

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

Viral haemorrhagic septicaemia

Version 1.0, 2005

AQUAVETPLAN is a series of technical response plans that describe the proposed Australian approach to aquatic animal disease incursions. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.

Primary Industries Ministerial Council

This disease strategy forms part of:

AQUAVETPLAN Edition 2

This strategy will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to:

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IMPORTANT NOTE: Important regulatory information is contained in the OIE *International Aquatic Animal Health Code (OIE 2004)* for viral haemorrhagic septicaemia, which is updated annually and is available on the internet at the OIE website:

http://www.oie.int/eng/normes/fcode/fcode2004/en_acode.htm

Further details are given in Appendix 1 of this manual.

DISEASE WATCH HOTLINES

These telephone numbers connect callers to the relevant state or territory officer to report concerns about any potential emergency disease situation. Anyone suspecting an emergency disease outbreak should use this number for immediate advice and assistance.

New South Wales	1800 043 536	Northern Territory	1800 720 002
Queensland	07 3830 8550	Victoria	136 186
South Australia	1800 065 522	Western Australia	1800 815 507
Tasmania	1800 005 555		

Preface

This disease strategy for the control and eradication of viral haemorrhagic septicaemia (VHS) in fish is an integral part of the **Australian Aquatic Veterinary Emergency Plan**, or **AQUAVETPLAN (Edition 2)**.

The 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 approved by:

- the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, at meeting 04 in May 2004;
- the Aquatic Animal Health Committee of the Primary Industries Standing Committee, at meeting 04 in June 2004; and
- the Primary Industries Standing Committee, at meeting 08 in March 2005.

Viral haemorrhagic septicaemia is listed by the OIE (World Organisation for Animal Health, formerly Office International des Epizooties) in the *International Aquatic Animal Health Code* (OIE 2004).¹

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**. The full list of AQUAVETPLAN manuals that may need to be accessed in an emergency is shown below:

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Disposal

Destruction

Enterprise Manual

Includes sections on:

- open systems

- semi-open systems

- semi-closed systems

- closed systems

Management manual

Control centres management

Aquatic Animal Diseases Significant to Australia: Identification Field Guide by Alistair Herfort, Department of Agriculture, Fisheries and Forestry, Canberra (Herfort 2004) is a source for some of the information about the aetiology, diagnosis and epidemiology of the disease and should be read in conjunction with this strategy.

This manual was drafted by Dr Paul Hardy-Smith, with the assistance of Professors Ron Hedrick and Barry Hill and Drs Craig Stephens and Mark Crane.

Scientific editing: Biotext Pty Ltd, Canberra.

¹ See http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005).

This manual was adapted from similar manuals in AUSVETPLAN, the Australian emergency plan for terrestrial animal diseases, and from the AQUAVETPLAN **Enterprise Manual**. The format and content have been kept as similar as possible to those 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 the permission to use the original AUSVETPLAN documents is gratefully acknowledged.

The text was amended at various stages of the consultation/approval process, and the policies expressed in this version do not necessarily reflect the views of all the members of the writing group. Contributions made by others not mentioned above are also gratefully acknowledged.

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

Government	Industry
Commonwealth of Australia	CSIRO Division of Livestock Industries
State of New South Wales	Tasmanian Salmonid Growers' Association
State of Queensland	Tuna Boat Operators Association
State of South Australia	Pearl Producers' Association
State of Tasmania	Australian Prawn Farmers Association
State of Victoria	Pet Industry Joint Advisory Council
State of Western Australia	RecFish Australia
Northern Territory	National Aquaculture Council
Australian Capital Territory	

The complete series of AQUAVETPLAN documents is available on the internet at: <http://www.affa.gov.au/AQUAVETPLAN> (Accessed 11 May 2005).

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1 Nature of the disease

Viral haemorrhagic septicaemia (VHS) is an infectious disease of freshwater and marine fish species. The causative agent, viral haemorrhagic septicaemia virus (VHSV), a rhabdovirus, was originally isolated from rainbow trout (*Oncorhynchus mykiss*) in Europe. In this species and in this region, the virus has caused significant mortality and economic loss.

VHSV can cause disease in a number of other freshwater and marine fish species in Europe and North America. This includes wild marine species such as pilchards (*Sardinops sagax*) and Pacific herring (*Clupea pallasii*). Isolates differ markedly in virulence and pathogenicity; clinical signs of disease were not observed in many fish species from which the virus has been isolated.

Outbreaks most often occur in susceptible fish populations at a water temperature of approximately 10°C. Mortality and morbidity have rarely been documented when water temperatures are above 15°C. The virus is also rarely isolated from fish living in waters above this temperature.

Serotyping, genotyping and challenge trials have confirmed significant differences between the different VHSV isolates in both structure of the genome and virulence. Separation of isolates into distinct genogroups or types is based mainly on geographic origin.

VHSV has never been isolated in Australia, or in any other country in the Southern Hemisphere.

VHS is listed on Australia's *National List of Reportable Diseases of Aquatic Animals* and is listed by the OIE (World Organisation for Animal Health, formerly Office International des Epizooties). Isolation of VHSV, with or without clinical signs of disease, must be reported.

1.1 Aetiology

VHS is caused by a single-stranded RNA virus belonging to the genus *Novirhabdovirus* within the Rhabdoviridae. Figure 1 shows a typical rhabdovirus.

The most common synonym for VHSV is Egtved virus, named for the Danish town where the disease was first recognised in rainbow trout. The virus is bullet shaped, is about 180 nm long and has a diameter of approximately 60 nm. Cohen and Lenoir (1974, cited in Wolf 1988) noted the extreme fragility of VHSV. The membrane glycoprotein of the envelope of VHSV is the major neutralising surface antigen.

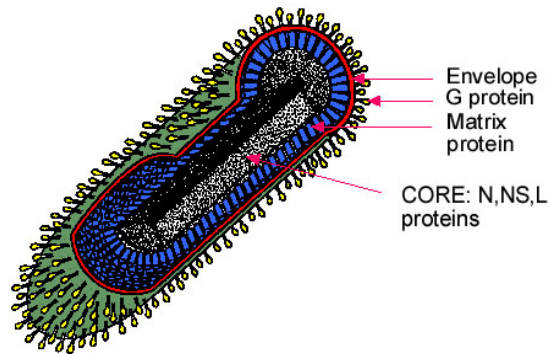


Figure courtesy of G Traxler

Figure 1 Schematic of a typical rhabdovirus

Using both serotyping and genotyping, significant differences between VHSV isolates from different regions have been documented in the literature. Categorisation of isolates is ongoing. The principal genogroups/genotypes are:

- **Type I** – Continental Europe freshwater group

Contains isolates considered highly pathogenic for rainbow trout (*Oncorhynchus mykiss*). Isolates from this group have also been reported to cause natural disease outbreaks in Northern pike (*Esox lucius*), graylings (*Thymallus thymallus*) and white fish (*Coregonus* spp).

- **Type II** – European marine group (principally from the North Sea)

Contains isolates considered less pathogenic for rainbow trout, but has been the cause of significant mortalities in turbot (*Scophthalmus maximus*).

- **Type III** – North American marine group

Contains isolates considered pathogenic for Pacific herring (*Clupea pallasii*). Isolates from this group have also been found in high titres in Pacific hake (*Merluccius productus*), pilchards (*Sardinops sagax*) and walleye pollock (*Theragra chalcogramma*) undergoing disease outbreaks.

