in the normal number of red blood cells.
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan. A series of technical response and control plans that outline the proposed Australian approach to an emergency aquatic animal disease incident. The plans provide guidance based on sound analysis, linking policy, strategy, implementation, coordination and emergency management components. *See also* AUSVETPLAN.
AUSVETPLAN	Australian Veterinary Emergency Plan. A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency management components.
CCEAD	Consultative Committee on Emergency Animal Diseases. AqCCEAD: Aquatic Consultative Committee on Emergency Animal Diseases.
Compensation	The sum of money paid by government to an owner for stock and/or property that is destroyed, possibly compulsorily, because of an emergency animal disease.
Control area	An area around the restricted area in which movement is controlled but not restricted. The control area is intended to reduce likelihood of the disease spreading beyond the restricted area.
Covert infection	A clinically inapparent infection that is transmissible and that may eventually lead to clinical disease.
CVO	Chief Veterinary Officer. The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. *See also* ACVO.
Dangerous contact animal	A susceptible animal that has been designated as being exposed to other infected animals or potentially infectious products following tracing and epidemiological investigation.

Dangerous contact premises or area	An area that has had a direct, and possibly infectious, contact with an infected premises or area. The type of contact will depend on the agent involved in the outbreak but, for example, may involve animal movements or net or equipment movements.
Declared area	A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include *restricted area, control area, infected premises, dangerous contact premises* and *suspect premises*.
Decontamination	A combination of physical and chemical procedures that are used to remove soiling and inactivate the target disease organism. Includes all stages of cleaning and disinfection.
Disease agent	A general term for a transmissible organism or other factor that causes an infectious disease.
Disinfectant	A chemical used to destroy or inactivate disease agents outside a living animal.
Disinfection	Procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies (after thorough cleansing) to premises, vehicles and other objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of fish carcasses and other contaminated objects by burial, burning, rendering, composting or some other process so as to prevent the spread of disease.
Ecchymotic haemorrhage	Bleeding or bruising in the skin or a mucous membrane in the form of small, nonelevated, round or irregular red, purple or blue spots. Larger than petechial haemorrhages.
ELISA	Enzyme-linked immunosorbent assay. A serological test to detect and measure the presence of antibody or antigen in a sample. The test uses an enzyme reaction with a substrate to produce a colour change when antigen–antibody binding occurs.
Emergency animal disease	A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications. *See also* Endemic animal disease, Exotic animal disease.
Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. *See also* Emergency animal disease, Exotic animal disease.

Enterprise	*See* Risk enterprise.
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with a disease.
Exophthalmia	Protrusion of the eyeball from the orbit, caused by disease or injury.
Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. *See also* Emergency animal disease, Endemic animal disease.
Fish by-products	Products of fish origin destined for industrial use (e.g. fishmeal).
Fish products	Fish meat products and products of fish origin (e.g. fish eggs) for human consumption or use in animal feeding.
Free area	An area known not to be contaminated with a given disease agent.
Haemorrhage	Escape of blood from a ruptured blood vessel; bleeding.
Inappetence	Lack of appetite.
Infected premises or area	The area in which the disease has been confirmed; likely to apply to an open system, such as an oceanic lease or a pond-based farm.
LDCC	Local Disease Control Centre. An emergency operations centre responsible for the command and control of field operations in a defined area.
Leukopenia	A decrease in the number of white blood cells in the blood.
Monitoring	Routine collection of data for assessing the health status of a population. *See also* Surveillance.
Movement control	Restrictions placed on the movement of fish, people and other things to prevent the spread of a pest or disease.
Nested RT-PCR	A two-step PCR process in which the second round identifies a DNA sequence 'nested' within the initial sequence, thus increasing the specificity. See *Polymerase chain reaction (PCR)* and *Reverse transcriptase-PCR (RT-PCR)*.
OIE Aquatic Code	OIE *Aquatic Animal Health Code* (OIE 2013), at http://www.oie.int/eng/normes/fcode/en_sommaire.htm. *See* Appendix 1 for further details.

