

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

Viral encephalopathy and retinopathy

Version 1.0, 2004

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

Primary Industries Ministerial Council

This disease strategy forms part of:

AQUAVETPLAN Edition 1

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

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IMPORTANT NOTE: Important regulatory information is contained in the OIE International Aquatic Animal Health Code for viral encephalopathy and retinopathy, which is updated annually and is available on the internet at the OIE website:

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

Further details are given in Appendix 2 of this manual.

DISEASE WATCH HOTLINES

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

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

Preface

This disease strategy for the control and eradication of viral encephalopathy and retinopathy (VER) is an integral part of the **Australian Aquatic Veterinary Emergency Plan**, or **AQUAVETPLAN (Edition 1)**.

The strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of VER in Australia. The strategy was approved by:

- the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, at meeting 04 in May 2004;
- the Aquatic Animal Health Committee of the Primary Industries Standing Committee at meeting 04 in June 2004; and
- the Primary Industries Standing Committee at meeting 07 in September 2004.

Viral encephalopathy and retinopathy is listed by the World Organisation for Animal Health, or Office International des Epizooties (OIE) in the *International Aquatic Animal Health Code*.¹

Detailed instructions for the field implementation of AUSVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the enterprise manual. The full list of AQUAVETPLAN manuals that may need to be accessed in an emergency is shown below:

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Disposal
Destruction
Disinfection

Management manuals

Control centres management

Enterprise manual

Including sections on:

- open systems
- semi-open systems
- semi-closed systems
- closed systems

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

This manual was drafted by a writing group comprising Dr Richard Miller and the late Dr Barry Munday of IDEXX Veterinary Pathology Services, and Dr Chris Baldock of AusVet Animal Health Services Pty Ltd. Scientific editing was by Biotext, Canberra.

¹ http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 4 November 2004).

This manual was adapted from similar manuals in AUSVETPLAN, the Australian emergency plan for terrestrial animal diseases, and from the AQUAVETPLAN **Enterprise Manual**. The format and content have been kept as similar as possible to those documents to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents is gratefully acknowledged.

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

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

Government

Commonwealth of Australia
State of New South Wales
State of Queensland
State of South Australia
State of Tasmania
State of Victoria
State of Western Australia
Northern Territory
Australian Capital Territory

Industry

Australian Barramundi Farmers Association
Queensland Aquaculture Industries Federation
Aquaculture Association of Queensland
Victorian Aquaculture Council
Tasmanian Aquaculture and Fisheries Institute
Northern Territory Seafood Council
CSIRO Division of Livestock Industries
Tasmanian Salmonid Growers' Association
Tuna Boat Operators Association
Pearl Producers' Association
Australian Prawn Farmers Association
Pet Industry Joint Advisory Council
RecFish Australia
National Aquaculture Council

The complete series of AQUAVETPLAN documents is available on the internet at: <http://www.affa.gov.au/AQUAVETPLAN> (Accessed 4 November 2004).

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1 Nature of the disease

Viral encephalopathy and retinopathy (VER), a disease caused by infection with a betanodavirus, occurs mainly in larval and juvenile marine finfish. It is a particular problem where stocking density is high and has caused severe losses in hatcheries. At least one species of betanodavirus, *Lates calcarifer* encephalitis virus (LcEV), occurs in Australia.

1.1 Aetiology

Fish nodaviruses are icosahedral, non-enveloped viruses with a commonly reported diameter of about 25 nm and a range of 20–34 nm. An electron-dense core of 13–21 nm, surrounded by a clear layer of about 5 nm, has been described by some authors. The virions may be membrane-bound by endoplasmic reticulum or be free in the cytoplasm, and may present as paracrystalline arrays (Glazebrook et al 1990, Breuil et al 1991, Bloch et al 1991, Boonyaratpalin et al 1996, Grotmol et al 1997a).

Cells containing virions have most often been identified as neurones, astrocytes, oligodendrocytes and microglia (Yoshikoshi and Inoue 1990, Bloch et al 1991, Grotmol et al 1997a), which is consistent with the presence of neural lesions and neurological signs in affected fish. However, in Atlantic halibut, Grotmol et al (1997a) also visualised virus particles in endothelial cells, pillar cells, lymphocytes attached to the endocardium, cardiac myocytes and epicardial cells, which again correlate with histopathological lesions reported by those authors (Munday et al 1992, Le Breton et al 1997).