A fourth genotype has been suggested. Snow et al (1999) found isolates from the Baltic Sea that differed from the freshwater and North Sea isolates.

Grouping of genotypes is generally geographic. One exception is the presence of an isolate belonging to Type II found in the North Pacific in wild Japanese flounder (*Paralichthys 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

The susceptibility of fish species to infection with VHSV and clinical signs of the disease vary significantly depending on the VHSV isolate, fish demographics (eg age, strain) and environmental variables (eg water temperature).

Rainbow trout is the most susceptible species to Type I isolates of VHSV and to the development of disease. In this species, epizootics have led to mortalities of 80–100% in fry weighing 0.3–3 g (Smail 1999). Isolates from Type II and Type III can also cause disease and mortality in fish (eg turbot and Pacific herring, respectively).

The range of fish species from which VHSV has been isolated both with and without signs of disease continues to grow. According to the OIE (2004) *International Aquatic Animal Health Code* (the OIE Aquatic Code; see Appendix 1) the virus has been isolated from at least 45 different species living both in marine and in freshwater environments. Appendix 2 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.

Most Australian fish species have not been tested for susceptibility to VHSV, so it is difficult to predict how VHS might manifest in Australia.

1.3 World distribution and occurrence in Australia

VHS virus:

- has never been isolated in Australia;
- has been isolated from freshwater fish species of many countries of continental eastern and western Europe and is considered endemic in these regions;
- has been isolated from many marine fish species in the northeast Pacific Ocean (from Alaska to California), the North Atlantic and the Baltic Sea, and from Japanese flounder in Japan; and
- has rarely been isolated from fish taken from areas where water temperatures were above 15°C.

1.4 Diagnostic criteria

An excellent review of the clinical signs and pathological changes of VHS is given in Smail (1999).

1.4.1 Clinical signs

In rainbow trout, acute, chronic and nervous forms of the disease have been identified, with a carrier state occurring in fish that survive (Ghittono 1965). In this state, virus can be isolated from persistently infected tissues, such as kidney and brain. Acute forms of the disease have also been observed in other species of fish, including sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) (Castric and de Kinkelin 1984, Schlotfeldt et al 1991). Virus multiplication in endothelial cells of blood capillaries, leukocytes, haematopoietic tissues and nephron cells underlies the clinical signs.

The infection of susceptible fish is often lethal because it impairs the osmotic balance. This occurs within the clinical context of oedema and haemorrhage. Note that these clinical signs are not pathognomonic for (unique to) VHS.

General

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

- loss of appetite
- haemorrhage at the base of fins, in the skin and in the eyes;
- exophthalmia (or 'pop eye') due to subretinal haemorrhaging; and
- ascites (markedly distended abdomen caused by abnormal accumulation of fluids, which may include blood).

Internal

- swelling and paleness of liver;
- swollen kidneys, which appear darker red in the early stages of disease (especially in the anterior kidney)
- in dead fish, the head and midsection of the kidney may be totally necrosed, although this sign is inconsistently reported in the literature;
- bloody ascitic fluid surrounding abdominal organs;
- oedema in muscles; and
- lack of food in the gastrointestinal tract.

Acute

In its acute form, VHS can cause rapid death. In rainbow trout the acute stage occurs from 2–30 days after experimental infection at 8–12°C. Clinical signs associated with this stage include:

- pale gills with or without petechiae (small red spots);
- flashing or corkscrewing;
- lethargy;
- darker than normal colour; and
- fish confined to edges of ponds/cages.

Chronic

Clinical signs might not be evident in the chronic form of VHS. However, the virus can be isolated from all major internal organs.

Nervous

In the nervous stage, there are very marked aberrations of swimming behaviour (ie constant flashing, tail-chasing and spiralling) caused by the effect of the virus on the brain. This is a feature of virulent freshwater strains of VHSV.

In marine species, VHSV has been isolated from the brain in experimental studies in cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*). However, a nervous form of the disease has not been observed in these species (David Smail, Senior Virologist, Marine Laboratory, Aberdeen, pers comm).

1.4.2 Pathology, histopathology and haematology

The OIE (2003) *Manual of Diagnostic Tests for Aquatic Animals* (the OIE Aquatic Manual; see Appendix 1) outlines tests for VHS.

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 (*S. maximus*) and Pacific herring (*C. pallasii*) (Munro 1996, Kocan et al 1997, Smail 1999, Isshiki et al 2001). Petechial haemorrhaging (small red spots) and ecchymotic haemorrhaging (bruising) have been observed in the peritoneum, on the swim bladder, adipose tissue, sexual organs and surface of the liver, and within the muscle. Haemorrhages have also been found in the epidural area in pike (*Esox lucius*).

A notable pathological feature observed in turbot was gross body swelling of infected fish due to fluid retention (Munro 1996).

Differentiation has been made with respect to clinical signs and the different forms of the disease (acute, chronic and nervous). No such differentiation has been made with respect to pathology.

Histopathology

Haemorrhaging is a feature of VHS. Microscopically, degenerative changes and necrosis are common presenting signs in many tissues (Wolf 1988).

The principal target is kidney, with severe damage (necrosis, degeneration) of haematopoietic tissue rather than excretory tissue. In more chronic cases, severe glomerular changes in the kidney resemble membranous glomerulonephritis in mammals. Lymphoid tissue necrosis leads to leukopenia.

In acutely affected fish, liver sinusoids are engorged with blood, and hepatocytes show extensive focal changes, including cytoplasmic vacuoles, pyknosis, karyolysis, lymphocytic invasion and occasionally intracytoplasmic and intranuclear inclusions (Stoskopf 1993). However, Wolf (1988) comments that distinctive or diagnostic inclusions are lacking in the liver. Smail (1999) notes that the liver shows widespread focal necrosis, degeneration of hepatocyte nuclei and granulation of chromatin.

Extravasation of blood may be found in skeletal muscle; however, muscle fibres and bundles are not damaged.

Ross et al (1994) noted a widespread necrosis and collapse of cardiac muscle in turbot. 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 necrotised (Isshiki et al 2001).

Importantly, pancreatic tissues show fewer and less destructive changes than other organs. 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 (*Esox 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).

Isshiki et al (2001) noted inclusion bodies in necrotic cells of the myocardium in infected Japanese flounder.

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

VHSV may be isolated during routine sampling of fish showing no clinical signs.

The screening procedure for VHS is based mainly on virus isolation in cell culture. Confirmatory testing is by immunological virus identification (eg neutralisation, immunofluorescence, enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase staining) or by reverse-transcriptase polymerase chain reaction (RT-PCR) techniques.

Fluorescence, ELISA, immunohistochemistry and RT-PCR are more rapid diagnostic methods for presumptive evidence of viral antigen in infected organ imprints or homogenates. These may be suitable for fish with overt disease (OIE 2003).

In infected fish, virus is most abundant in the kidney, spleen and heart.

Currently, the only laboratory capable of confirming VHSV in Australia is the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong. Methods used for the detection and identification of VHSV at AAHL and procedures for the correct submission of specimens are given in Appendix 3.

The presence of VHSV can be confirmed in a submitted sample within days, with the time needed dependent 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.

