FINAL REPORT

Aquatic Animal Health Subprogram – Viral Haemorrhagic Septicaemia (VHS) – A Disease Strategy Manual

P. Hardy-Smith
Panaquatic Health Solutions

June 2004

FRDC Project No. 2002/640

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Author Paul Hardy-Smith

Title Aquatic Animal Health Subprogram – Viral Haemorrhagic Septicaemia (VHS) – A Disease Strategy Manual

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CONTENTS

2002/640	**Viral Haemorrhagic Septicaemia (VHS) – A Disease Strategy Manual**

PRINCIPAL INVESTIGATOR: Dr P. Hardy-Smith
Panaquatic Health Solutions

ADDRESS: Level 1, 496 Bridge Road
(PO Box 98)
Richmond, VIC, 3121
Australia
Ph: +61 3 9429 5090 Fax: + 61 3 9429 5094
Email: panaquatic@ozemail.com.au

Outcomes achieved to date:

The final draft of Viral Haemorrhagic Septicaemia (VHS) – A Disease Strategy Manual is now complete. Chapter One of this Manual describes the disease. Chapter Two discusses the Principles for Control of this disease, and Chapter Three details the Preferred Control Policy that will be used if VHS is detected in Australia.

The preparation of this Manual has involved consultation with industries and governments in States where an outbreak of this disease could occur i.e. Tasmania, Victoria, New South Wales, South Australia and Western Australia. Some States have larger populations of susceptible fish species than others, and the degree of consultation relative to each State has reflected this.

During the consultation process, the disease has been discussed and outbreak scenarios considered. This has therefore raised the level of understanding and awareness of this disease with government and industries in these States. This is considered a significant outcome achieved to date.

In addition, some of the key personnel responsible for the control of aquatic animal disease in States with susceptible fish populations have reviewed the draft Manual (in some cases more than once) and forwarded useful and pertinent comments to the Principal Investigator. Having such personnel consider the disease and control options has been proactive in preparing Australia for a possible incursion of this disease.

The draft Manual has been reviewed by a number of international peers. Such collaboration is important in fostering relations that may become very useful should VHS ever be detected in Australia

Non technical summary:

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 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 pallasi*). The virus has also been isolated from many fish species where no clinical signs of disease were observed.

Outbreaks most often occur in susceptible fish populations at a water temperature of approximately 10ºC. Death of fish caused by this disease has rarely been documented when water temperatures are above 15ºC. The virus itself is also rarely detected in fish above this water temperature.

There are a number of different groups ('genotypes') into which isolates of the virus can be categorised. These groups are predominately based on geographic origin.

There are fish species in that are known to be susceptible to this disease, and many species where susceptibility is uncertain.

VHSV has never been detected in Australia, or in any other country in the Southern Hemisphere. However, the level of monitoring and surveillance of some of the fish species in temperate Australian waters, particularly of wild species, is not sufficient to rule out the possibility that VHSV may already be present in one or more Australian fish species. This is considered unlikely, but possible.

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

The first line of defence against importing VHSV from another country is the continued implementation of preventative customs and quarantine measures. These measures are constantly being reviewed with significant consideration being given to international trade agreements and understandings.

If VHSV is detected in Australia, aquatic animal health authorities could be faced with any of a number of scenarios, which range from:

- The isolation of VHSV from wild fish showing no clinical signs of disease; to
- An outbreak of clinical VHS in farmed and wild rainbow trout.

There are essentially three broad options available to control the disease. These options are:

1. ***Eradication***
   - Eradication of VHSV from Australia. This is the highest level of control measure and cost.
2. ***Containment, control and zoning***
   - Involves containment of VHSV to areas with enzootic infection, prevention of further spread and protection of uninfected areas.
3. ***Control and mitigation of disease***
   - The implementation of management practices that decrease the incidence and severity of clinical outbreaks.

The preferred policy is to eradicate VHSV if it were detected in Australia (Option 1), especially if clinical disease is associated with the outbreak(s). Depending on circumstances, this may not be feasible e.g. if the virus is identified in wild fish stocks.

If eradication is not feasible, the preferred policy will be either Option 2 or 3.

Epidemiological information on which to base a decision may initially be limited. The adoption of a policy does not preclude adopting a different policy as more information becomes available e.g. eradication may be chosen as a long term policy even when containment, control and zoning is chosen in the first instance.

The Director of Fisheries and/or the CVO of the State/Territory in which the disease occurs and/or virus is detected will be responsible for deciding which control option(s) is chosen, and for implementing disease control measures in accordance with relevant legislation.

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* where possible of predators and/or scavengers (e.g. birds) gaining access 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* where possible to define and maintain infected and VHS free zones;
- *Restocking* with older, less susceptible fish or less susceptible species unlikely to develop clinical disease;
- *A public awareness campaign* to facilitate cooperation from aquaculturalists and the community.

Acknowledgments

The Principal Investigator acknowledges the contribution of Dr Ron Hedrick, Dr Craig Stephens and Dr Mark Crane for their help in reviewing the initial drafts of the document. The Principal Investigator also acknowledges the helpful advice and input from Dr Barry Hill, and the help of key stakeholders from around Australia who provided comments and critique during the preparation of the Manual and to those that reviewed the final draft of the Manual. The assistance of the library staff and Ms Nette Williams at AAHL was also greatly appreciated as was the support provided by members of the Aquatic Animal Health Subprogram.

Background

In the May 2000 Budget, the Federal Government announced its *Building a National Approach to Animal and Plant Health* program. This initiative seeks to maintain Australia's status as a sought after supplier of high quality, 'clean, green' agricultural produce. Within this initiative, funds were made available to Agriculture, Fisheries and Forestry – Australia (AFFA) as administered funds for the Program *Emergency Management Planning* for aquatic animal diseases. As per an Agreement between AFFA and the Fisheries Research and Development Corporation (FRDC), these monies are administered by the FRDC on AFFA's behalf. The FRDC's vehicle for delivery is the FRDC Aquatic Animal Health Subprogram.

During December 2001 and January 2002, stakeholders from industry and governments in Australia nominated their priorities for projects under this Program. On 15 February 2002, the Subprogram's Steering Committee and Scientific Advisory Committee met to evaluate the nominations. Through this process, the Viral Haemorrhagic Septicaemia (VHS) – *Disease Strategy Manual* was approved as a priority. This Manual is one of a total of nine disease strategy and Operational manuals approved as priorities through this process.

These Manuals will form part of a series that are being developed under *Australia's National Strategic Plan for Aquatic Animal Health* (AQUAPLAN) and collectively will be known as AQUAVETPLAN

Need

Viral Haemorrhagic Septicaemia (VHS) is exotic to Australia. This disease, caused by a rhabdovirus, Viral Haemorrhagic Septicaemia Virus (VHSV), is listed by the OIE (Office International des Epizooties). Listed diseases are considered to be of socio-economic and/or public health importance within countries and are significant in the international trade in aquatic animals and aquatic animal products. Overseas VHS is a major cause of mortality in a number of fish species of both the freshwater and marine environment.

There are a number of fish species known to be susceptible to infection with VHSV being farmed in this country (e.g. rainbow trout) and occurring in the wild (e.g. pilchards). There are a number of other species, both wild and farmed, for which the susceptibility is unknown but which could potentially be found to be susceptible to infection with VHSV.

Aquaculture is growing in Australia and the value of commercial finfish production of fish that are susceptible to this disease is increasing (ABARE 2001). So too is the range of species being cultured in this country that could be at risk were there to be an outbreak of this disease. This disease could also have a significant impact on wild fish species.

It is difficult to predict how an outbreak of VHS may occur in Australia. Minimising the potential for importing product that could possibly carry the virus is being addressed elsewhere (AFFA 2001).

In any emergency management strategy, it is important to have in place options for dealing with an outbreak of the disease to minimize the impact should it occur. Speaking from the applicant's own experience, it is also highly beneficial that such options have been considered and agreed to by relevant stakeholders before the emergency occurs.

While the Federal Government encourages a detection and eradication culture based on constant vigilance and a readiness to tackle any emergency, prior to this Manual there were no accepted national guidelines for dealing with an outbreak of an exotic viral disease in finfish in Australia.

Hence the need to develop this Disease Strategy Manual that includes information on all potentially susceptible species, describes details about the disease, response options and the preferred, nationally agreed upon approach to its control.

Objectives

1. To develop a consensus between government and industry on a preferred control policy for VHS should an outbreak of this disease occur in Australia
2. Preparation of a stakeholder endorsed final version of VHS - Disease Strategy Manual for submission to AQUAPLAN Business Group (ABG)/Scientific Advisory Committee (SAC) which incorporates this preferred control policy. This manual will enhance the capability of both terrestrial and aquatic animal health professionals to identify and efficiently manage an emergency response in the event of a suspect or confirmed incursion of VHS in Australia.