Fish nodaviruses are now classified in the *Betanodavirus* genus, within the family Nodaviridae (Ball et al 2000), as distinct from the insect nodaviruses, which are alphanodaviruses. The official virus species names are barfin flounder nervous necrosis virus (BFNNV), *Dicentrarchus labrax* encephalitis virus (DIEV), Japanese flounder nervous necrosis virus (JFNNV), *Lates calcarifer* encephalitis virus (LcEV), redspotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV) and tiger puffer nervous necrosis virus (TPNNV). Two further tentative species names are Atlantic halibut nodavirus (AHNV) and Malabar grouper nervous necrosis virus (MGNNV). However, there are many unnamed species, and the taxonomy of fish nodaviruses is complex and confusing.

1.2 Susceptible species

Worldwide, clinical VER has been reported in at least 32 marine fish species in 16 families (Munday et al 2002). In Australia, the main cultured species involved has been barramundi, which suffers mass mortality of larval and juvenile fish (Munday et al 1992). However, two instances of natural infection in freshwater fish have been diagnosed in Australia: Hyatt found typical histological lesions and viral particles in the brains of Australian catfish outside cages holding barramundi in fresh water (A Hyatt, Principal Research Scientist, CSIRO Livestock Industries, November 2003, pers comm); and Moody isolated a nodavirus from clinical cases of VER in sleepy cod in fresh water but not in contact with barramundi (N Moody,

Virologist, Queensland Department of Primary Industries and Fisheries, November 2003, pers comm). Experimentally, VER has been produced in the following Australian freshwater species: Macquarie perch, Murray cod, silver perch (Glazebrook 1995), golden perch, sleepy cod and Barcoo grunter (I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm). However, it appears that the freshwater species are less susceptible to infection than barramundi (I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm).

Grotmol et al (1997b) reported that Atlantic salmon suffering from the vascular disease designated cardiac myopathy syndrome had viral particles resembling betanodaviruses in affected tissues. However, Tasmanian Atlantic salmon with vascular lesions have not been found to be infected with nodaviruses (B Munday, Emeritus Professor, University of Tasmania, October 2002, pers comm).

1.3 World distribution and occurrence in Australia

VER has been reported from all continents with the exception of Africa, but the majority of reports have come from those regions undertaking intensive culture of marine species. The preponderance of groupers, sea bass and flatfish among the affected species is particularly apparent (Munday et al 2002).

While these viruses have apparently spread within the natural ranges of affected species as a result of commerce, VER has also been reported in these species in countries where they do not naturally occur and to which they have been exported. These species may have been infected by endemic strains of nodaviruses, but the epidemiological evidence suggests that the seed stock may have carried exotic strains of the viruses.

In Australia, clinical VER has been recorded at sites as far apart as Queensland, the Northern Territory and Tasmania (Munday et al 2002). The available information suggests that nodaviruses are widespread in the Australian marine environment but only cause clinical disease in cultured fish. Translocated VER in barramundi has been diagnosed in South Australia in September 2004 (reported on the Primary Industries and Resources South Australia website ²).

Betanodavirus infections are known to be endemic in Queensland and the Northern Territory and are probably present in Tasmania. Based on this distribution, and the ubiquity of these viruses worldwide, it is probable that they occur in most Australian marine waters. Even so, there are valid reasons for controlling the spread of infection between geographical areas and even between farms, so it is necessary for aquaculturists to ascertain current regulations before relocating susceptible species. In most cases, laboratory tests (see below) will be required before fish can be translocated between states or from endemic (marine) to non-endemic (freshwater) environments.

² <http://www.pir.sa.gov.au/pages/showcase/media/2004/10.09.nodavirus.htm>
(Accessed 3 November 2004).

1.4 Diagnostic criteria

Presumptive diagnosis of VER can be made in endemic zones on the basis of clinical signs but should always be confirmed by appropriate laboratory procedures.

To date, diagnosis for regulatory purposes has been mainly by histology, which is a low-sensitivity procedure that can also be of inadequate specificity if undertaken by inexperienced diagnosticians. Histology supported by electron microscopy and/or immunocytochemistry is more efficient but not practicable for routine diagnosis. Without doubt, the use of appropriate molecular techniques will overcome many of the problems inherent in these methods.

1.4.1 Clinical signs

Most fish are affected as larvae or juveniles, at which stage losses tend to be very high (see Table 1). However, in recent years, significant mortalities have occurred in older fish up to harvest size, especially in European sea bass (Le Breton et al 1997), groupers (Fukuda et al 1996, A Le Breton, Fish Health Consultant, November 2003, pers comm), and Atlantic halibut (Aspehaug et al 1999). In the case of sea bass and groupers, there appears to be an association between high water temperatures and the occurrence of disease in older fish (Le Breton et al 1997, Tanaka et al 1998). Because groupers are being developed as aquaculture species in Australia, these observations are important.