Carriers of VHSV can be difficult to detect and are a major problem in the control of this disease. Research suggests that low temperatures are required for the virus to break latency. For example, VHSV was isolated from a population of infected rainbow trout in the winter when water temperatures were low. However, the virus could not be isolated from the same infected population during autumn, which may indicate that the amount of virus in the fish had decreased to a

nondetectable level or that the virus had been eliminated by a temperature-dependent immune response occurring during the summer period (Vestergard Jørgensen 1982). The authors considered it unlikely that the population sampled in September could have been different from that sampled in winter.

Having a carrier state in fish that will at times be undetectable will affect the positive predictive value of any sampling procedure developed.

Detection of VHSV in the environment

VHSV can be cultured from both fresh and salt water, but such isolation can be difficult due to large dilution factors. In testing for infectious haematopoietic necrosis (a related rhabdovirus), methods have been developed to concentrate water samples to increase the sensitivity of virus isolation (Mulcahy et al 1983, Watanabe et al 1988).

Transport of specimens

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, specimens will then be forwarded AAHL for emergency disease testing.

1.4.4 Differential diagnosis

VHSV and the disease VHS should be confirmed by laboratory testing where there is significant mortality and morbidity of fish (either in fresh or marine water), together with petechial haemorrhaging in tissues such as liver and muscle. Observed neurological signs, such as spiralling, will reinforce the urgency of submitting samples for laboratory testing.

Table 1 shows a number of important differential diagnoses for VHS.

Table 1 Differential diagnoses for VHS

Disease or disorder	Pathogen	In Australia	Fish species affected	Clinical signs	Diagnosis
Enzootic haematopoietic necrosis	Enzootic haematopoietic necrosis virus	Yes	Redfin perch, salmonids	Haemorrhage, necrosis; epizootics in redfin	Cell culture Immunodiagnosics Histopathology PCR
Infectious haematopoietic necrosis	Infectious haematopoietic necrosis virus	No	Salmonids	Haemorrhage	Cell culture Immunodiagnosics Histopathology PCR
Infectious pancreatic necrosis	Infectious pancreatic necrosis virus	No ^a	Salmonids	Extended abdomen, spiralling, high mortality	Cell culture Immunodiagnosics Histopathology PCR
Bacterial septicaemia	Generally gram-negative bacteria	Yes	All	Lethargy, reddening, ulcers/abscesses	Bacterial isolation associated with clinical signs
Infection with rickettsia-like organisms	Rickettsia-like organism	Yes	Salmonids	Congestion, petechiation, anaemia, ascites	Clinical signs PCR
Whirling disease	<i>Myxobolus cerebralis</i>	No	Salmonids (esp. rainbow trout)	'whirling', deformities, esp. rainbow trout	Identification of myxospore in cartilage PCR
Electrocution	–	Yes	Any	Significant haemorrhaging in musculoskeletal system	History Skeletal pathology
Osmotic stress	–	Yes	Post-transfer salmon smolts	Bloody ascitic fluid	History of recent transfer Non-culture of pathogens

PCR = polymerase chain reaction

^a While acute infectious pancreatic necrosis virus does not occur in Australia, a related aquatic birnavirus is endemic to parts of Tasmania.

1.5 Resistance and immunity

Innate fish defence mechanisms for 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; and
- interferon production.

1.5.1 Innate immunity

Antiviral cytotoxic cells have been demonstrated in fish (Ellis 2001). These cells are capable of destroying infected cells even before the entire viral genome has been expressed to produce new infective particles.

Interferon production has been demonstrated clearly in rainbow trout exposed to VHSV; production peaks at around 3 days after infection (Dorson et al 1994).

It is likely that this rapid innate response helps to provide some degree of protection until the active (acquired) immune defences are able to respond.

1.5.2 Adaptive immunity

Survivors of VHS have been shown to be resistant to reinfection. Neutralising antibodies have been demonstrated in recovering trout. This antibody response can take a variable time to develop. In 130 g trout, the response time was approximately 4–10 weeks (Olesen et al 1986, 1991). Temperature has a profound effect on the development of active immunity.

It has been postulated that VHSV antibodies are both neutralising (ie reacting with a few epitopes on the glycoprotein of the virus) and non-neutralising (ie directed against virus protein), and that non-neutralising antibodies persist in fish for a longer time than neutralising antibodies (Olesen et al 1991).

1.5.3 Vaccination

Currently, there are no commercially available VHS vaccines. DNA-based vaccine technology is being researched.

1.6 Epidemiology

1.6.1 Virus transmission and incubation period

Natural infections occur by horizontal transmission of waterborne virus. The virus gains entry through the gills of the fish (Neukirch 1985) and possibly through the skin (Yamamoto et al 1992). Multiplication may take place at the site of entry; alternatively, the virus may pass through without primary multiplication, which then occurs in the endothelial cells of the vascular system, mainly of the kidney, spleen and brain.

Pathology to cells lining the circulatory system has been noted 48 hours after infection (Smail 1999). Necrosis of liver hepatocytes occurs by day 4 after infection (Evensen et al 1994).

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. Shedding was found to peak at days 4–5. At this time, each infected herring was, on average, shedding virus at a rate of more than $10^{6.5}$ PFU/hour (Kocan et al 1997). It is likely that much of this shedding was in the urine (Neukirch 1985). Virus does not appear to be shed in the faeces. It is probable that some shedding occurs from other areas (eg skin, mucus, gills) in clinically diseased fish, including ulcers in fish such

as cod and haddock (Smail 2000). As far as the author is aware, no published research has quantified the amount of virus shed from carrier fish after a significant period (months to years) of infection, although some shedding is suspected during this period (David Smail, Senior Virologist, Marine Laboratory, Aberdeen, pers comm).

1.6.3 Persistence of virus

The European freshwater isolates of VHSV are ether, heat and acid (at pH 3) labile. 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. However, this may also be due to the initial virus concentrations in fish tissues: if low, as found in fish that appear healthy, then freezing might effectively eliminate detectable virus; if high, as in dead and dying fish, then freezing may reduce, but not eliminate, detectable virus (Hedrick et al 2003).

Many factors can affect virus survival in the environment (quoted in Toranzo and Hetrick 1982), so survival will vary significantly according to the conditions. These factors include:

- temperature (as noted above);
- salinity;
- solar radiation;
- presence of chemical pollutants;
- bacterial antagonism; and
- suspended solids.

VHSV can survive in both freshwater and marine environments. The North American strain of VHSV could be recovered for up to 40 hours in natural filtered seawater. The addition of ovarian fluid or foetal bovine serum prolonged this to 72 and 96 hours respectively. Toranzo and Hetrick (1982) showed that infectious haematopoietic necrosis virus, a related fish rhabdovirus, survived longer in fresh water at 15°C (25 days for a 3-log₁₀ reduction) than in salt water at the same temperature (14 days). Freezing at –20°C maintains infectivity for several years (Wolf 1988).

Mori et al (2002) reported a significant reduction in VHSV titre in untreated seawater compared to sterilised or filtered (0.22 µm) seawater, particularly at temperatures of 15°C or higher, suggesting considerable inactivation due to the action of bacteria or other microorganisms.

While VHSV has been isolated from ovarian fluid and eggs at spawning, it is unlikely that vertical transmission of the virus occurs (Wolf 1988). Adequate disinfection with an iodophore will rapidly inactivate any virus on the egg surface.

Birds may spread the virus from farm to farm either by physically carrying infected fish or by eating infected fish at one farm and regurgitating them at another. However, 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.