Methods

1. Current literature on the disease was reviewed. This review was done in collaboration with the Australian Animal Health Laboratory (AAHL), using the facilities available to AAHL for such review.
2. Consultation with international fish health professionals involved in research on and/or managing of this disease and disease outbreaks. Professionals included Dr Ron Hedrick, of the Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Professor Barry Hill, Chief Adviser for Fish and Shellfish Health for the UK Ministry of Agriculture, Fisheries and Food (MAFF) and Dr Craig Stephens, a veterinary fish epidemiologist currently working in Canada.
3. Preparation of the initial draft of Sections 1 (nature of the disease) and 2 (Principles of control and eradication) of the Manual. This was prepared after reviewing the process used in developing the first Disease Strategy Manual (the AQUAVETPLAN Furunculosis Disease Strategy Manual) to identify what were considered the pros and cons of this process. The positive aspects were, where possible, incorporated into the preparation of this Manual.
4. Distribution of the initial draft to a considerable number of industry and government stakeholders (as identified in consultation with AFFA) and to nominated peers (e.g. R. Hedrick, B. Hill, C. Stephens). Draft was distributed electronically. Feedback was not as extensive as hoped from the industry and government stakeholders. As a suggestion, limiting the number of stakeholders to whom the draft was sent and individually discuss the review with them prior to forwarding the draft may have helped in ensuring a greater number of reviews. Following up with those that did take the time to review the document was also important. This was done in a later step (Step 7 below) and worked very well.

5. A number of workshops were held with relevant government and industry stakeholders in Tasmania, Victoria and South Australia. Appendix A contains a copy of the agenda for these workshops. The aim of these workshops was to go through draft sections 1 and 2 and gain consensus for section 3 (response options for the control of the disease and the preferred control option(s)). The workshops involved discussing feasible scenarios surrounding the detection of VHS in Australia[1]. Stakeholders in Western Australia, Australian Capital Territory and New South Wales were individually consulted at this stage.
6. The draft Section 3 (Preferred Control Policy in Australia) was then prepared. A small number of key stakeholders (including the Government aquatic animal veterinarians in Tasmania, Victoria and South Australia, the Project Leader of the AAHL Fish Diseases Laboratory, Ramesh Perera of Biosecurity Australia) reviewed the draft. Time availability has been a critical factor for these reviewers; fortunately most have completed the review and forwarded comments back to the Principal Investigator.
7. The Final Draft for Endorsement was then prepared and the final report written in preparation for circulation.

Results/Discussion

The first key objective of this project has now been completed. Consensus has been reached on the preferred control policy for VHS should this disease be detected in Australia. Unlike the preferred control policy in many terrestrial animal diseases (e.g. Rabies, Foot and Mouth disease) the preferred policy was not simply to take all measures to eradicate the disease. While it was agreed that this was the **ideal** policy, it was also agreed that the actual policy adopted depended on the circumstances surrounding the outbreak/detection. Eradication was considered to not always be an option. The chosen policy could also change as more information became available during the outbreak/detection investigation.

The second key objective, stakeholder endorsement of the final version of VHS – A Disease Strategy Manual for submission to AQUAPLAN Business Group (ABG)/Scientific Advisory Committee (SAC) has been completed.

Benefits and adoption

This project has ensured that there is now a working VHS - Disease Strategy Manual in place. This specifically details the disease, known susceptible and potentially susceptible species, response options and the preferred, nationally agreed-upon approach to its control should an outbreak occur. Federal and State authorities adopting this Manual now have a guide to how deal with an outbreak of this disease in Australia. This will help considerably in the effective management and control of the outbreak.

The submission of the final document is the culmination of a significant process. This process, as outlined in "Methods" above, involved considerable consultation with relevant government and industry stakeholders who would be affected by such an outbreak and who will be in positions of responsibility should such an outbreak occur. Hence there is already a significant increase in the understanding and awareness of this disease, and the possible options for dealing with an emergency. Benefits have also resulted from the planned workshops with key stakeholders and their inclusion in the review process. This has ensured they have ownership of the Manual which will significantly benefit the response.

[1] As the Principal Investigator was also preparing the draft Manual on whirling disease, where relevant this disease was also discussed to minimise the time required by stakeholders to attend these workshops.

The Manual also highlighted the fact that VHSV isolates can be categorised into a number of different groups, and not all these groups contain isolates that cause disease. This is important to remember when considering a control option.

Having this Manual available benefits the private sector that farm commercially susceptible species by potentially minimizing the impact of the disease on such industries should an outbreak occur. The exact value of this benefit is difficult to calculate, but as value of these industries grow, so too does the potential impact.

Having this Manual should also minimize the impact of the disease on potentially susceptible wild finfish species e.g. trout and native fish populations.

Further Development

This Manual is a working document. It is recommended that it be reviewed on a regular basis to ensure information is kept up to date. Ideally the Manual would be reviewed annually.

It is the opinion of the Principal Investigator that the current format of the Aquavetplan manuals should also be reviewed.

The manual has been prepared for formal endorsement by Australia's Aquatic Animal Health Committee and Primary Industries Standing Committee.

Planned outcomes

The Project's outputs have contributed to the planned outcomes as identified in **Benefits and Adoption** above.

Conclusion

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. VHSV can cause disease in other fish species inhabiting both the marine and freshwater environment and has caused significant mortality and economic loss overseas.

VHSV has also been isolated from many fish species in which there were no signs of disease.

There are fish species in Australia that are known to be susceptible to VHSV, and many species where susceptibility is uncertain. VHSV has never been isolated in Australia, and it is hoped that VHSV never will be isolated here. If it is isolated, there now is a Manual that should ensure a far more rapid, comprehensive and accepted approach to control of the disease, and minimise its impact.

KEYWORDS: **Viral Haemorrhagic Septicaemia, VHS, VHSV, Strategy Manual, Control**

Appendix A:

Basic agenda of workshops held with relevant industry and government stakeholders to discuss whirling disease and Viral Haemorrhagic Septicaemia (VHS)

10.00AM Welcome and a brief summary of the Aquavetplan manuals, and how they fit into the Commonwealth Aquaplan strategy (PH-S)

10.15AM Summary of the current status and strategy in the State to fish health emergencies (local speaker)

10.30AM Brief summary of Whirling Disease and consideration of 2 scenarios where Whirling Disease is isolated in the State:

1) Confirmation of *M. cerebralis* myxospores in Atlantic salmon that have been routinely sampled for export purposes at a commercial hatchery. No evidence of clinical signs of disease.

2) Anglers reporting deformities in wild trout in one of the States lakes, and further investigation confirming the presence of *M. cerebralis* myxospores in the cartilage of these affected trout.

Areas to consider are:

- Eradication OR
- Containmnent, control and zoning OR
- Control and mitigation of disease.

What would you like to see happen in this State?

11.30AM Brief summary of Viral Haemorrhagic Septicaemia and consideration of 2 scenarios where VHSV is isolated in this State:

1. VHSV isolated from Atlantic salmon in saltwater as part of routine testing – no clinical signs of disease. For the purposes of this discussion the area of isolation was given as a particular region in the State.

2. VHSV isolated from Atlantic salmon broodstock in freshwater at spawning – accompanied by some haemorrhaging. Some morbidity and mortality.

Again areas to consider are:

- Eradication OR
- Containmnent, control and zoning OR
- Control and mitigation of disease.

What would you like to see happen in this State?

12.30PM Wrap up and lunch.

Appendix B:

Aquatic Animal Health Subprogram – Viral Haemorrhagic Septicaemia (VHS) – A Disease Strategy Manual

AUSTRALIAN AQUATIC ANIMAL DISEASE EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy Manual

VIRAL HAEMHORRHAGIC SEPTICAEMIA

CONTENTS

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 pallasi*). 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 10ºC. Mortality and morbidity has rarely been documented when water temperatures are above 15ºC. The virus is also rarely isolated from fish living in waters above this temperature.

Serotyping, genotyping and challenge trials have confirmed significant differences in both structure of the genome and virulence between the different VHSV isolates. Separation of isolates into distinct genogroups or types is predominately based 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 World Organisation for Animal Health (Office International des Epizooties, or OIE). 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 (Viral Haemorrhagic Septicaemia Virus –VHSV) belonging to the genus of *Novirhabdovirus* within the *Rhabdoviridae*. 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 with a diameter of approximately 60 nm and length around 180nm. VHSV has been noted as being extremely fragile (Cohen and Lenoir, 1974 cited in Wolf, 1988). The membrane glycoprotein of the envelope pf VHSV is the major neutralising surface antigen. Figure 1 is a depiction of a typical rhabdovirus.