In general, the clinical signs relate to the lesions present in the brain and retina: there are abnormalities of movement, swim bladder control, sight and colouration. In most species, there is uncoordinated swimming, especially spiral swimming and darting, although flatfish tend to have a looping swimming pattern and to rest belly-up (Glazebrook et al 1990, Yoshikoshi and Inoue 1990, Bloch et al 1991, Breuil et al 1991, Mori et al 1991, Mori et al 1992, Boonyaratpalin et al 1996, Grotmol et al 1997a). Swim bladder hyperinflation has been reported in barramundi, European sea bass and striped jack (Breuil et al 1991; Mori et al 1992; B Munday, Emeritus Professor, University of Tasmania, October 2002, pers comm). Larval barramundi and halibut become paler (Glazebrook et al 1990, Grotmol et al 1997a), whereas groupers, juvenile halibut, European sea bass and turbot become darker. Some fish become thin from anorexia, but the main outcome is mass mortality, especially of larvae (Munday and Nakai 1997).

Table 1 Important clinical features of viral encephalopathy and retinopathy of larval and juvenile fish

	Earliest occurrence of disease	Usual onset of disease	Latest occurrence	Usual mortality rate	Highest mortality rate
Barramundi / Asian sea bass	9 dph	15–18 dph	≥ 24 dph	50–100%/month	100% in <1 month
European sea bass	10 dph	25–40 dph	Age 12 months and older	10%/month	
Redspotted grouper	14 dph (7–8 mm tl)	9–10 mm tl	<40 mm tl	80%	Up to 100%
Brownspeckled grouper		20–50 mm tl		50–80%	
Striped jack	1 dph	1–4 dph	<20 dph (8 mm tl)	100%	
Japanese parrotfish	6–25 mm tl		<40 mm tl		Up to 100%
Halibut		60–70 dph	adult		Up to 100%
Japanese flounder	35 dph (17–18mm tl)	25 mm tl		100%	
Turbot	<21 dph		Bodyweight 50–100 mg		Up to 100%

dph = days post-hatch; tl = total length

1.4.2 Histopathology

For histological diagnosis of VER, whole larvae or small juvenile fish may be submitted to the laboratory alive or fixed in formal saline. For larger fish, the brain, eyes and spinal cord (if possible) should be submitted in formal saline.

At the light microscope level, histopathological findings (characterised by vacuolation and necrosis of the central nervous system) are remarkably consistent between the various species. In general, the anterior brain is more severely affected than the posterior brain and spinal cord. Larval fish are more severely affected than juveniles. The most characteristic lesions present as vacuoles in the grey matter of the brain; these appear to be intracytoplasmic, but their exact position cannot always be determined.

Other lesions noted include pyknosis (degeneration of the cell nucleus), shrinkage and basophilia of affected cells (Yoshikoshi and Inoue 1990; I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm), focal pyknosis and karyorrhexis (fragmentation of the cell nucleus) of neural cells, granularity of the neuropil and the presence of mononuclear cell infiltrates (Grotmol et al 1995; I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm). Cerebral blood vessel lesions have been described by Munday et al (1992), who found eosinophilic, periodic acid Schiff (PAS) positive material in the walls of blood vessels, and Le Breton et al (1997), who reported swelling of the endothelium. Basophilic, intracytoplasmic inclusions – approximately 1 µm in diameter in Japanese parrotfish (Yoshikoshi and Inoue 1990) and barramundi (Glazebrook et al 1990); 2–5 µm in European sea bass (Breuil

et al 1991); and of unspecified size in brownspotted grouper (Boonyaratpalin et al 1996) – have been reported in brain cells. Persistently infected but asymptomatic juvenile halibut were found to have focal aggregates of macrophage-like cells containing viral particles scattered throughout the brain and retina (Nilsen et al 2001). Anderson (I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm) reported mild vacuolation and the presence of macrophage-like cells in the brains of asymptomatic, experimentally infected Barcoo grunter.

Where the eye has been examined, retinal lesions have been described in all species. Vacuolation involves the cellular components of the retina, especially the bipolar and ganglionic nuclear layers (Munday et al 1992), although small vacuoles can be found in the rod and cone layer (Grotmol et al 1995). Grotmol et al (1997a) have also described an ophthalmitis involving both the anterior and posterior chambers in some fish; the cell types involved were lymphocytes and macrophages. Similar lesions were found by Anderson (I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm) in experimentally infected Barcoo grunter.

Grotmol et al (1997a) described a mild endocarditis and degeneration and necrosis of the gill pillar cells in affected halibut.

1.4.3 Laboratory tests

A number of laboratory procedures of varying sensitivity and specificity can be used to detect nodavirus infections in clinically infected or inapparently infected fish. The nested polymerase chain reaction (PCR) being developed by the Oonoonba Veterinary Laboratory (Fisheries Research and Development Corporation, FRDC project 2001/626; Moody et al 2004) will probably become the standard technique for certification of species likely to be infected with nodaviruses.