1.6.4 Sources of VHSV

VHSV has a widespread distribution overseas in a variety of wild and cultured fish that inhabit freshwater and marine environments. It is likely that many Australian temperate fish species (eg pilchards) would be susceptible to infection with this virus, and could become carriers.

1.6.5 Factors influencing transmission

Age and size

Age is a significant factor in determining severity of disease in rainbow trout. Fish weighing 0.3–3.0 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).

Temperature

Water temperature is an important factor in the propagation and spread of VHSV. In temperatures above 15°C, virus shedding from infected fish and survival of the virus outside the fish host are significantly reduced.

VHS is considered a cold-water disease, with a temperature range of 2–12°C. Transmission of the virus occurs readily over this range. Temperatures above 15°C inhibit virus growth. However, Castric and de Kinkelin (1984) conducted research suggesting an upper threshold for in vivo infections of marine fish between 18°C and 20°C. Different isolates of this virus in different fish species may show variance in temperature tolerance.

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

Infectious dose

Infectious doses that have caused clinical disease in rainbow trout, turbot and herring have been quoted in the literature. Units of measurement vary, for example $10^{3.5-4.5}$ PFU/mL and 10^5 TCID₅₀/mL (King et al 2001, Kocan et al 1997).

The dose required to infect individual fish of different species will vary considerably, depending on many of the factors discussed above. These doses should therefore be used as a guide only. Kocan et al (1997) suggest that the minimum dose of VHSV needed to initiate infection of juvenile herring by waterborne exposure for one hour may be as low as $10^{1.5-2.5}$ PFU/mL. However, they refer to a laboratory-based experiment with one-hour exposures, and not all the fish in the tanks exposed to the low dose succumbed to infection.

Because of the significant levels of shedding of virus from individual fish, the virus could very quickly spread horizontally in a schooling species, such as sardine or herring, resulting in VHS outbreaks (Kocan et al 1997).

Species

Stone et al (1997) suggest that all marine fish might be susceptible to infection with VHSV. It is possible that if VHSV is introduced into Australia, a broad range of temperate marine and freshwater fish species in this country may be susceptible. Whether infection of Australian native species would lead to clinical disease is unknown. There is experimental evidence that some fish species are refractive to infection (see Appendix 2). Because these trials did not use all strains of VHSV, caution must be used when considering the susceptibility of any species of fish to infection with the virus.

1.6.6 Inactivation of the virus

VHSV is a relatively fragile virus and is quickly inactivated by chlorine and iodophor disinfectants. Table 2 summarises the potential disinfectants and dose rates that will inactivate the virus. Ozone is also likely to inactivate VHSV, but dose rates are not yet available.

Table 2 Inactivating agents for VHSV

Physical agents	
Heat	VHSV is completely inactivated at 45°C for 60 minutes or 60°C for 15 minutes
UV light	Water must be treated to a dose of $1-3 \times 10^3 \mu\text{W s cm}^{-2}$
Chemical agents^a	
Chlorine	Complete inactivation in less than 5 minutes at a dose rate of 200 mg/L
Iodine	Complete inactivation in less than 5 minutes at a dose rate of 25 mg/L
NaOH	Complete inactivation in less than 5 minutes at a dose rate of 10 g/L
Quaternary ammonia	Complete inactivation in less than 5 minutes at a dose rate of 10 mg/L

UV = ultraviolet

^a Inactivation is affected by factors such as amount of suspended solids, salinity, organic load.

2 Principles of control and eradication

2.1 Introduction

Viral haemorrhagic septicaemia (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, but the susceptibility of many species is uncertain.

Possible scenarios for the isolation of VHS virus (VHSV) in Australia include:

- from Atlantic salmon/rainbow trout in salt water or fresh water as part of routine testing, with no clinical signs of disease;
- from Atlantic salmon/rainbow trout in salt water or fresh water, accompanied by haemorrhaging, increasing morbidity and mortality;
- from wild fish around tuna/yellowfin cages;
- from southern bluefin or yellowfin tuna showing no clinical signs of disease, or showing morbidity and mortality (possible but unlikely);
- from pilchards during a pilchard die-off;
- from wild marine fish other than pilchards, with or without clinical signs of disease; and
- from 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 isolated in Australia:

- **Eradication**

The scale of eradication may be national (eradicate VHSV from Australia), local (eradicate from a local trout farm) or somewhere in between (eradicate from a region or state).

- **Containment, control and zoning**

Containment, control and zoning includes measures to exclude VHSV from defined geographic areas and unaffected populations (eg by quarantine) and contain the virus to areas with enzootic infection.

- **Control and mitigation of disease**

Control and mitigation measures are aimed at 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** and **Control Centres Management Manual**. The **Enterprise Manual** details state/territory legislation relating to disease control and eradication.

Response measures could involve any or all of the following:

- early detection and identification of VHSV and of 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 the extent of such presence;
- 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, equipment and other means of mechanical spread of the virus;
- good communication between all relevant government and industry stakeholders; and
- a publicity campaign.

2.2.1 Quarantine and movement controls

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

- the establishment of specified areas (see Figure 2)
 - *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* – non-infected area (this area is 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 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 between different river systems and different marine zones in designated areas;

- restrictions or bans on the use and movement of equipment within and between marine and freshwater areas; and
- controls on access of predators, such as birds, to potentially infective material (eg fish carcasses, hatchery tanks).

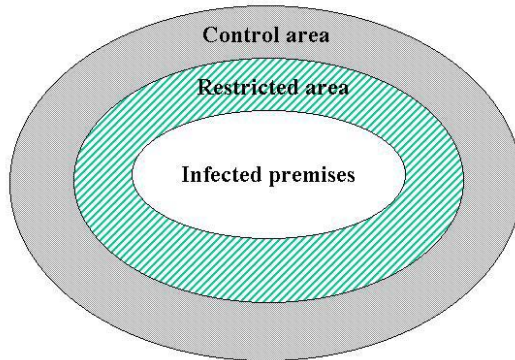


Figure 2 Establishment of specified areas to control viral haemorrhagic septicaemia

Some practices that would be affected by such actions include:

- live fish transportation between and within freshwater operations (including broodstock);
- live fish transportation between freshwater and saltwater operations;
- fish harvesting (wild and farmed) and transportation to processing plants;
- discharge of processing plant effluent;
- transportation of consumer-ready products; and
- disposal of dead fish.

The imposition of restrictions can significantly help in the early stages of control of a disease outbreak. Imposing restrictions also buys time while the true extent of the problem is assessed.

If VHSV is isolated, it may be difficult to determine the size of the specified areas. In 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. The virus was successfully eradicated from the farm (Munro 1996). The 20-km radius was the distance at which virus concentration fell below one infectious virion per cubic metre of seawater (with the assumption that natural factors did not inactivate the virus).

With infectious salmon anaemia (ISA) virus, it was found that the risk of infection increased by a factor of eight if the site was closer than 5 km to another ISA-positive site (Jarp and Karlsen 1997).

The extent of restrictions should take into account 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 production systems, there is virtually no control over the aquatic environment. Fish are contained in cages moored in estuaries or sheltered areas of the sea. Cages and nets can become damaged, thereby allowing fish to escape into the wild. There is significant interaction between wild fish and the farmed fish. The author has observed significant numbers of wild Pacific herring (*Clupea pallasii*) inside cages of farmed Atlantic salmon (*Salmo salar*). 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 the fish from the water.