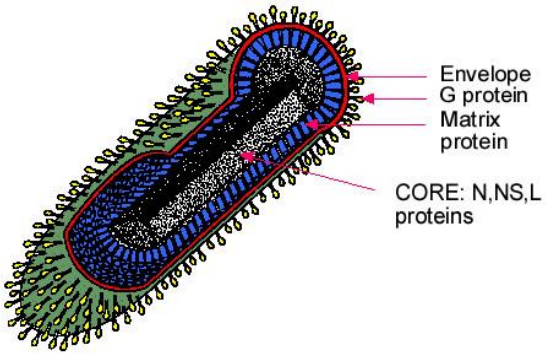


Figure 1 - schematic of a typical rhabdovirus (courtesy of G.Traxler)

Using both serotyping and genotyping, significant differences between VHSV isolates from different regions have been documented in the literature. Categorisation of isolates is still 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 species*).

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

  Contains isolates considered less pathogenic to 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 pallasi*). 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 or North Sea isolates.

Grouping of genotypes is generally geographic in nature. 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 (e.g. age, strain) and environmental variables (e.g. 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 0.3-3g (Smail 1999). With respect to viral isolates from the other genotypes, isolates from both **Type II** and **Type III** can cause disease and mortality in fish (e.g. Turbot and Pacific herring, respectively).

The range of fish species from which VHSV has been isolated both with and without signs of disease is still growing. According to the OIE (2003) the virus has been isolated from at least 45 different species living in both marine and freshwater environments. A list of fish species from which VHSV has been isolated (not necessarily accompanied by clinical signs of disease) and in which clinical signs of disease has been observed is given in Appendix A.

It is difficult to predict how VHSV might manifest in a new ecosystem or region. Predicting effects if introduced into Australia is speculative given variation in species susceptibility and viral strain effects. For example, a marine isolate of VHSV increased in virulence after a number of passages through rainbow trout (Snow and Cunningham, 2000). This reinforces the fact that RNA viruses are considered highly mutable, and capable of shifts in pathogenicity (Steinhauer and Holland, 1987).

1.3 World distribution and occurrence in Australia

VHSV :

- 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 enzootic in these regions;
- Has been isolated from many marine fish species in the North American part of the Pacific Ocean (from Alaska down to California), the North Atlantic, 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.

Wolf (1988) notes "one cannot assume…that VHS does not occur in countries where it has not yet been reported". The lack of finding VHSV in negative regions may be as much a reflection of the nature and intensity of surveillance for the virus as it is a reflection of the true epidemiology of the virus.

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 *(S. 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 species is often lethal, due to the impairment of the osmotic balance. This occurs within the clinical context of oedema and haemorrhage.

Acute:

In the acute form, VHS can cause rapid onset of mortality. Experimentally, in rainbow trout the acute stage occurs between days 2 - 30 post infection at 8-12ºC. Clinical signs associated with this stage include:

- Pale gills with or without petechiae;
- Peracute flashing or corkscrewing;
- Lethargy, darker than normal colouration, with fish confined to edges of pond/cages;

Chronic:

Clinical signs may not be associated with the chronic form of VHS. The virus can though be isolated from all major internal organs.

Nervous:

In this stage there are very marked aberrations of swimming behaviour i.e. constant flashing, tail-chasing and spiralling and is associated with tropism of the virus to 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 *(S. maximus),* a nervous form of the disease has not been observed in these species (David Smail, Senior Virologist, Marine Laboratory, Aberdeen, personal communication).

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:

- Haemorrhage at the base of fins, skin and in the eyes;
- Exophthalmia (due to subretinal haemorrhaging);
- Markedly distended abdomen (due to ascitic fluid +/- blood).

Internal:

- Swelling and paleness of liver;
- Swollen kidneys which appear darker red in the early stages of disease (especially in the anterior kidney). As the disease progresses head and midsection may be totally necrosed in dead fish though this sign is inconsistently reported in the literature;
- Bloody ascitic fluid surrounding abdominal organs;
- Oedema within musculature;
- Loss of appetite
- Lack of food in gastrointestinal tract.

1.4.2 Pathology

A prominent feature of this disease 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. pallasi*) (Kocan *et al* 1997, Smail 1999, Isshiki *et al* 2001, Munro 1996). Petechial/ecchymotic haemorrhaging has been observed in the peritoneum, on the swim bladder, adipose tissue, sexual organs, surface of 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.

1.4.2.1 Histopathology:

Haemorrhaging is a feature of the disease. 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 necrotized (Isshiki 2001).

Importantly, pancreatic tissues show fewer and less destructive changes than other organs. This is in contrast to infectious pancreatic necrosis (IPN) and infectious haematopoietic necrosis (IHN), 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).

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

1.4.2.2 Haematology:

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

1.4.3 Laboratory tests

1.4.3.1 Laboratory diagnosis

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 to the Australian Animal Health Laboratory (AAHL) for exotic disease testing. AAHL is located in Geelong, Victoria.

The screening procedure for VHS is based mainly on virus isolation in cell culture. Confirmatory testing is by immunological virus identification e.g. neutralisation, immunofluorescence, enzyme-linked immuno-sorbent assay (ELISA) and immunoperoxidase staining or by reverse-transcriptase polymerase chain reaction (RT-PCR) based 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, 2003b).

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

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

Currently, the only laboratory capable of confirming VHSV in Australia is AAHL. Methods used for the detection and identification of VHSV at this laboratory and procedures for the correct submission of specimens are given in Appendix C.

The presence of VHSV can be confirmed in a submitted sample within days depending on the original virus titre in the sample. Determining the genotype to which the isolate belongs can also be done within days. Genotyping can help to more quickly determine where the isolate may have come from and significantly help an epidemiological investigation.

Carriers of VHSV can be difficult to detect and are of major concern with 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, VHSV could not be isolated from the same infected population during spring and autumn (Vestergard Jørgensen 1982a).

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

1.4.3.2 Detection of VHSV in the environment:

VHSV can be cultured from both fresh and saltwater, however such isolation can be difficult due to the large dilution factors. Methods have been developed to concentrate

water samples to increase the sensitivity of virus isolation (in this case IHN) (Mulcahy *et al* 1983, Watanabe *et al* 1988).

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 the freshwater or marine environment) together with petechial haemorrhaging in tissues such as liver and muscle. Neurological signs, such as spiralling, would reinforce the urgency of submitting samples for laboratory testing.

Table 1 shows a number of important differential diagnoses for this disease.

Table 1 Differential Diagnoses

Disease/Disorder	Pathogen	In Australia?	Fish species affected	Clinical signs	Diagnosis
Enzootic Haematopoietic Necrosis (EHN)	EHNV	Yes	Redfin perch, salmonids	Haemorrhage, necrosis, epizootics in redfin	Cell culture/immunodiagnostic/ histopathology, PCR
Infectious Haematopoietic Necrosis (IHN)	IHNV	No	Salmonids	Haemorrhage	Cell culture/immunodiagnostics/ histopathology, PCR
Infectious Pancreatic Necrosis (IPN)	IPNV	No	Salmonids,	Extended abdomen, spiraling, high mortality	Cell culture/immunodiagnostics/ histopathology, PCR
Bacterial septicaemia	Generally gram negative bacteria	Yes	All	Lethargy, reddening, ulcers/abcesses	Bacterial isolation associated with clinical signs
Infection with rickettsia-like organisms (RLOs)	*Rickettsia-like organism*	Yes	Salmonids	Congestion, petechiation, anaemia, ascites	Clinical signs, PCR
Whirling Disease	*Myxobolus cerebralis*	No	Salmonids (esp. rainbow trout)	“whirling”, deformities esp. rainbow trout	Identification of myxospore in cartilage, PCR
Electrocution	Not applicable	Yes	Any	Significant haemorrhaging in musculoskeletal system	History, skeletal pathology
Osmotic stress	Not applicable	Yes	Post transfer salmon smolts	Bloody ascitic fluid	History of recent transfer/non culture of pathogens

1.5 Fish defence mechanisms, resistance and immunity potential

1.5.1 Innate immunity

Innate defence mechanisms include:

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

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.

IFN production has clearly been demonstrated in rainbow trout exposed to VHSV; production peaks at around 3 days post 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 Active immunity

Survivors of VHS have been shown to be resistant to reinfection. Neutralizing antibodies have been demonstrated in recovering trout. This antibody response can take a variable time to develop. In 130g trout, the response time was approximately 4-10 weeks (Olesen and Jørgensen, 1986, Olesen *et al* 1991). Temperature has a profound effect on the development of active immunity. It has also been postulated that VHSV antibodies are both neutralising (i.e. antibodies reacting with a few epitopes on the glycoprotein of the virus) and non-neutralising (i.e. antibodies directed against virus protein) and that non-neutralising antibodies persist in fish for a longer time than neutralizing 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 entry and incubation period

Natural infections occur by horizontal transmission of waterborne virus. The virus gains entry through the gills of the fish (Neukirch 1984) and possibly skin (Yamamoto *et al* 1992). Multiplication may take place at the site of entry; alternatively it may pass through without primary multiplication. This 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 post 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 (e.g. skin, mucus, gills) in clinical diseased fish (e.g. ulcers in fish such as cod and haddock). Unlike infectious pancreatic necrosis virus (IPNV), there is no shedding of VHSV from carriers for much of the year.