Light microscopy of central nervous system and eyes

For light microscopy, the appropriate specimens are live fish or central nervous tissue and eyes fixed in formal saline.

Light microscopic examination of haematoxylin and eosin (H&E) stained sections brain and retina sections from the infected fish will show mild to severe vacuolation, usually more pronounced in the forebrain. Sometimes, intracytoplasmic inclusions up to 5 µm may be seen. Such lesions may provide presumptive diagnosis of VER but for verification, viral antigen should be demonstrated by indirect fluorescent antibody test (IFAT) or immunoperoxidase staining using a polyclonal antibody against a nodavirus.

As vacuolation of the central nervous system and/or retina do not occur in all subclinically infected fish, light microscopy has relatively low sensitivity when used for certification purposes. Also, its specificity varies with the extent and severity of lesions; of course, this can be improved by the use of IFAT or immunoperoxidase techniques.

Electronmicroscopy

Electronmicroscopy is mainly useful for supporting a diagnosis of clinical VER and for research purposes. Suitable specimens are live fish, or central nervous system tissue and/or eyes fixed in glutaraldehyde. The laboratory should be asked to specify the exact method of fixation required.

Rapid demonstration of nodavirus virions can be made with negative staining techniques. This will demonstrate that the virus has the appropriate size and shape, but does not fully confirm nodavirus. Positive staining for transmission electronmicroscopy provides more detail and allows even greater assurance that a nodavirus is involved, but again is not fully confirmatory and is more of a research tool.

Molecular techniques

Specimens to be used in molecular testing should be live whole fish, frozen fish or appropriate tissues. Samples preserved in absolute alcohol should be suitable for routine diagnosis.

Molecular techniques, particularly PCR, have become the main diagnostic method for fish nodaviruses mainly because they can detect tiny quantities of viral RNA (ribonucleic acid) in any tissue. The mainstay of this approach has been the PCR for a target sequence of 430 bases of SJNNV RNA2. However, the sensitivity of this assay varies with the strain of nodavirus. To overcome this, more specific primers have been designed for a number of betanodaviruses, including LcEV (Glazebrook 1995; R Reuter, Pathologist, Iddex Laboratories, October 2003, pers comm).

More recently, Dalla Valle et al (2000) reported that a nested PCR for DIEV was 10- to 100-fold more sensitive than reverse transcriptase PCR (RT-PCR) and enabled diagnosis using blood and sperm as well as nervous and ovarian tissues. Similarly, studies involving natural and experimental nodaviral infections in Australian barramundi demonstrated the superiority of a nested RT-PCR, based on the primers described by Thiery et al (1999), over the basic RT-PCR (Moody, unpublished, and FRDC project 2001/626 ((Moody et al 2004).

It is anticipated that the nested RT-PCR, with its high sensitivity and specificity, will become the main technique for diagnosing betanodavirus infections in Australia, especially for certification purposes. However, because the virus may be only transiently present in the gonads, a combination of PCR and the enzyme-linked immunosorbent assay (ELISA) described below has the best chance of detecting infection in carrier fish, particularly in broodstock.

Virus culture

Samples for cell culture are, ideally, live whole fish; otherwise, chilled or frozen tissues can be used. Cell culture has high sensitivity and specificity.

A number of cell lines are now available for the culture of betanodaviruses. The striped snakehead cell line (SSN-1) originally developed by Frerichs et al (1996) has been shown to be permissive for 17 isolates of fish nodaviruses, encompassing the RGNNV, SJNNV, TPNNV and BFNNV types (Iwamoto et al 1999). Recently, Iwamoto et al (2000) reported that a clone of this cell line, designated E-11, is particularly useful for qualitative and quantitative analyses because of its stable,

clear cytopathic effect (CPE) expression and production of high levels of virus. This cell line has been imported by the Australian Animal Health Laboratory (AAHL).

Chi et al (1999) and Lai et al (2001) have developed cell lines from the groupers *Epinephelus coioides* and *E. awoara* that support grouper nervous necrosis viruses, but the lines have not been tested for other types of fish nodaviruses.

Permissive cell lines have also been developed from barramundi/Asian sea bass tissues (Chong et al 1990, Chang et al 2001). Within the FRDC project 2001/626 (Moody et al 2004), one of the aims is to develop such a cell line for use outside AAHL.

In the past, cell culture was used mainly for research, but it has shown promise as a means of expediting diagnosis of betanodavirus infections in a combined cell culture-PCR technique (Iwamoto et al 2001).