Semi-closed systems

Semi-closed 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, and preventing inflow or outflow of water may have adverse effects. Output water control and treatment to control VHSV are possible in theory, but are usually not economically viable. In Scotland, the virus was successfully eradicated from a pump-ashore tank farm. In that outbreak, effluent water was disinfected before release (Munro 1996).

Fish input and output may be controlled. Fish inputs into freshwater farms may be from on-site hatcheries or from other freshwater or marine farms (eg broodstock). Fish are also able to enter farm waterways and possibly ponds via intake water from the water source. Movement restrictions could significantly interrupt farm management practices and production.

VHSV has been successfully eliminated from fish hatcheries in Denmark (Jørgensen 1980). The principal method was to empty, disinfect and keep dry for at least one month all VHS-infected trout farms using water from the same stream, starting with the farm at the top of the stream and progressively working downstream. The farms were then restocked with VHS-free fish. There was no destruction of wild fish.

Closed systems

It is possible to isolate a closed system, such as an aquarium. Therefore, preventing the spread of VHSV from such a system is possible.

Zoning

Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN **Zoning Policy Guidelines**.² Zoning may be possible if VHSV is isolated from a single culture facility. Zoning may also be possible if the virus has been carried into a freshwater environment from a marine source (eg through feeding of marine trash fish to freshwater cultured fish), even if the virus is known to be present in wild marine species.

² See <http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717> (Accessed 16 June 2005).

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

2.2.2 Tracing

Depending on where VHSV is isolated, tracing of fish, fish products, people and equipment may be difficult. 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.

A thorough and comprehensive epidemiological investigation requires trained personnel with adequate time and resources.

Immediate tracing steps to aid in the epidemiological investigation include:

- *trace-back* of all movements of the 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, or both?
- *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 – eg broodstock, smolts, fish used for restocking and harvested wild fish;
- fish products – fish for consumption, 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 PCR (see Section 1.4.3) may also be useful in identifying whether or not the isolated VHSV matches known overseas strains. This could help in determining 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 as 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. All the measures listed in Section 2.2.1 will need to be considered.

2.2.3 Surveillance

Surveillance is a critical element in any control strategy. Surveillance can be costly and may require the following resources:

- field personnel;
- laboratory personnel;
- administrative assistance;
- equipment and instruments; and
- diagnostic reagents.

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

2.2.4 Treatment of infected fish

There are currently no treatments for viral diseases in fish.

2.2.5 Destruction and disposal of fish

Destruction of infected fish will eliminate a major potential source of virus, reduce the virus load in the surrounding environment and reduce the risk of infection of other wild or farmed fish.

If possible, measures to minimise the spread of virus while fish are destroyed should be implemented.

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 isolated from fish in those facilities (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 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, see the AQUAVETPLAN **Destruction**³ and **Disposal**⁴ operational procedures manuals.

³ See <http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906> (Accessed 16 June 2005).

⁴ See <http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5> (Accessed 16 June 2005).

2.2.6 Treatment of fish products and byproducts

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

VHSV is not a resilient virus, but will survive reasonably well at low temperatures. Freezing will not destroy the virus, but repeated freeze-thaw cycles will reduce the overall virus titre.

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 during the preclinical phase of infection.

Using appropriate controls, it may be feasible to harvest and safely process those fish without signs of disease. In Scotland, in the control of an outbreak of VHSV in turbot, 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. This removed the organs that were most likely to contain the highest titre of virus (Munro 1996). The eradication strategy on this farm was successful.

2.2.7 Decontamination

Successful disinfection requires effective cleaning before the disinfection process. Drying and sunlight will effectively destroy the VHSV in a matter of hours, and the virus is readily inactivated by a number of disinfectants (see Section 1.6.6).

Processing plants handling infected or potentially infected fish are potential sources of the pathogen. If emergency harvest is carried out and some of the fish show clinical signs, there may be high virus titres in processing plant effluent. Titres of infectious haematopoietic necrosis virus, another rhabdovirus, have been shown to be approximately $1.3\text{--}4.3 \times 10^3$ PFU/mL (PFU = plaque forming unit) in processing water when the fish being processed were from an infected site. Such water requires disinfection before release into an aquatic environment.

Because of differences between farming enterprises, disinfection protocols for freshwater facilities may need to be determined case by case. This will involve the farm manager, the state/territory CVO and/or the director of fisheries. The disinfection protocol should take into consideration the factors outlined in Section 1.6, including:

- the source and location of infection;
- the type of enterprise (eg facilities using well water versus those using water from river, lake or stream);
- the design of the site and its proximity to other waterways;
- the protocol's environmental impact; and
- the availability of approved, appropriate and effective disinfectants.

Environmental considerations

Large numbers of dead fish can be the source of unpleasant odours and can be unsightly if not covered.

In decontamination operations, all legislation and regulations concerning the disposal or discharge of chemicals and cleaning agents into the environment must be observed.

2.2.8 Vaccination

There are currently no commercial vaccines available for VHSV.

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 (eg dogs) from accessing infected fish, including from disposal sites.

2.2.10 Restocking

VHSV can infect many species, but strains of VHSV differ significantly in their virulence in different species. Rainbow trout is considered the most susceptible fish species to the European freshwater strain of VHSV.

If a highly virulent strain of VHSV is isolated in Australia accompanied by significant signs of disease, restocking with a different species of fish should be considered. Restocking will only be considered after the initial outbreak has been dealt with.

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; and
- eating fish that may have been exposed to VHSV is not considered a health risk.

A media kit should be distributed quickly to ensure that the media can help reduce any potential public fear or perception of risk.

If VHSV is isolated from a marine species such as pilchards, it may be associated with significant mortality, as has been observed in wild marine fish overseas. Although the virus may not be proven as a cause of the mortality, it nevertheless may be associated with it. Many of these fish may wash up on beaches and be of concern to the public. It is important that the public has the confidence that something constructive is being done about the problem, and that there is someone or some authority taking responsibility for the investigation.

Some states (for example, Victoria⁵ and Tasmania) have developed manuals outlining the procedures to be followed in the event of an outbreak of a disease such as VHS, and detailing the roles and responsibilities of the various organisations, departments and personnel in such an outbreak.

2.3 Feasibility of specific options for control in Australia

This section discusses the feasibility of control of VHSV if the virus is isolated in Australia. Feasibility 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.

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; and,
- 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 both 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 and tuna farming operations; and
- overseas 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). VHSV was successfully eradicated from a pump-ashore turbot farm in Scotland (Munro 1996).

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

⁵ See FRDC (2002).

Unexposed fish

If there is doubt about whether fish have been exposed to VHSV, they should be treated as exposed.

Unexposed fish may be destroyed as a precaution in order to remove a potential source of disease before exposure and infection occur. However, if exposure can be prevented in the first place, there will be no further propagation of disease 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 whether exposure can be prevented and destruction avoided.

Exposed or potentially exposed, but clinically normal fish

Immediate destruction of exposed fish prevents further virus propagation by decreasing the infectious load on a site and minimising the spread of infection.

In a VHS eradication program, normal or controlled grow-out 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. Dead fish can be more difficult to remove from a facility than live fish.