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.

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

- Temperature (as commented above);
- Salinity;

- Solar radiation;
- Presence of chemical pollutants;
- Bacterial antagonism; and,
- Suspended solids.

VHSV can survive in both the 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 (IHNV), a related fish rhabdovirus, survived longer in freshwater at 15°C (25 days for a 3-$\log_{10}$ reduction) compared to saltwater 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 VHSV titre in untreated seawater compared to sterilised or filtered (0.22μm) 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 this virus occurs (Wolf, 1988). Adequate disinfection with an iodophore will rapidly inactivate any virus adherent to the egg surface.

Birds carrying infected fish can spread the virus from farm to farm. 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 (e.g. pilchards) would be susceptible to infection with this virus, and could become carriers.

1.6.5 Factors influencing transmission:

1.6.5.1 Age:

Age is a significant factor in determining severity of disease in rainbow trout. Fish weighing 0.3-3.0g are most susceptible. Mortality at 9-12°C in fish of this age with virulent isolates of VHSV is 80-100%. In fingerlings and growers mortality is significantly lower, given the same conditions (Smail, 1999).

1.6.5.2 Temperature:

VHS is considered a cold-water disease, with a temperature range of 2-12°C. Transmission of the virus readily occurs over this range. Temperatures above 15°C are inhibitory to virus growth. However, Castric and de Kinkelin (1984) conducted research suggesting an upper threshold for *in vivo* infections of marine fish between 18 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 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).

1.6.5.3 Exposure dose:

Exposure doses that have caused clinical disease in rainbow trout, turbot and herring have been quoted in the literature. Units of measurement vary e.g. $10^{3.5\text{-}4.5}$PFUml^{-1} , 10^{5}TCID$_{50}$ml^{-1}(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 be used as a very rough guide only.

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

1.6.5.4 Species:

Stone *et al* (1997) suggested that perhaps all marine fish are susceptible to infection with VHSV. While there may be no accompanying disease, it is assumed that if VHSV was introduced into Australia, there is a broad range of temperate marine and freshwater fish species in this country from which would be susceptible to infection. Experimentally there is evidence that some fish species are refractive to infection (see Appendix A). As not all strains of VHSV were used in these trials, caution must used when considering the susceptibility of any species of fish to infection with VHSV.

1.6.6 Chemical/physical treatments:

VHSV is a relatively fragile virus, and is quickly inactivated by chlorine and iodophor disinfectants. The following summarises the potential disinfectant and dose rates that will inactivate this virus:

Physical agents[1]:

Heat:	VHSV is completely inactivated at 45°C for 60 minutes or 60°C for 15 minutes
UV	Water must be treated to a dose of 1-3 X $10^{3}\mu$W s cm^{-2}

Chemical agents:

Chlorine	Complete inactivation in less than 5 minutes at a dose rate of 200mg/L*
Iodine	Complete inactivation in less than 5 minutes at a dose rate of 25mg/L*
NaOH	Complete inactivation in less than 5 minutes at a dose rate of 10g/L*
Quaternary ammonia	Complete inactivation in less than 5 minutes at a dose rate of 10mg/L*

* depending on conditions such as amount of suspended solids, salinity, organic load

[1] Ozone is also likely to inactivate the virus. Specific dose rates will be included when available.

2 Principles of control and eradication

2.1 Introduction

VHSV has caused significant mortality and economic loss in cultured and wild fish species overseas. There are fish species in Australia that are known to be susceptible to this disease, and many species where susceptibility is uncertain.

The first line of defence against importing VHSV from another country is the continued implementation of preventative customs and quarantine measures. These measures are reviewed on an on-going basis to ensure they are appropriate to changing circumstances, such as changes to end-use practices or health status of source populations.[2].

If VHS occurs in Australia, aquatic animal health authorities could be faced with any of the scenarios listed in **Appendix B**. Scenarios range from:

- The isolation of VHSV from wild fish showing no clinical signs of disease; to
- An outbreak of clinical VHS in farmed and wild rainbow trout.

Each scenario may require a different control strategy. This will be influenced by the circumstances surrounding the isolation of VHSV. The list in Appendix B is not exhaustive and takes no account of the different strains of VHSV. Rather the list is there to provide the reader with some idea of the scope of possible incursions.

2.2 Methods to prevent spread and/or eliminate pathogens

There are essentially three disease control strategies that could be adopted if VHSV were isolated in Australia:

1. *Eradication*

   The scale of eradication may be national (e.g. eradicate VHSV from Australia) or local (e.g. eradicate VHSV from a local trout farm).

2. *Containment, control and zoning*

   This includes measures to exclude VHSV from defined geographic areas and unaffected populations (e.g. by quarantine) and containment of the virus to areas with enzootic infection.

3. *Control and mitigation of disease*

[2] The reader is referred to The Australian Quarantine Inspection Service (AQIS) 1999 Import Risk Analysis (IRA) on Non-Viable Salmonids and Non-Salmonid Marine Fish (http://www.affa.gov.au/corporate_docs/publications/pdf/market_access/biosecurity/animal/finalfinfish.pdf) and to the more recent AFFA Biosecurity memo 2003-15 - *Importation of pilchards (Sardinops sagax) for direct introduction into natural waters. Biosecurity policy review of viral haemorrhagic septicaemia virus (VHSV)* (http://www.affa.gov.au/corporate_docs/publications/word/market_access/biosecurity/animal/2003/2003-15a.doc) for further information in this area.

Measures are aimed at managing the frequency and severity of disease episodes in infected populations to within acceptable levels.

The basic principles of eradication and other control responses are described in the AQUAVETPLAN **Enterprise Manual** and the AQUAVETPLAN **Control Centre Management Manual**. The AQUAVETPLAN **Enterprise Manual** contains State/Territory legislation relating to disease control and eradication.

Control measures could involve any or all of the following measures:

- Early detection and identification of VHSV and whether there is 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 as quickly as possible whether virus is present in wild fish populations and the extent of such presence;
- Seizure, quarantine or destruction of infected fish (may not always be possible or warranted);
- Tracing, seizure and quarantine or destruction of potentially infected fish (may not always be possible or warranted);
- Movement controls over fish and fish products;
- Movement control 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;
- Prevention of viral spread (if possible) by controlling stock and water movement. This will not be possible if VHSV is isolated from wild marine fish;
- Conducting a publicity campaign.

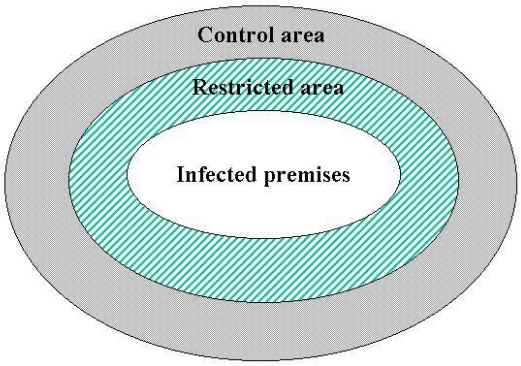
Water temperature is an important consideration with respect to propagation and spread of VSHV. In temperatures >15°C both virus shedding from infected fish and survival of the virus outside the fish host are significantly reduced.

2.2.1 Quarantine and movement controls

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

1. The establishment of specified areas[3] (see Figure 2) i.e.
    - *declared area* — includes restricted area and control area
    - *restricted area* — area around 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);

Figure 2 Establishment of specified areas to control VHS



2. Bans on the movement of live fish out of restricted areas into areas where VHSV is considered absent;
3. Restrictions or bans on releasing fish into river or freshwater lake systems or marine zones in designated areas;
4. Restrictions or bans on the movement of fish between different river systems and different marine zones in designated areas;
5. Restrictions or bans on the use and movement of equipment within and between marine and freshwater areas;
6. Controlling access of predators such as birds to potentially infective material (e.g. fish carcasses, hatchery tanks).

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;

[3] See AQUAVETPLAN **Enterprise Manual, Section A** for more details (http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=00499893-720A-4F46-9CB9D85A876398B7)

- 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 implementation 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 20km of the infected premises were deemed suspect and placed under movement controls. The virus was successfully eradicated from the farm (Munro 1996). The 20km radius was the point where virus concentration was less than one infectious virion per cubic metre of seawater (with the assumption that natural factors did not inactivate virus).