Serology

Screening for specific antibodies by ELISA has been used for selection and monitoring of broodfish in striped jack (Mushiake et al 1992), barfin flounder (Watanabe et al 2000) and European sea bass (Breuil et al 2000). However, with the advent of sensitive nested RT-PCRs, diagnosis can be achieved using minimal amounts of tissue (Dalla Valle et al 2000; N Moody, Virologist, Queensland Department of Primary Industries and Fisheries, November 2003, pers comm). For screening of broodstock, where the virus may be only transiently present in the gonads, a combination of ELISA and PCR tests offers the best chance of detecting infection.

Transport of specimens

Specimens should initially be sent to the state or territory diagnostic laboratory. Because VER is an endemic disease, there is no requirement to forward specimens to the CSIRO Australian Animal Health Laboratory (CSIRO-AAHL), Geelong, for emergency disease testing.

1.4.4 Differential diagnosis

The following diseases are listed on the OIE technical disease card³ for VER to be considered in a differential diagnosis:

- streptococcosis
- amyloodiniosis
- environmental stress factors (high ammonia and nitrate levels).

1.4.5 Treatment of infected animals

There is no treatment available for animals infected with VER.

³ <http://www.oie.int/aac/eng/Publicat/Cardsenglish/2.2.02.%20VER%20September%202000.DOC> (Accessed 3 November 2004).

1.5 Resistance and immunity

In recent years, great advances have been made in our understanding of teleost immunity, although it is still not as well understood as mammalian immunity (Watts et al 2001). Innate or nonspecific immunity is present as the first line of defence against pathogens, whereas the more specific active immunity takes a variable time to develop (depending on ambient temperature) and is not developed in many larval fish.

1.5.1 Innate immunity

Nonspecific immunity comprises constitutive physical (eg skin) and chemical (eg lysozyme) factors, which are always present, and inducible factors (eg inflammatory mediators and acute phase proteins), which are subject to up-regulation during an active response (Watts et al 2001). Nonspecificity is a powerful attribute allowing tremendous versatility and may have a more significant role in fish than in mammals, especially as the specific immune response is slower in fish, particularly at temperatures less than optimal for the particular species.

Innate immunity is very important in VER because most outbreaks of the disease occur in larval and juvenile fish.

1.5.2 Active immunity

Specific or adaptive immunity is involved in responses to pathogens and other antigens that result in the formation of specific antibodies or cell-mediated immunity. These responses are delayed and subject to ambient temperature (Watts et al 2001). Active immunity has been shown experimentally to protect against VER (Nakai 2000).

1.5.3 Passive immunity

Passive immunity in fish may occur when antibodies are transferred from broodfish to larvae via yolk or by injection of homologous or heterologous immune serum. There is some hope that this approach may be useful in VER, as Tanaka et al (2001) showed that betanodavirus treated with immune fish serum was less infectious than virus treated with normal fish serum.

1.5.4 Vaccination

Studies on vaccination against betanodaviruses have only recently commenced and have focused on using recombinant coat proteins (Tanaka et al 2001, Yuasa et al 2002). These have provided encouraging results and may be suitable for those species in which the disease sometimes expresses itself relatively late (eg some groupers, European sea bass). However, vaccines may need to be tailored for specific situations because not only may one genotype of betanodavirus not protect against another, but one strain of a particular genotype may not protect against another strain (Tanaka et al 2001, Yuasa et al 2002).

1.6 Epidemiology

The epidemiology of VER in striped jack is well established, and it is reasonable to expect that the disease behaves similarly in other species (Munday et al 2002). The disease appears to spread through a variable degree of vertical transmission, followed by lateral transmission.

1.6.1 Persistence of agent

The resistance of betanodaviruses to environmental conditions such as pH 2–9 (Frerichs et al 1996) and storage in seawater at 15°C for more than a year (Frerichs et al 2000) increases the likelihood of lateral transmission. However, transmission by aerosols does not appear to occur (M Heasman, Senior Research Scientist, New South Wales Department of Primary Industries, November 2003, pers comm), and there are no data relating to transmission on fomites.

As the virus can survive for one year at 15°C, betanodaviruses can potentially survive in dead fish. Fish products and byproducts may therefore allow the spread of infection to uninfected areas. Betanodaviruses are inactivated at a temperature of 60°C for 30–60 minutes (Arimoto et al 1996, Frerichs et al 2000).

1.6.2 Modes of transmission

Vertical transmission

The potential for vertical transmission can be gauged from the fact that Arimoto et al (1992) detected the virus in 65% of striped jack broodfish. Also, Breuil et al (2000) reported that 17% of wild and 18% of farmed European sea bass were seropositive by ELISA, and Huang et al (2001) found that 9% of commercial barramundi were seropositive. It is important to realise that the viruses may not reside in the reproductive organs at all times and are more likely to be found there after stressful procedures, such as repeated spawning (Nguyen et al 1997).