Clinically diseased fish

Immediate removal, destruction and disposal of all diseased and dead fish is essential. These 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 such a situation, 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 will make control and containment of the virus very difficult. The extent of VHSV both geographically and biologically will need to be investigated to help determine whether zoning is feasible. Environmental conditions in many parts of Australia are not suitable for the establishment of VHSV, and this may make zoning easier.

If VHSV is isolated from fish in a freshwater establishment, there is the possibility of the virus being 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 (Jorgensen 1974).

Unexposed fish

Containment and control options for unexposed fish are the same as those outlined for eradication in 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 will rely on movement restrictions on exposed or potentially exposed fish to prevent infection spreading to uninfected zones. The feasibility of implementing a zoning program will depend 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 byproducts.

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. In fish that have been infected with VHSV, evisceration will remove the organs likely to have the highest titre of virus. Freezing and thawing fish products will reduce virus titre 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 no treatments for VHS, and fish that survive an outbreak can continue to be a source of infection. Destruction is the only option.

2.3.3 Control and mitigation of disease

In a control and mitigation program, the aim may simply be to reduce the frequency of 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 a single-stranded RNA virus. RNA viruses show more mutability than DNA viruses (Steinhauer and Holland 1987). 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.

If farmed fish are allowed to grow out for harvest, there should be a complete break between emptying the farm and restocking, as this will help break the VHSV cycle in the facility.

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 throughout much of Europe, in North America and in Japan. Isolating VHSV in Australia would not necessarily mean that trade in fish products would be seriously affected.

Many overseas countries require imports such as fertilised fish eggs (embryos) to be certified free from VHSV. This export trade might still be possible despite some

parts of Australia being considered infected with the virus, especially if such products come 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 Australian Quarantine and Inspection Service should be contacted for current information regarding export market requirements.

Domestic markets

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, restrictions will be less stringent.

3 Policy and rationale

3.1 Overall policy

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

The choice of response option will be decided by the director of fisheries and/or the chief veterinary officer (CVO) of the state/territory in which the outbreak occurs, following initial epidemiological investigations.

There are three possible response options:

- ☞ *eradication*, with the aim of returning Australia to freedom from VHS;
- ☞ *containment, control and zoning* to confine VHS virus to enzootic areas, prevent further spread and protect uninfected areas; or
- ☞ *control and mitigation* of 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 first chosen may be changed as more information becomes available; for example, eradication may eventually be chosen as a long-term policy even when containment, control and zoning was the first choice.

Strategies that may be used under these control options include:

- ☞ *quarantine and movement controls* on fish, fish products and things in declared areas to prevent spread of infection;
- ☞ *prevention of access* by predators and/or scavengers (eg 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 infected fish hosts, 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; and
- ☞ a *public awareness campaign* to encourage cooperation from aquaculturalists and the community.

The director of fisheries and/or the CVO of the state or territory in which the disease occurs and/or the virus is isolated will be responsible for deciding which control option is chosen, and for implementing disease control measures in accordance with relevant legislation.

If VHS, with or without disease, is confirmed in Australia, the director of fisheries and/or the CVO of the state/territory in which the isolation occurs will be responsible for implementing disease control measures in accordance with relevant legislation. The aquatic Consultative Committee on Emergency Animal Diseases (aqCCEAD, comprising representatives of the state/territory and Australian governments and representatives of the affected industries) will be convened to discuss options for response 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.

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.

3.2 Overview of response options

The control option to be adopted will be decided following 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.

The flow chart in Figure 3 gives some possible scenarios and is designed to help in 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 (eg in a closed system such as an aquarium or in a fully recirculating system); and
- 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 feasible and chosen as the preferred control option when:

- VHSV is isolated from wild or farmed fish confined to a specific geographical 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; and
- eradication is not considered to be an option.

Where containment, control and zoning is chosen as the initial option, it may later evolve into a policy of control and mitigation of disease.

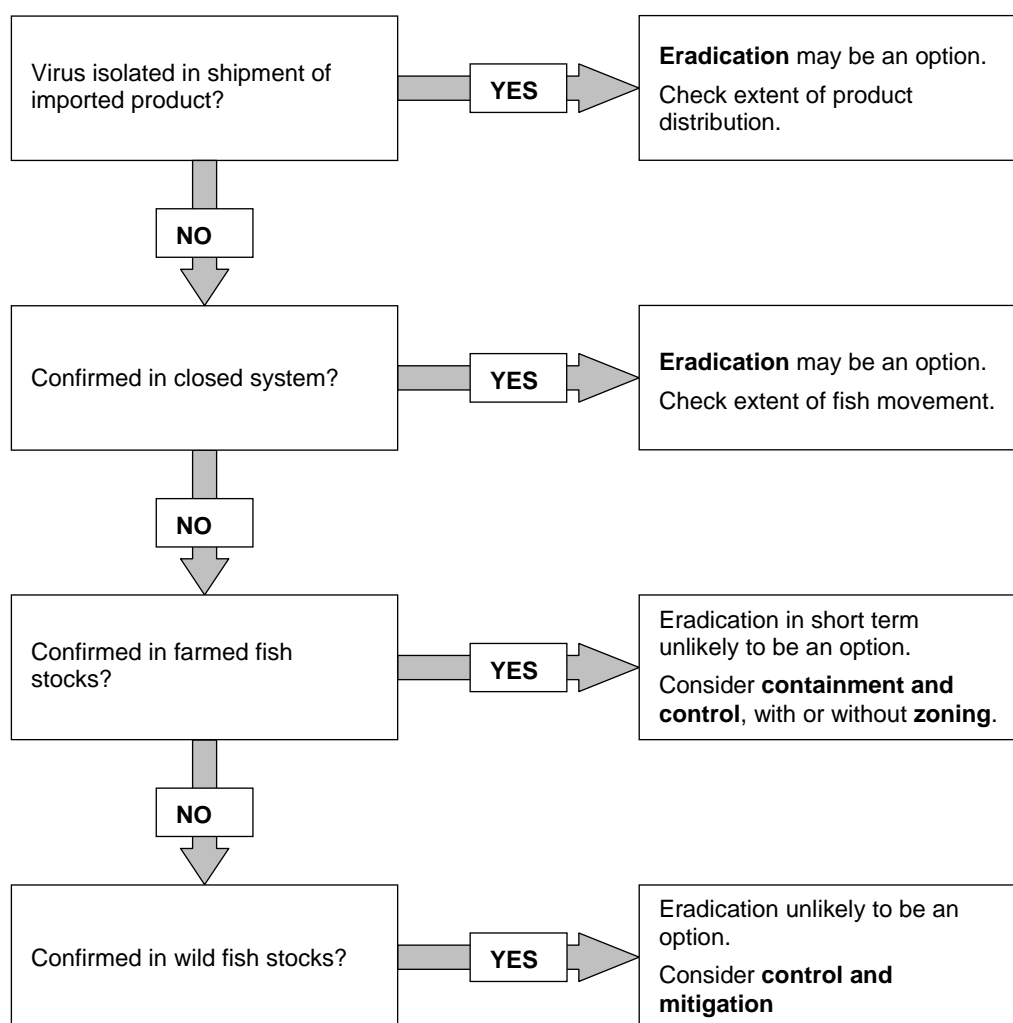


Figure 3 Decision flow chart

Below are some key criteria for the adoption of a policy option. These criteria are not exhaustive and are given only as a guide.