With Infectious Salmon Anaemia virus (ISAV), it has been found that the risk of infection increased by a factor of 8 if the site was situated closer than 5km to another ISA-positive site as compared to the risk if the site was more than 5km away (Jarp and Karlsen, 1997).

The extent that such restrictions are imposed should be weighed against the fact that the virus could already be widespread in the region in which it has been isolated. Therefore rapid determination of the nature and extent of the problem is important for the decision making process.

2.2.1.1 Zoning

Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN **Zoning Policy Guidelines**.[4] This document is based on terrestrial diseases, though many of the principles still apply.

Zoning may be possible if VHSV is isolated from a single culture facility or in imported fish. Zoning may also be possible if the virus has been carried into a freshwater environment from a marine source (e.g. through feeding of marine trash fish to freshwater cultured fish), even if it is known to be present in wild marine species.

If VHSV were to become 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.

2.2.1.2 Semi-open systems

There is virtually no control over the aquatic environment of semi-open systems. 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 fish kept in cages. The author has had experience in isolating VHSV from farmed Atlantic salmon (*Salmo salar*) coming from a cage in which there was a significant population of Pacific herring (*Clupea pallasi*). Overall morbidity and mortality in the salmon was not significant.

[4] http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717

The only way to prevent release of virus into the surrounding environment from infected fish in a semi open system is to remove the fish from the water.

2.2.1.3 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 are possible in theory for controlling the virus, but are usually not viable economically. In Scotland VHSV was successfully eradicated from a pump ashore tank farm. In this outbreak effluent water was disinfected prior to release (Munro 1996).

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

VHSV has been successfully eliminated from fish hatcheries in Denmark (Jørgensen 1980). The principle method used was to empty, disinfect and keep dry for at least one month all VHS-infected trout farms in a stream, starting at the top of the stream. The farms are then restocked with VHS-free fish. There was no destruction of wild fish.

2.2.1.4 Closed systems

It is possible to isolate a closed system, such as an aquarium. Hence preventing the spread of VHVS from that system is possible.

2.2.2 Tracing

Tracing of fish, fish products, people and equipment may be difficult depending on where VHSV is isolated. Some facilities culturing fish are involved in restocking programs where there is extensive movement of live fish. Other facilities move fish products daily to distant markets. Often very little is known of wild fish movements.

A thorough and comprehensive epidemiological investigation requires trained personnel capable of spending the time to conduct such an investigation.

Immediate tracing steps to aid in the epidemiological investigation include:

1. Tracing back all movements of the infected fish to help establish the origin of the outbreak. This will show whether the infected fish were exposed to VHSV at the current location, or had been imported into the infected location carrying the virus, or both;
2. Tracing forward all contacts with infected fish, premises and sites (to establish the current location and potential spread of infection) i.e. where did fish, water and equipment from the positive facility go within the period of infection/exposure?

Tracing should include:

- Fish e.g. broodstock, smolts, fish used for restocking purposes and harvested wild fish;

- Fish products - fish for consumption, effluent and waste products from slaughter and processing, especially skeletal elements.
- Water - input and output;
- Equipment/vehicles/personnel. VHSV is not a robust virus, so it would not be difficult to ensure these items do not carry live virus.

Diagnostic tools such as Polymerase Chain Reaction (PCR) may also be useful in identifying whether or not the VHSV isolated matches known overseas strains of the virus. This could help in determining the most likely route of entry of the virus.

2.2.2.1 Neighbouring fish populations

With respect to 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.

If VHSV is isolated from a freshwater facility, there may be the potential to quarantine such a facility to prevent the spread of the virus to both neighbouring farm sites and fish populations. All measures considered in section 2.2.1 would need to be considered. Wild fish must always be considered as potential carriers of the virus.

2.2.3 Surveillance

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

1. Field personnel
2. Laboratory personnel.
3. Administrative assistance.
4. Equipment and instruments.
5. Diagnostic reagents.

Confirmation of VHSV must be done at AAHL, Geelong. In the development of a surveillance and monitoring program, the capacity of AAHL to handle samples and conduct testing must be considered.

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

If destruction of fish were ordered it would be done to eliminate a major potential source of virus. The objective is to reduce the virus load in the surrounding environment and reduce the risk of infection of other wild or farmed fish.

Destroying fish is a major undertaking and needs to be carefully considered. There are currently no compensation schemes to cover any losses incurred in such destruction hence the farmer may have to bear the costs of such destruction. An industry (or the farmer) may decide that destruction of fish is worthwhile to protect the industry as a whole.

Destruction of wild fish is not feasible or practical. In North America, large numbers of Pacific salmon growing in enhancement hatcheries were destroyed after VHSV was isolated from fish in these hatcheries (Meyers and Winton 1995). Subsequently VHSV was determined to be enzootic in this region.

Destruction of large quantities of fish requires considerable resources to handle such large quantities of dead fish. For example, boats/trucks capable of effectively containing potentially infective material and composting/rendering facilities/burial sites to take the dead fish are required. Measures to minimize spread of virus to allow time to destroy and dispose of fish need to be implemented if possible. For more details see the **AQUAVETPLAN Operational Procedures Manuals: Destruction** and **Disposal.**[5].

2.2.6 Handling and management of fish products and by-products

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

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

Environmental conditions in many parts of Australia would not be suitable for the establishment of VHSV.

As virus can be very difficult to detect in carrier fish, it is expected that the virus titre in fish showing no clinical signs would be low. It may be possible to harvest and process those fish without signs of disease with minimal risk of spreading VHSV. In Scotland, in the control of an outbreak of VHSV in turbot market size fish were eviscerated on site, the viscera destroyed by burning, and the remaining carcass 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

VHSV is readily inactivated by a number of disinfectants (see section 1.6.6). Like all successful disinfection procedures, effective cleaning should precede the disinfection stage. Drying and sunlight will effectively destroy the pathogen in a matter of hours.

Potential sources of the pathogen are processing plants handling infected or exposed fish. 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 another rhabdovirus, IHNV, have been shown to be approximately 1.3-4.3x10^3 PFU/ml in processing water when the fish being processed were from an infected site. Such water requires disinfection prior to release into an aquatic environment (see Section 1.6.6).

Due to differences in farming enterprises, disinfection protocols of freshwater facilities may need to be determined on an individual basis. This will involve the farm manager, the State/Territory CVO and/or Director of Fisheries. The protocol should take into consideration factors outlined in Section 1.6, including:

- The source and location of infection;

[5] Destruction Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906
Disposal Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5

- The type of enterprise (e.g. facilities using well water versus those using groundwater from river, lake or stream);
- The design of the site and its proximity to other waterways;
- Current disinfection/treatment protocols;
- Environmental impact of the disinfectant protocol; and
- Availability of approved, appropriate and effective disinfectants.

2.2.8 Environmental considerations

Disposal of large numbers of dead fish can be the source of unpleasant odours, and if not covered be unsightly. Predators can transport infected fish to uninfected areas. In addition, all legislation and regulations concerning the disposal or discharge of chemicals and cleaning agents into the environment must be observed.

From overseas research virus replication is inhibited at temperatures above 15°C. Viral shedding and survival may be limited above this temperature. In summer freshwater and marine water temperatures in many parts of Australia are higher than 15°C.

2.2.9 Vaccination

There are currently no commercial vaccines available for VHSV.

2.2.10 Predator/scavenger 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. Hence suitable precautions should be taken to prevent birds and mammalian predators/scavengers (e.g. dogs) from accessing to infected fish. This includes disposal sites. In addition, predator fish and mammals can also remove infected material from sea cages.

2.2.11 Restocking measures

VHSV can infect many species. However, different 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. It will also only be considered once the initial outbreak has been dealt with.

2.2.12 Public awareness

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

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

A media kit should be distributed quickly to ensure the media can help reduce any potential public fears or perceived risks.

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. Though 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 that is taking responsibility for the investigation[6].

Some states (e.g. Victoria, Tasmania) have developed Manuals outlining what procedures would be followed in an outbreak of a disease such as VHSV. These Manuals also detail roles and responsibilities of the various organizations, departments and personnel in such an outbreak.

2.3 Feasibility of control in Australia

The following section discusses the feasibility of the control of VHSV if isolated in Australia. Feasibility will depend on the circumstances surrounding 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 the following:

(i) The infection is widespread
(ii) The outbreak has no point source and is unable to be contained; and,
(iii) Infection is present or potentially present in wild fish species in freshwater or marine environments.