Lateral transmission

Lateral transmission may be affected by stocking density, ambient temperature and the virulence of the particular nodavirus in the exposed species. Arimoto et al (1993) found that, for striped jack, VER did not spread when the ratio of infected to naive fish was 1:100 or less. Clinical disease in older groupers appears to be associated with elevated water temperatures (Tanaka et al 1998).

Cross-infectivity of nodaviruses

A major concern in Australia is the possibility that barramundi nodavirus may be translocated to freshwater environments with juveniles used for aquaculture in inland saline waters. Variable susceptibility to LcEV has been demonstrated or reported for Australian catfish (A Hyatt, Principal Research Scientist, CSIRO Livestock Industries, November 2003, pers comm), Macquarie perch, Murray cod, silver perch (Glazebrook 1995), golden perch, sleepy cod and Barcoo grunter (I Anderson, Principal Veterinary Pathologist, Queensland Department of Primary Industries and Fisheries, October 2003, pers comm). Therefore, it is imperative to ensure that VER does not become established in inland river systems.

2 Principles of control and eradication

2.1 Introduction

An outbreak of viral encephalopathy and retinopathy (VER) in Australia would be a serious threat to the fish farming industry. A number of different control measures could be used to minimise its impact, depending on the circumstances of the outbreak. This section provides background information to enable the choice of the most appropriate response following detection of VER.

There are two broad response options:

- *eradication* – elimination of the betanodavirus(es) responsible for the outbreak (this option has the highest cost and most stringent control measures); and
- *containment, control and zoning* – containment of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas.

Because at least one species of betanodavirus is endemic to a wide area of Australia, eradication is only likely to be attempted on a single enterprise basis within this area. Compulsory eradication may be imposed in non-endemic areas and, conceivably, could involve multiple premises. In other situations, control measures will be recommended or implemented to reduce the impact of the disease and minimise further spread. To minimise the risk of disease transmission, translocation of susceptible fish between states and territories and between endemic and non-endemic areas will require competent certification of the fish being moved.

The basic principles of eradication and other control responses are described in the **Enterprise Manual** and the **Control Centres Management Manual**, and include:

- rapid detection and identification of infection;
- rapid definition of the nature and extent of the problem;
- rapid definition and implementation of control measures;
- prevention of spread by controlling stock and water movement, within and between farms; and
- maintenance of good management practices and high standards of hygiene.

The choice between eradication and control will depend on the following factors:

- *Location and presence or absence of reservoirs of infection*

Because betanodaviruses appear to be capable of infecting many marine and freshwater species, the presence of large populations of fish in receiving waters is likely to militate against successful eradication. In such

circumstances, continual monitoring of the wild fish population would be necessary to confirm eradication.

- *Chance of successful eradication*

Eradication is more likely if VER is detected early in an isolated group of fish, in a facility releasing no, or very little, untreated water. The fewer of these conditions met, the lower the chance of successful eradication.

- *Acceptable level of risk of future spread of infection*

In an endemic area, the risk associated with allowing the grow-out of infected stock might be low. In a non-endemic area, grow-out would not be allowed without a change in the policy of the controlling authority.

- *Short-term costs of control and disruption to production*

Fortunately, betanodavirus infections are usually introduced with larvae or juveniles of relatively low individual value. If older fish are infected, they can be salvaged for human consumption. However, individual enterprises are likely to suffer losses from interruptions to production caused by depopulation.

- *Long-term costs of production, with or without the presence of the pathogen; and*

- *Long-term costs of control should the pathogen become endemic.*

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

The quarantine and movement restrictions that should be implemented immediately upon suspicion of VER in a non-endemic area are:

- establishment of specified areas (Figure 1; see the **Enterprise Manual, Section A** for more details)
 - declared area – includes restricted area (RA) and control area (CA)
 - restricted area – area around the infected premises or site
 - control area – a buffer between the RA and free areas
 - free area – non-infected area, which is not considered a declared area and may include large parts of Australia in which the presence or absence of VER remains unassessed;
- quarantine of the infected premises/facilities;
- bans on the movement of live fish out of infected areas; and
- restrictions or bans on the use and movement of equipment within and between river systems and fish farms.