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

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 will 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, will be initiated immediately to determine:

- which genogroup/type the isolate is most likely to belong to – this will help in identifying the possible source of the virus, and possible extent of disease;
- how widespread the virus may be, both geographically and biologically (ie the range of susceptible fish species that may be infected) – until adequate data become available, it should be assumed that any fish populations that might have been exposed to the virus have been exposed; and
- how to prevent any further virus spread from the infected location, if prevention is deemed possible.

It can be very difficult to isolate the virus from carrier fish. This must be taken into consideration when designing and conducting the epidemiological investigation and assessing the results. The focus should be on fish in waters colder than 15°C.

3.3.2 Quarantine and movement controls

Quarantine and movement controls (see 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 for VHS.

3.3.4 Vaccination

There are currently no commercially available vaccines for VHSV.

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 ensure no further spread of the virus and to protect 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 already be present in wild fish populations. Humane destruction of cultured fish in such circumstances will minimise virus load in the area, but not eliminate the virus.

See the AQUAVETPLAN **Destruction Operational Procedures Manual** for details of destruction operations.⁶

⁶ See <http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906> (Accessed 16 June 2005).

3.3.6 Treatment of fish products and byproducts

The treatment of fish products and byproducts must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product.

Harvested fish should be eviscerated 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 what to do with these fish will depend on the control option chosen (see Section 2.2.6).

Any harvesting or processing equipment used must be treated as contaminated and disinfected accordingly (see the AQUAVETPLAN **Disposal Operational Procedures Manual**).⁷

3.3.7 Vector control

To limit spread of the virus in the initial response, effective control is essential to prevent predators and scavengers (eg birds, 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 effective communication with stakeholders and the public. A vital aspect of the response program will be to address the concerns of the public (especially such groups 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 holds no health risk for humans (see also Section 2.2.11).

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 in Australia, its isolation will be reported to the OIE. In this event, industries exporting fish products, such as fertilised eggs, will need to confirm the requirements of countries importing Australian product. Fish exported from temperate areas of Australia are usually eviscerated, so major impacts on such exports are not expected.

⁷ See <http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5> (Accessed 16 June 2005).

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

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

The Australian Quarantine and Inspection Service should be contacted for the most current information about export market 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 (see Section 2.3.4).

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 level. Criteria for proof of freedom at each level are given in the OIE Aquatic Code (OIE 2004).

3.6 Funding and compensation

There is currently no cost-sharing arrangement in place for aquatic animal diseases.

Appendix 1 *OIE International Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals*

OIE Aquatic Code

The objective of the OIE (2004) *International Aquatic Animal Health Code* (the OIE Aquatic 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/export services, epidemiologists and all those involved in international trade.

The current edition of the OIE Aquatic Code (7th edition) was published in 2004 and is available on the OIE website at:

http://www.oie.int/eng/normes/fcode/a_index.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 2.1.5 Viral haemorrhagic septicaemia

OIE Aquatic Manual

The purpose of the OIE (2003) *Manual of Diagnostic Tests for Aquatic Animals* (the OIE Aquatic Manual) 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 across the globe.

The current edition of the OIE Aquatic Manual (4th edition) was published in 2003 and is available on the OIE website at:

http://www.oie.int/eng/normes/fmanual/A_summry.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 2.1.5 Viral haemorrhagic septicaemia

OIE Disease Technical Cards

The purpose of the OIE *Disease Technical Cards* is to provide a summary of information relevant to each disease, its characteristics, diagnosis and control.

The *Disease Technical Cards* are available on the OIE website at:

http://www.oie.int/aac/eng/cards/en_diseasecard.htm
(Accessed on 11 May 2005)

The following card is relevant to this manual:

Viral haemorrhagic septicaemia

Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at:

http://www.oie.int/eng/normes/en_acode.htm
(Accessed on 11 May 2005)

Appendix 2 Species susceptibility to viral haemorrhagic septicaemia virus

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

Atlantic cod (<i>Gadus morhua</i>)	Atlantic salmon (<i>Salmo salar</i>)
Blue whiting (<i>Micromesistius poutassou</i>)	Brook trout (<i>Salvelinus fontinalis</i>)
Brown trout (<i>Salmo trutta</i>)	Dab (<i>Limanda limanda</i>)
English sole (<i>Parophrys vetulus</i>)	Eulachon (<i>Thaleichthys pacificus</i>) (smelt)
Flounder (<i>Platichthys flesus</i>)	Golden trout (<i>Salmo aquabonita</i>)
Grayling (<i>Thymallus thymallus</i>)	Haddock (<i>Gadus aeglefinus</i>)
Halibut (<i>Hippoglossus hippoglossus</i>)	Herring (<i>Clupea harengus</i>)
Hybrid (rainbow trout x coho salmon) (<i>Oncorhynchus mykiss</i> x <i>O. kisutch</i>)	Japanese flounder (<i>Paralichthys olivaceus</i>) (hirame)
Lake trout (<i>Salvelinus namaycush</i>)	Lesser argentine (<i>Argentina sphyraena</i>)
Norway pout (<i>Trisopterus esmarki</i>)	Pacific cod (<i>Gadus macrocephalus</i>)
Pacific hake (<i>Merluccius productus</i>)	Pacific herring (<i>Clupea pallasii</i>)
Pacific mackerel (<i>Scomber japonicus</i>)	Pacific salmon (<i>Oncorhynchus</i> spp)
Pacific sardine (<i>Sardinops sagax</i>)	Pike (<i>Esox lucius</i>)
Plaice (<i>Pleuronectes platessa</i>)	Poor cod (<i>Trisopterus minutus</i>)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Rockling (<i>Rhinonemus cimbricus</i>)
Sand lance (<i>Ammodytes hexapterus</i>)	Sea bass (<i>Dicentrarchus labrax</i>)
Shiner perch (<i>Cymatogaster aggregata</i>)	Surf smelt (<i>Hypomesus pretiosus</i>)
Three-spine stickleback (<i>Gasterosteus aculeatus</i>)	Turbot (<i>Scophthalmus maximus</i>)
Walleye pollock (<i>Theragra chalcogramma</i>)	White fish (<i>Coregonus</i> spp)
Whiting (<i>Merlangius merlangus</i>)	

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

Japanese flounder (<i>Paralichthys olivaceus</i>) (hirame)	Pacific hake (<i>Merluccius productus</i>)
Pacific herring (<i>Clupea pallasii</i>)	Pacific sardine (<i>Sardinops sagax</i>)
Pike (<i>Esox lucius</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Turbot (<i>Scophthalmus maximus</i>)	

Species challenged with at least one VHSV isolate (generally Type I) and found not to be susceptible^a

Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	Coho salmon (<i>Oncorhynchus kisutch</i>)
Common carp (<i>Cyprinus carpio</i>)	Eurasian perch (<i>Perca fluviatilis</i>)
Goldfish (<i>Carassius auratus</i>)	Roach (<i>Leuciscus rutilus</i>)
Tench (<i>Tinca vulgaris</i>)	

^a This does not signify that these species are not susceptible to other VHSV isolates.

Appendix 3 Detection and identification of viral haemorrhagic septicaemia virus

The following methods are used for the detection and identification of viral haemorrhagic septicaemia virus (VHSV) at 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.

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 AAHL, pooled tissues may be stored (on ice but not frozen) in transportation medium.

Samples should not be frozen prior to processing but should be maintained between 4°C and 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 post-collection should be frozen in the temperature range –70°C to –80°C.