This is due to:

- The ability of VHSV to spread and establish reservoirs of infection in wild fish populations;
- The ability of VHSV to infect many different species of fish in both freshwater and saltwater;
- The ability of VHSV to infect fish but remain undetectable;
- The lack of a full understanding of how the pathogen survives in the aquatic environment;
- The ability of infected wild fish to transmit and establish 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 (both semi-open and semi-closed systems); and experience from overseas that eradication of an aquatic pathogen is unsuccessful 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 e.g. 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, then the following must be considered:

[6] Whether or not local councils decide to remove dead fish from the beaches is outside the scope of this manual. This decision will need to be based on aesthetics and public health (from putrefying organic matter, not from the fact that VHSV is present) rather than on disease control grounds. Removal of large numbers of dead wild fish is unlikely to have any effect on controlling the spread of the disease.

2.3.1.1 Unexposed fish:

If there is doubt as to whether fish have been exposed to VHSV or not, they should be treated as exposed.

The principle behind the prompt removal of unexposed fish is to decrease the chance of spread of infection to these fish stocks and prevent propagation of the disease. If exposure to VHSV can be prevented, there is no risk of these fish becoming infected and hence no need for removal. If exposure to VHSV cannot be prevented, then these fish remain at risk of infection whilst they remain alive. If they do become infected, they will become multipliers of the virus.

2.3.1.2 Exposed or potentially exposed, clinically normal fish:

Destruction of fish immediately prevents further virus propagation. This is an option as it is very effective at decreasing the infectious load on a site and minimising the spread of infection. Normal or controlled grow-out is only an eradication option if there is no possibility that during the grow-out period that the pathogen will spread beyond the *declared area*.

Emergency harvesting may depopulate an area as quickly as destruction and removal of the fish[7] depending on the number of fish involved.

2.3.1.3 Clinically diseased fish:

Immediate removal, destruction and disposal of all diseased and dead fish is essential to the success of an eradication strategy. It is likely that in a given population of fish, a number will be showing some clinical signs of disease, and others will show no signs. In such situations all fish in the population should be treated as diseased. These fish, along with infectious waste, are the main source of VHSV in the environment. Burial sites should be chosen carefully to ensure there is no contact with waterways or predators.

2.3.2 CONTAINMENT, CONTROL AND ZONING

The detection of VHSV in wild fish from the marine environment 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 or not zoning is feasible.

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 practiced for many years without measures being taken to remove the wild fish populations (Jorgensen, 1974).

2.3.2.1 Unexposed fish

Control options for unexposed fish are the same as those outlined for eradication in Section 2.3.1. The implementation of a zoning program and associated control measures to maintain uninfected zones would be necessary.

[7] The author has had experience in destruction versus emergency harvesting of fish. Dead fish can be more difficult to remove from a facility than live fish.

2.3.2.2 Exposed or potentially exposed, clinically normal fish

A successful zoning program for farmed fish will rely on the implementation of movement restrictions on exposed or potentially exposed fish that prevent infection spreading to uninfected zones. The feasibility of implementing such a 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 several factors including movement restrictions required on fish, people, vehicles, boats and market access for the fish products and by-products.

In a declared area, normal or controlled grow-out and slaughter may be feasible without further spread of infection. Harvested fish must be processed to the degree required for the designated market. In fish that have been infected with VHSV, evisceration of fish will remove organs likely to have the highest titre of virus. Freezing and thawing fish products will reduce virus titre if present in that product.

2.3.2.3 Clinically diseased fish

These fish, along with infectious wastes, constitute the greatest risk for spreading the infection to uninfected zones. There are no treatments for these fish. Destruction is the only option to totally remove this risk. Fish that survive an outbreak can act as a source of infection for other fish.

2.3.3 CONTROL AND MITIGATION OF DISEASE

If VHSV is isolated, the chosen control strategy may simply be to reduce the frequency of existing disease to levels biologically and/or economically justifiable or otherwise of little consequence. 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 benefits derived. VHSV may be isolated from fish with no clinical signs of disease.

In North America, a long term management strategy where VHSV is enzootic was adopted (Meyer and Winton 1995). A committee [8] meets every 6 months to discuss fish disease issues and formulate cooperative disease policies when necessary.

VHSV is a single stranded RNA virus. RNA viruses show increased mutability compared with DNA viruses. If an isolate of VHSV is isolated in Australia and is associated with no signs of clinical disease, it is possible that further passage of such an isolate in a species such as Atlantic salmon *could* lead to an increase in virulence. Removal of all potentially infected fish from that location will limit the possibility of this happening. If fish are allowed to grow out for harvest, there should be a complete break between emptying a farm and restocking as this will help break the VHSV cycle in farmed fish.

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.

[8] The Pacific Northwest Fish Health Protection Committee (PNFHPC).

2.3.4.1 Export and domestic markets

VHSV is enzootic throughout much of Europe, in North America and Japan. VHSV is listed by the Office International des Épizooties (OIE, World Organisation for Animal Health). Isolating VHSV in Australia does not necessarily mean that trade in fish products will be seriously affected. Many overseas countries require imports such as fertilised fish eggs (embryos) to be certified free from VHSV. This may still be possible especially if such commodities originate in a VHSV free zone even though other parts of Australia are considered infected with VHSV.

It is likely that evisceration of fish prior to export will satisfy international trade requirements in fish harvested from a region positive for VHSV.

For the most current information regarding export market requirements, contact AQIS[9].

2.3.4.2 Domestic markets

A cautious approach is required for the salvage of exposed or potentially exposed product for the domestic market. Decisions regarding the release of fish or fish products to the domestic market will depend on the control strategy implemented. Evisceration will remove organs most likely to contain the highest titre of virus in infected fish. Environmental conditions in many parts of Australia would not be suitable for the establishment of VHSV.

Requirements for the release of exposed or potentially exposed fish product to the domestic market will be less stringent if VHSV becomes enzootic in Australia. If areas of Australia remain free of VHSV, restrictions may be advantageous to maintain freedom in such areas.

[9] http://www.affa.gov.au/outputs/quarantine.html

3 Preferred control policy in Australia

3.1 Overall policy for Viral Haemorrhagic Septicaemia

VHS is reportable in Australia and is an OIE listed disease.

The preferred policy is to eradicate VHSV if it were isolated in Australia, especially if clinical disease is associated with the outbreak(s). Depending on circumstances, this may not be feasible e.g. if the virus is identified in wild fish stocks.

If eradication is not feasible, the preferred policy will be:

- ***containment, control and zoning*** **to areas with enzootic infection, prevention of further spread and protection of uninfected areas; or**
- ***control and mitigation of disease*** **by implementing management practices that decrease the incidence and severity of the disease.**

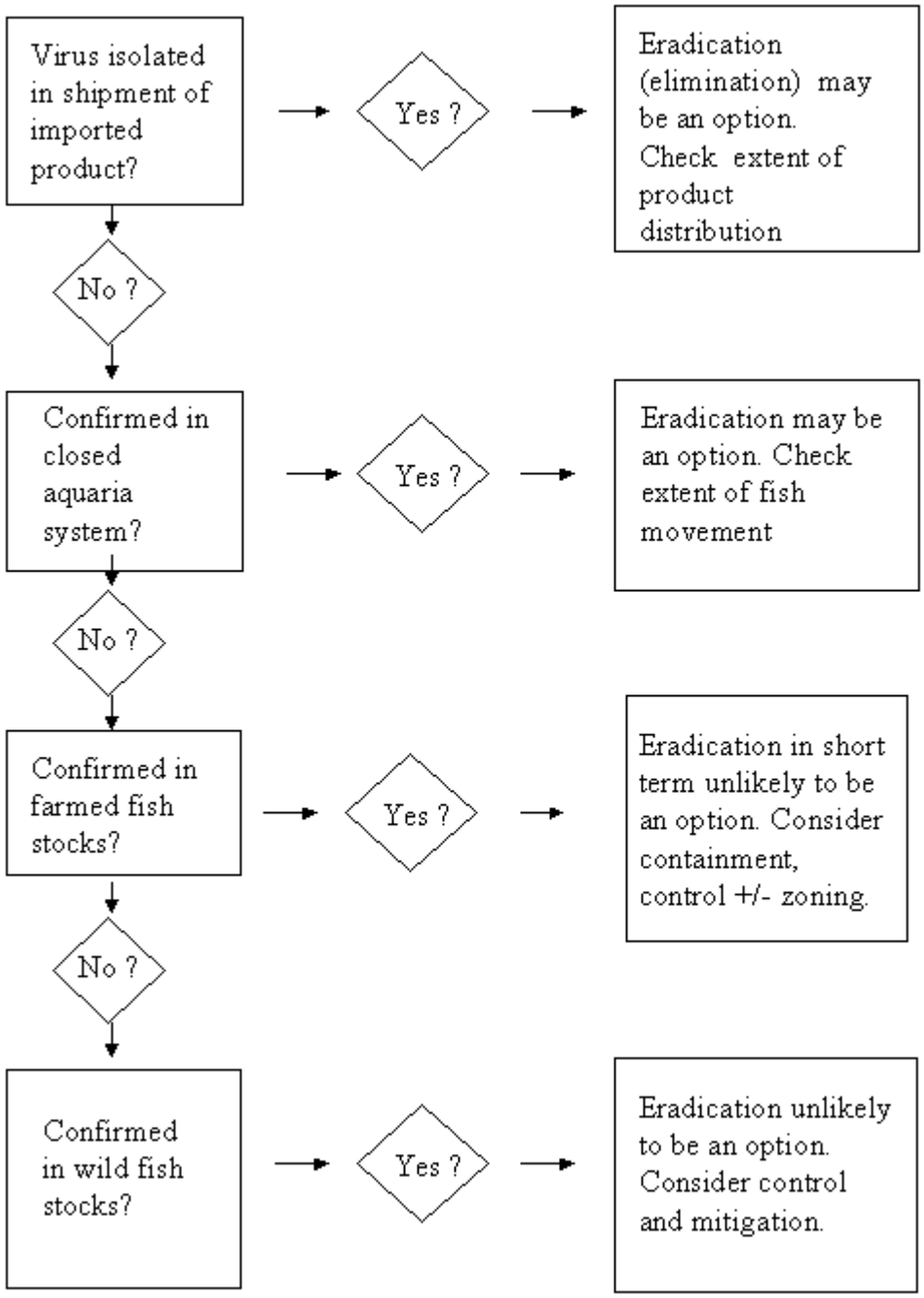
Epidemiological information on which to base a decision may initially be limited. The initial adoption of a policy does not preclude adopting a different policy as more information becomes available e.g. eradication may be chosen as a long term policy even when containment, control and zoning is chosen in the first instance.