OIE Aquatic Manual	OIE *Manual of Diagnostic Tests for Aquatic Animals.* Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines). Published annually online at http://www.oie.int/eng/normes/fmanual/A_summry.htm. *See* Appendix 1 for further details.
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Owner	Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).
PCR	Polymerase chain reaction. A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also *Reverse transcriptase-PCR (RT-PCR)* and *Nested RT-PCR*.
Petechial haemorrhage	Tiny, flat, red or purple spots in the skin or mucous membranes caused by bleeding from small blood vessels.
Premises or area	Any land, building or structure whatsoever or wheresoever situated where animals, animal product, animal pathogen, biological preparation or agricultural produce or any other thing that might carry animal pathogen is kept, stored, sold, prepared or dealt with in any manner whatsoever. A production site, which may range from an aquarium to an aquaculture lease in the open ocean.
Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or demonstrating positive antibody titres) at a given point in time.
qRT-PCR	Quantitative RT-PCR, also known as real time RT-PCR or kinetic RT-PCR. A method that allows detection and measurement of the number of copies of PCR templates generated during each cycle of the PCR process.
Quarantine	Legal restrictions imposed on places, fish, vehicles, or other objects, limiting movement.
Restricted area	The area around an infected premises (or area), likely to be subject to intense surveillance and movement controls. It is likely to be relatively small. It may include some dangerous contact premises (or area) and some suspect premises (or area), as well as enterprises that are not infected or under suspicion. Movement of potential vectors of disease out of the area will, in general, be prohibited. Movement into the restricted area would only be by permit. Multiple restricted areas may exist within one control area.

Risk enterprise	A defined livestock or related enterprise which is potentially a major source of infection for many other premises. Examples include hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots and garbage depots.
RT-PCR	Reverse transcriptase polymerase chain reaction. A PCR method for amplifying a defined piece of ribonucleic acid (RNA). The RNA strand is first reverse transcribed into its DNA complement, followed by amplification of the resulting DNA. See *Polymerase chain reaction (PCR), Nested RT-PCR* and *qRT-PCR*.
Standing Council on Primary Industries (SCoPI)	SCoPI is the subsumed parts of the two previous ministerial councils, the Primary Industries Ministerial Council (PIMC) and the Natural Resource Management Ministerial Council (NRMMC). SCoPI aims to pursue and monitor priority issues of national significance affecting Australia's primary production sectors which require a sustained and collaborative effort across jurisdictions; and address key areas of shared Commonwealth, state and territory responsibility and funding for Australia's primary production sectors.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). *See also* Specificity.
Septicaemia	Systemic disease associated with the invasion and persistence of pathogens, or their toxins, in the bloodstream.
Serotype	A subgroup of a specific microorganism identified by the antigens carried (as determined by a serological test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). *See also* Sensitivity.
State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible fish	Fish that can be infected by a particular disease agent.

Suspect fish	Fish that may have been exposed to an emergency disease (such exposure may prompt quarantine and intensive surveillance, but not necessarily pre-emptive slaughter); *or* fish not known to have been exposed to a disease agent but showing clinical signs requiring further diagnostic investigation to confirm cause.
Suspect premises or area	Temporary classification of premises containing suspect fish. After rapid resolution of the status of the suspect fish contained on it, a suspect premises is reclassified either as an infected premises (and appropriate disease control measures taken) or as free from disease. The reason for the suspicion varies with the agent and may involve expression of clinical signs or observations of increased mortality.
Thrombocytopenia	Reduced number of thrombocytes in the blood.
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.
Vaccination	Inoculation of healthy individuals with antigens of disease-causing agents to stimulate a host immune response to provide protection from disease.
Vaccine	Modified strains or antigenic components of disease-causing agents which are manufactured specifically to protect against a pathogen. They are typically applied to fish by injection or immersion.
Vector	A living organism that transmits an infectious agent from one host to another. A *biological* vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A *mechanical* vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.
Zoning	The process of defining disease-free and infected areas.

Abbreviations

AAHL	Australian Animal Health Laboratory
ACVO	Australian Chief Veterinary Officer
AFDL	AAHL Fish Disease Laboratory
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
AUSVETPLAN	Australian Veterinary Emergency Plan
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	Chief Veterinary Officer
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FRDC	Fisheries Research and Development Corporation
G	Glycoprotein of VHSV
IHN	Infectious haematopoietic necrosis
ISA	Infectious salmon anaemia
L	Polymerase of VHSV
M	Matrix protein of VHSV
N	Nucleoprotein of VHSV
NV	Non-viral protein of VHSV
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
P	Phosphoprotein of VHSV
PCR	Polymerase chain reaction
PFU	Plaque forming units
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
RNAi	RNA interference

RT-PCR	Reverse transcriptase polymerase chain reaction
ssRNA	Single-stranded ribonucleic acid
TCID	Tissue culture infective dose
VHS	Viral haemorrhagic septicaemia
VHSV	Viral haemorrhagic septicaemia virus

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