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 used for testing. Tissue samples obtained during non-spawning 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:

<i>Fish size (length)</i>	<i>Tissues</i>
<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

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, AFDL will use a range of fish cell lines in an attempt to isolate the virus.

Based on international protocols, 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 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 that 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 2001).

Identification

Immunocytochemistry

Virus identification by various immunoassays has become a standard procedure for viruses where specific antibodies are available. At AFDL, immunocytochemistry using an immunoperoxidase test is favoured. Briefly, 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 (eg 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. Following incubation, excess conjugate is removed by washing, a substrate (eg with 3 amino-9-ethylcarboxyole) is added and colour is allowed to develop. Finally, following 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 (eg Qiagen AVL buffer) containing guanidinium isothiocyanate.

Nucleic acid is obtained from cell-free samples using the QIAamp Viral RNA extraction kit (QIAGEN cat no. 52904) or from tissues using the RNeasy Viral RNA extraction kit (QIAGEN cat no. 74904). cDNA is prepared from the viral RNA using a standard protocol that has been adopted for all RNA agents. Following production of cDNA, a PCR is then conducted using primers based on those of Stone et al (1997):

5' gtccccagggatgatgncc 3'

5' AGTCCCCAGGGATGATGNCC 3'

nested PCR set:

5' cacgagtacccgttcttccc 3'

5' AGTCCCCAGGGATGATGNCC 3'.

Sequencing of PCR products is required for definitive diagnosis. In addition, sequence information will assist in strain identification.

References

Papers relevant to testing for VHSV include Blanchard (1993); Crane et al 2000, 2001; Department of Fisheries and Oceans (Canada) (1984); European Union (2001); Hill (1976); Stone et al (1997); Thoesen (1994); OIE (2003) Aquatic Manual.

Glossary

Aquatic Animal Health Committee	A committee comprising representatives of the Australian government, Australian state and territory governments, the major aquaculture, wild capture, aquarium and recreational fishing industries and a CSIRO representative. The committee provides advice to Primary Industries Ministerial Council on aquatic animal health matters, focusing on technical issues and regulatory policy. <i>See also</i> Primary Industries Ministerial Council
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. <i>See also</i> Chief veterinary officer
AQUAVETPLAN	<i>Australian Aquatic Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. <i>See also</i> AUSVETPLAN
AUSVETPLAN	<i>Australian Veterinary Emergency Plan.</i> 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 plans.
Chief veterinary officer (CVO)	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. <i>See also</i> Australian Chief Veterinary Officer
Compensation	The sum of money paid by government to an owner for stock that are destroyed and property that is compulsorily destroyed because of an emergency animal disease.
Control area	A buffer between the restricted area and areas free of disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may reduce in size. The shape of the area may be modified according to circumstances, eg water flows, catchment limits etc. In most cases, permits will be required to move animals and specified product out of the control area into the free area.
Covert infection	Clinically inapparent infection that is transmissible and that may eventually lead to clinical disease.

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	Area that has had a direct, and possibly infectious, contact with an infected premises/area. The type of contact will depend on the agent involved in the outbreak but, for example, may involve animal movements or net/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 <i>restricted area</i> , <i>control area</i> , <i>infected premises</i> , <i>dangerous contact premises</i> and <i>suspect premises</i> .
Decontamination	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 disease agents outside a living animal.
Disinfection	The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies to premises, vehicles and other objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of fish carcasses and things by burial, burning or some other process so as to prevent the spread of disease.
Ecchymotic haemorrhages	Bleeding or bruising in the skin or a mucous membrane in the form of small round spots or paintbrush-like red/purplish discolouration.
Enzyme-linked immunosorbent assay (ELISA)	A serological test designed 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. <i>See also</i> Endemic animal disease, Exotic animal disease
Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. <i>See also</i> Emergency animal disease, Exotic animal disease

Enterprise	See Risk enterprise
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with the 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 byproducts	Products of fish origin destined for industrial use (eg fishmeal).
Fish products	Fish meat products and products of fish origin (eg eggs) for human consumption or use in animal feeding.
Free area	An area known to be free of the disease agent.
Haemorrhage	Bleeding from a ruptured blood vessel.
Hyperaemia	An excess of blood in an area.
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.
Leukocytopaenia	Abnormally reduced numbers of white cells (leukocytes) in the bloodstream.
Local disease control centre	An emergency operations centre responsible for the command and control of field operations in a defined area.
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 disease.
Nested RT-PCR	A double-stage PCR process where the second round identifies a DNA sequence 'nested' within the initial sequence, thus increasing the specificity. See <i>Polymerase chain reaction (PCR)</i> and <i>Reverse transcriptase-PCR (RT-PCR)</i>
OIE Aquatic Code	OIE <i>International Aquatic Animal Health Code</i> (OIE 2004). Published on the internet at: http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005). See Appendix 1 for further details

OIE Aquatic Manual	OIE <i>Manual of Diagnostic Tests for Aquatic Animals</i> (OIE 2003). Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines). The current edition is published on the internet at: http://www.oie.int/eng/normes/fmanual/A_summry.htm (Accessed 11 May 2005). 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).
Petechial haemorrhage	Tiny, flat, red or purple spots in the skin or mucous membranes caused by bleeding from small blood vessels.
Polymerase chain reaction (PCR)	A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also <i>Reverse transcriptase-PCR (RT-PCR)</i> and <i>Nested RT-PCR</i>
Premises or area	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 positive antibody titre) at a given point in time.
Primary Industries Ministerial Council	The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Agriculture and Resource Management Council of Australia and New Zealand).
Quarantine	Legal restrictions imposed on a place, fish, vehicles, or other things, 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.
Reverse transcriptase-PCR (RT-PCR)	A highly sensitive technique for the detection and quantitation of mRNA (messenger RNA) by reverse transcription to DNA followed by PCR. See <i>Polymerase chain reaction (PCR)</i> and <i>Nested RT-PCR</i>

Risk enterprise	A defined livestock or related enterprise, which is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots and garbage depots.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). <i>See also</i> Specificity
Sentinel fish	Fish of known health status monitored to detect the presence of a specific disease agent.
Septicaemia	The invasion and persistence of pathogens in the bloodstream.
Serosanguinous fluid	Fluid composed of blood and serum.
Serotype	A subgroup of microorganisms identified by the antigens carried (as determined by a serology test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). <i>See also</i> 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 with a particular disease.
Suspect fish	Fish that may have been exposed to an emergency disease such that its quarantine and intensive surveillance, but not pre-emptive slaughter, is warranted. <i>or</i> Fish not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis.
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; however, it may involve clinical signs or increased mortality.

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 weakened or attenuated strains of disease-causing agents to provide protection from disease.
Vaccine	Modified strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
Vector	A living organism that transmits an infectious agent from one host to another. A <i>biological</i> vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A <i>mechanical</i> 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
AFDL	AAHL Fish Diseases Laboratory
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
aqCCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
AUSVETPLAN	Australian Veterinary Emergency Plan
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Department of Agriculture, Fisheries and Forestry (Australian Government)
DNA	Deoxyribonucleic acid; cDNA = complementary DNA
EAD	emergency animal disease
ELISA	enzyme-linked immunosorbent assay
ISA	infectious salmon anaemia
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PCR	polymerase chain reaction
PFU	plaque forming unit
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
TCID	tissue culture infective dose
VHS	viral haemorrhagic septicaemia
VHSV	VHS virus

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