The Director of Fisheries and/or the CVO of the State/Territory in which the disease occurs and/or virus is isolated will be responsible for deciding which control option(s) is chosen, and for implementing disease control measures in accordance with relevant legislation.

The Director of Fisheries and/or the CVO of the State/Territory in which the isolation(s)/outbreak(s) occurs will make ongoing decisions on follow-up disease control measures in consultation with the aquatic Consultative Committee on Emergency Animal Diseases (CCEAD), the State/Territory and Commonwealth governments and representatives of the affected industry(s).

The flowchart diagram depicted in **Figure 3** (page 28) gives **some** possible scenarios and is designed to help in the initial decision making process. This decision may need to be made on very limited epidemiological information. As more information becomes available, there may be modification to the initial response.

Figure 3 - Decision flowchart[10]



As indicated by this diagram, the three control options that may be chosen to control VHSV are:

[10] Note that in this flowchart "farmed fish" refers to fish farmed in semi open or semi closed systems

- *Eradication* — eradication of VHSV from Australia (highest level of control measure and cost).
- *Containment, control and zoning* — containment of the virus to areas with enzootic infection, prevention of further spread and protection of uninfected areas.
- *Control and mitigation of disease* — the implementation of management practices that decrease the incidence and severity of clinical outbreaks (lowest level of control measure and cost).

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* where possible of predators and/or scavengers (e.g. birds) gaining access 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* where possible to define and maintain infected and VHS free zones;
- *restocking* with older, less susceptible fish or less susceptible species unlikely to develop clinical disease;
- *a public awareness campaign* to facilitate cooperation from aquaculturalists and the community.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel during the various stages of activation upon suspicion of an incidence of VHS in Australia, refer to the AQUAVETPLAN **Control Centre Management Manual**.

3.2 Initial response

While determining the extent of the outbreak(s) or spread of virus and which overall control strategy option to adopt, the following measures should be taken to minimise or prevent further impact or spread of disease.

3.2.1 Epidemiological investigation

A comprehensive epidemiological investigation, including tracing and surveillance, will be initiated immediately on suspicion or confirmation of VHSV in Australia. This investigation will determine:

(i) 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.

(ii) How widespread the virus may be, both geographically and biologically (e.g. the range of susceptible fish species that may be infected). Prior to adequate data becoming available, it should be assumed that any fish populations that potentially have been exposed to the virus have been;

(iii) How to prevent any further virus spread from the infected location if this 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 an epidemiological investigation, and assessing the results of such investigations. Focus should be on fish populations resident in waters where temperatures are less than 15°C.

3.2.2 Quarantine and movement controls

Quarantine and movement controls must be implemented on anything capable of transmitting the virus (see section 2.2.1).

Control areas (Section 2.2.1) should be established if the virus or the disease has been isolated from fish in an area conducive to control. It should be acknowledged that only limited epidemiological information on which to make a decision may be available. Control area boundaries can be refined as more information becomes available.

3.2.3 Treatment of fish

There are no treatments for VHSV so this is not an option.

3.2.4 Vaccination of fish

There are currently no commercially available vaccines for VHSV.

3.2.5 Destruction of fish

The decision to destroy fish must be made based on the circumstances surrounding the outbreak(s).

It is not possible to clearly define when and when not fish should be destroyed. Virus shedding from clinically diseased fish is likely to be high. An outbreak(s) associated with significant clinical disease may warrant humane destruction of fish. Such destruction should ensure no further spread of the virus, and will help to protect other neighbouring fish populations. If the outbreak occurs in natural waterways, it is possible that by the time a control strategy is implemented the virus is already present in wild fish populations. Humane destruction of cultured fish in this circumstance will minimise virus loading in the area, but not eliminate virus. See the **AQUAVETPLAN Operations Procedure Manual - Destruction** for details[11].

The decision on whether or not to destroy unexposed or potentially exposed fish showing no clinical signs will again depend on the circumstances surrounding the outbreak(s).

3.2.6 Treatment of fish products and by-products

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

Harvested fish can be safely frozen until a definitive diagnosis is made on whether the fish are infected. The freeze/thaw cycle will reduce virus titre. Harvested fish should be eviscerated to remove organs likely to contain the highest titre of virus.

A decision on what to do with these fish will depend on which control option is chosen (see section 2.2.5).

[11] http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906

Any harvesting or processing equipment used must be treated as contaminated and disinfected accordingly (see AQUAVETPLAN **Operational Procedures Manual: Disposal**[12]

3.2.7 Predators/scavengers

To limit spread of the virus in the initial response effective predator control, to prevent predators and scavengers (e.g. birds, rodents) eating or carrying infected carcasses away from the infected premise(s), is essential.

3.2.8 Education and media

When investigating an outbreak, developing and maintaining good public relations is critical. Diseases such as VHS have the potential for sensationalism, especially considering the significant coverage the disease has had in Europe. Promoting the true facts about the disease and the outbreak(s), in a clear and concise way, is important to minimise this and the time required to deal with media. This is time that could be better spent controlling the outbreak. It is important reassure the public that the authorities are taking all measures to control the situation.

It must be clearly stated that VHSV is not considered to be a health risk for humans.

3.3 Control options

The preferred control option to be adopted will be decided following or during the initial response to the outbreak of VHS and/or isolation of VHSV. While it is important to be decisive in the initial choice, it is also important that the decision is dynamic. The policy must be able to evolve with changing circumstances as more information becomes available. Below are some key criteria for the adoption of a policy option. These criteria are not exhaustive, and are given to serve as a guide.

3.3.1 Option 1 - Eradication

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

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

If VHSV is isolated in imported product and there has been limited or no further product distribution[13] eradication is also the preferred option.

For full details of measures to be taken if eradication is chosen as the preferred control option, see Section 2 of this manual.

3.3.2 Option 2 - Containment, control and zoning

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

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[12] http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5

[13] For example, isolating VHSV in a shipment of imported pilchards will possibly be a scenario where eradication is less feasible than isolation of VHSV in imported rainbow trout fillets.

1. VHSV is isolated from wild or farmed fish confined to a specific geographical location – this may take a comprehensive monitoring and surveillance program to determine.
2. There is clinical disease associated with the outbreak(s) but it is determined that the outbreak(s) is confined to a specific geographical region where virus containment is possible and when eradication is not considered an option.
3. VHSV is isolated in product that has a limited distribution (e.g. wild fish imported for use in a specific geographical location).

Where containment, control and zoning is chosen as the initial option, it is possible that this may evolve to control and mitigation of disease.

For full details of measures to be taken if containment, control and zoning is chosen as the preferred control option, see Chapter 2 of this manual.

3.3.3 Option 3 - Control and mitigation of disease

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

1. VHSV is considered widespread in wild fish stocks and/or farmed fish stocks and distributed widely in an area(s) where zoning would be difficult.
2. There is no possibility of limiting the spread of the virus.