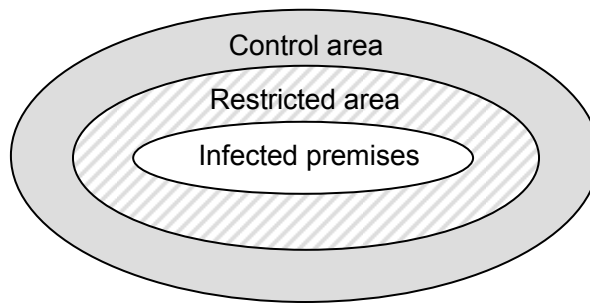


Figure 1 Establishment of specified areas to control VER

The following practices must be considered when implementing any control strategies:

- transportation of live fish between and within freshwater and marine operations;
- movement of equipment between farms, river systems and marine sites;
- fish harvesting and transportation to processing plants;
- discharge of processing plant effluent;
- transportation of consumer-ready products; and
- disposal of dead fish.

The feasibility of restrictions and bans and the extent to which they are imposed will depend on the location of infection, the location and type of enterprises affected and the response option chosen (ie eradication or control).

Zoning

Zoning for VER is possible but may be difficult once the disease is introduced into a previously non-endemic zone. Covertly infected fish populations can become established and are very difficult to detect without the use of destructive measures. Reservoirs of infection can become established in wild fish populations, and these reservoirs are unlikely to be successfully eradicated. Once established in the marine environment or a river system, or in a migratory wild fish population, infection is easily spread. Zoning may include non-infected (free) zones, control zones (in which infection occurs but is actively controlled) and endemic zones (in which minimal disease control is undertaken).

Principles of zoning for infected and non-infected zones in Australia are outlined in the *AQUAPLAN Zoning Policy Guidelines* (AFFA 2002). To support a zoning policy, a corresponding surveillance program for VER would also be required.

Semi-open systems

In semi-open production systems, in which fish are held in cages moored in estuaries or other sheltered areas, there is virtually no control over the aquatic environment. Impermeable liners may be placed temporarily around sea cages (eg

to administer treatments for diseases); however, this prevents oxygen-rich water reaching the fish and waste products being diluted out, and such liners would not be a feasible method of long-term VER control. Treatment of fish in semi-open systems will affect the surrounding environment, and this must be taken into consideration before disinfectant treatments are used.

Fish kept in marine cages may only be controlled to a limited extent. Cages can become damaged, thereby allowing fish to escape into the wild. Some small wild fish species, attracted by food, can swim in and out of the cages at any time. Larger wild fish species can become trapped inside cages if they swim into them when they are small, remain there and grow too large to escape. Large fish can also get caught inside cages during net changeover. These potential means of spreading infection must be considered in the control of VER.

Semi-closed systems

Semi-closed systems allow slightly more control over water than semi-open systems, but farms vary in the extent to which input and output water can be contained. These systems are not designed to be self-contained, so preventing inflow or outflow of water may have adverse effects. Semi-closed farms generally have limited facilities to hold or treat water, and while output water control and treatment are possible in theory, on most farms such treatment would not be feasible. For example, the organic matter content in farm effluent is too high for ozone treatment.

Fish input and output may be controlled, but 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. Fish are also able to enter farm waterways and, possibly, ponds in intake water from rivers.

Some inland pond systems may constitute virtually closed systems because they are filled with groundwater, and there is no effluent except after harvest, if at all.

2.2.2 Tracing

The information gathered from tracing will help determine the most appropriate response action. Immediate steps are to trace-back all contacts with infected fish, premises and sites, to establish the origin of the outbreak, and to trace-forward all contacts to establish the current location and potential spread of infection.

The following items must be traced:

- fish – broodstock, larvae, juveniles etc;
- fish products – eggs, fish for consumption, effluent and waste products from slaughter and processing;
- water – input and output;
- vehicles – fish transport vehicles, feed trucks, visitors' cars, boats;
- materials – fish cages, nets, other floating installations, tools and instruments; and

- personnel – farm workers, sales and feed-supply representatives, tradespeople, veterinarians, scientists, technicians and visitors.

Neighbouring fish populations

Neighbouring fish farms and processing plants may be, or become, infected. Maps showing the location of neighbouring fish farms, processing plants and waterways, and hydrographic data are necessary to monitor the potential spread of the pathogen. The location of susceptible fish species and vectors should also be noted both upstream and downstream of the infected site. Further sources of infection may be identified if a number of facilities share common water.

For information on the location of farming establishments and wild fish populations at risk of infection, the relevant state/territory fisheries or agriculture agency should be contacted (see the **Enterprise Manual, Appendix 5** for contact details).

2.2.3 Surveillance

Surveillance, by screening for clinical signs and by laboratory testing, is necessary to:

- define the extent of infection;
- detect new outbreaks;
- establish RAs and CAs to which quarantine and movement restrictions are applied;
- establish disease-free and infected areas or zones for a VER zoning program; and
- monitor the progress and success of an eradication strategy.