For full details of measures to be taken if control and mitigation of disease is chosen as the preferred control option, see Section 2 of this manual.

3.4 Trade and industry considerations

Australia's status with respect to VHSV will change if the virus is isolated here.

3.4.1 Export markets

If VHSV is isolated in Australia it will be reported to the OIE. Industries exporting fish products such as fertilised eggs will need to confirm the requirements of importing countries with respect to Australia being declared VHSV positive. Exported fish product from temperate areas of Australia that is exported is usually eviscerated during processing so there should be no impact on the export of this product.

Increased monitoring and surveillance with comprehensive sampling of fish populations[14] within affected industries may be required to satisfy freedom of disease requirements for the importing countries.

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

For the most current information regarding export market requirements, contact AQIS[15].

[14] The main industry exporting eggs already has a comprehensive monitoring and surveillance program in place.

[15] http://www.affa.gov.au/outputs/quarantine.html

3.4.2 Domestic markets

A cautious approach is required for the salvage of exposed or potentially exposed product for the domestic market. Decisions regarding the release of fish or fish products to the domestic market will depend on the control strategy implemented.

3.5 Criteria for Proof of Freedom

Proof of freedom from VHSV may be important for trade. Proof of Freedom can be given at the aquaculture establishment, zone and country level. Criteria for Proof of Freedom at each level are given in the World Animal Health Organisation, or OIE (Office Internationale des Epizooties) Aquatic Animal Health Code (OIE, 2003a).

3.6 Funding and compensation

Currently there is no requirement for compensation to be paid to farms where destruction of fish is ordered. This is being reviewed.

APPENDIX A

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

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

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

Rainbow trout *(Oncorhynchus mykiss)*
Pike *(Esox lucius)*
Turbot *(Scophthalmus maximus)*
Pacific herring (*Clupea pallasi)*
Pacific sardine *(Sardinops sagax)*
Pacific hake (*Merluccius productus)*
Japanese Flounder *(Paralichgthys olivaceus)* (hirame)

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

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

* this does not signify that these species are not susceptible to other VHSV isolates

APPENDIX B

Possible scenarios for isolation of VHSV in Australia

1.	VHSV isolated from Atlantic salmon/rainbow trout in **saltwater** as part of routine testing – no clinical signs of disease
2.	VHSV isolated from Atlantic salmon/rainbow trout in **freshwater** as part of routine testing – no clinical signs of disease
3.	VHSV isolated from Atlantic salmon/rainbow trout in **freshwater** – accompanied by haemorrhaging. Increasing morbidity and mortality.
4.	VHSV isolated from Atlantic salmon/rainbow trout in **saltwater** – accompanied by haemorrhaging. Increasing morbidity and mortality.
5.	VHSV isolated from imported pilchards or baitfish
6.	VHSV isolated from wild fish around tuna/yellowfin cages
7.	VHSV isolated from tuna(SBT/YFT*) showing no clinical signs of disease
8.	VHSV isolated from tuna (SBT/YFT*) showing morbidity and mortality
9.	VHSV isolated from pilchards during a pilchard die-off
10.	VHSV isolated from wild marine fish besides pilchards – no clinical signs of disease
11.	VHSV isolated from wild marine fish besides pilchards – clinical signs of disease
12.	VHSV isolated from wild freshwater fish – no clinical signs of disease
13.	VHSV isolated from wild freshwater fish – clinical signs of disease
14.	VHSV isolated from imported species of aquarium fish

* Southern Bluefin Tuna/Yellowfin Tuna

APPENDIX C

Detection and identification of viral haemorrhagic septicaemia virus (VHSV)

The following methods are used for the detection and identification of viral haemorrhagic septicaemia virus (VHSV) at CSIRO Australian Fish Diseases Laboratory (AAHL).

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 (for transport of specimens to Geelong) specimens will then be forwarded to the AAHL, for exotic disease testing.

Collection of tissue samples is done according to international protocols and is summarised below.

Tissues or fluids from affected fish may be pooled in one container containing transportation medium at a ratio of 1 part tissue (weighing a minimum of 0.5g) to 5 parts medium, representing one pooled sample. Pooled tissues may be stored (on ice but not frozen) in transportation medium during transportation to AAHL.

Samples should not be frozen prior to processing but should be maintained between 4-10^{O}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 storage must be at 4^{O}C. Samples to be assayed after 72 hours post-collection should be frozen in the temperature range -70^{O}C to -80^{O}C.

Tissues to be examined will be dependent 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:

Fish size (length)	Tissues
<4cm	**entire fish (remove yolk sac if present)**
4-6cm	**entire viscera including kidney**
>6cm	**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, AAHL Fish Diseases Laboratory 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, 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 which are then incubated at $15^{O}C$ over a period of several days to allow the development of any viral cytopathic effect (CPE) 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 which will bind to specific epitopes if present. Excess primary antibody is removed by washing and a secondary biotinylated antibody (e.g. biotinylated anti-rabbit Ig if the primary antibody was raised in rabbits) is added. After an incubation period excess secondary antibody is removed by washing and streptavidin-peroxidase conjugate is added. Following incubation, excess conjugate is removed by washing, a substrate (e.g. AEC) 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 which is recognised by the primary antibody will yield a positive colour reaction (Crane *et al*., 2000).

Immunohistochemistry

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

PCR

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

Nucleic acid is obtained from cell-free samples using the QIAamp Viral RNA extraction kit (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 was 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.

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GLOSSARY

AQUAVETPLAN	A series of documents that describe the Australian response to exotic aquatic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
AUSVETPLAN	A series of documents that describe the Australian response to exotic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
Control area[a]	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.
Dangerous contact premises or area[a]	That which 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]	An area that has been subjected to a legal declaration and includes both a restricted area and a control area.
Decontamination	Includes all stages of cleaning and disinfection.
Disinfectant	An agent used to destroy microorganisms outside a living animal.
Disposal	Sanitary removal of fish carcases 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.
ELISA	Enzyme linked immunosorbent assay — 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.
Exophthalmia	Protrusion of the eyeball from the orbit, caused by disease or injury.

Fish by-products	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[a]	An area known to be free of the disease agent.
Haemorrhage	The escape of blood from a ruptured vessel, causing bleeding.
Hyperaemia	An excess of blood in an area.
Inappetence	Lack of appetite.
Infected premises or area[a]	The area in which the disease has been confirmed. Definition of an 'infected area' is more likely to apply to an open system such as an oceanic lease.
Leukocytopenia	Abnormally reduced numbers of white cells (leukocytes) in the bloodstream.
Mitigation	Reduction in severity, eg mitigation of the impact of disease is to decrease the severity of the impact of the disease.
Movement control	Restrictions placed on movement of fish, people and things to prevent spread of disease.
Petechial haemorrhage	Tiny flat, red or purple spots in the skin or mucous membranes caused by bleeding from small blood vessels.
PCR	A diagnostic technique involving the production of millions of copies of a specific target DNA segment *in vitro*.
Premises or area[a]	Production sites that may range from an aquarium to an aquaculture lease in the open ocean.
Quarantine	Legal restrictions imposed on a place, fish, vehicle, or other things, limiting movement.
Restricted area[a]	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.
Sentinel fish	Fish of known health status monitored for the purpose of detecting the presence of a specific exotic disease agent.
Septicaemia	The invasion and persistence of pathogens in the bloodstream.

Serosanguineous fluid	Fluid composed of blood and serum.
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 species	Fish that can be infected with the disease.
Suspect premises or area[a]	Where the emergency disease is suspected but not yet confirmed. The reason for the suspicion varies with the agent, however it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, people or things that may be implicated in the spread of disease.
Vector	A living organism that transmits an infection from one host to another. A *biological* vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A *mechanical* vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.
Zoning	The process of defining disease-free and infected zones.
Zoonotic disease	Disease transmissible from animals to humans.

[a]Due to the nature of the aquatic environment and of aquatic animal disease, these areas may be difficult to define and may need to be revised as further information is obtained about the nature of the agent and the extent of the disease.

ABBREVIATIONS

AAHL	Australian Animal Health Laboratory
AFDL	AAHL Fish Diseases Laboratory (formerly AFHRL)
AFHRL	Australian Fish Health Reference Laboratory
AQIS	Australian Quarantine and Inspection Service
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	Chief Veterinary Officer
ELISA	enzyme linked immunosorbent assay
IFAT	indirect fluorescent antibody test
IHN	infectious haematopoietic necrosis
IPN	infectious pancreatic necrosis
ISA	Infectious Salmon Anaemia
OIE	Office International des Épizooties (World Organisation for Animal Health)
PCR	polymerase chain reaction
PFU	Plaque Forming Unit
SCFA	Standing Committee on Fisheries and Aquaculture
TCID	Tissue Culture Infective Dose

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