2.2.4 Destruction of fish

Slaughter must be both hygienic and humane. There must be no spillage of infectious waste. Increased viral shedding may occur if fish are stressed at slaughter, so the least stressful methods should be used.

There are many different methods for anaesthetising and/or slaughtering fish, all of which have limitations. Methods used include the following:

- chemical anaesthesia – fish are herded into a liner, then exposed to an anaesthetic solution in water before slaughter (Aqui-S is the only anaesthetic currently approved for this use in Australia, and has no withholding period);
- ice slurry;
- stunning (with or without subsequent bleeding); and
- carbon dioxide narcosis (with or without subsequent bleeding).

The most appropriate method of slaughter depends on the following factors:

- size and number of fish;
- deadline for slaughter, which depends on the pressure of infection and the risk of further spread;
- destination of the slaughtered fish (ie for human consumption or for disposal);
- slaughter facilities, including the site, equipment and methods available; and
- experience and availability of personnel.

2.2.5 Treatment of fish products and byproducts

Betanodaviruses can potentially survive in dead fish (virus can survive for one year at 15°C), and fish products and byproducts may therefore allow dissemination of infection to uninfected areas. Betanodaviruses are inactivated at a temperature of 60°C for 30–60 minutes (Arimoto et al 1996, Frerichs et al 2000).

Betanodaviruses are transmitted vertically, but viable eggs may be rendered virtually free of virus by surface disinfection with ozone. Recommended disinfection doses and rates are 0.1µg/mL residual ozone for 2.5 minutes for striped jack (Arimoto et al 1996), 4µg/mL for 30 seconds for halibut (Grotmol and Totland 2000), 1µg/mL for 1 minute for striped trumpeter (S Battaglone, Aquaculture Section Leader, Tasmanian Aquaculture and Fisheries Insititute, October 2003, pers comm) and 0.4 µg/mL for 2 minutes for barramundi (G Schipp, Senior Aquaculture Researcher, Northern Territory Department of Business, Industry and Resource Development, October 2003, pers comm).

2.2.6 Disposal of animal products and byproducts

Disposal must be immediate to decrease infection pressure on the site. See the **Operational Procedures Manual** for details. Burial sites must be chosen carefully to ensure that there is no contact with waterways or vectors.

2.2.7 Decontamination

Effective decontamination of equipment, materials, tanks and buildings requires thorough cleaning before disinfection. Because of differences between farming enterprises, disinfection protocols may need to be determined case by case by the farm manager, the state/territory chief veterinary office (CVO) and/or the director of fisheries. The protocol should take into consideration:

- source and location of infection;
- type of enterprise (marine or freshwater farm or processing plant);
- construction materials of the buildings/structures on the site;
- design of the site and its proximity to other buildings or waterways;
- current disinfection protocols;
- environmental impact of the disinfectant protocol; and
- availability of approved, appropriate and effective disinfectants.

Betanodaviruses are remarkably resistant to many disinfectants. Recommended treatments are 50 ppm final concentration of sodium hypochlorite, benzalkonium chloride or iodine for 10 minutes at 20°C. However, the presence of organic matter calls for higher concentrations for longer periods.

Since betanodaviruses can survive in seawater for 12 months and in freshwater for up to six months, decontamination of earth ponds and farm water channels requires the removal of the upper 10–15 cm of sludge and treatment with slaked lime to give a pH of 11–12 (Arimoto et al 1996, Frerichs et al 2000).

Environmental considerations

Control of VER needs to take account of the following environmental considerations:

- Discharge of infected or potentially infected effluent into catchment areas or natural waterways will lead to further spread of infection and could lead to the establishment of reservoirs of infection in wild fish populations and waterways.
- Disinfectants could affect the environment, especially if they are used in larger than normal quantities or concentrations, as is possible in a disease control operation. The local environmental protection agency may need to be consulted (see the **Enterprise Manual**).
- The destruction and disposal of infected carcasses or material will have an impact on the environment. This impact must be minimised while ensuring that there is no dissemination of infection.

2.2.8 Vector control

Control of vectors is essential to prevent further spread of the disease. The following potential vectors should be considered:

- *Birds* – sea pens, open-air tanks and ponds may attract birds and must be covered (eg using nets or tank roofs) to prevent birds gaining access and transmitting infection.
- *Fomites* – strict hygiene measures include disinfection of boots, nets and other equipment.
- *Wild fish* – where possible, contact between wild fish and farmed fish must be prevented. This is not possible in marine farming operations.

2.3 Feasibility of specific options for control in Australia

2.3.1 Eradication

Eradication of VER under Australian conditions may be compulsory, such as when betanodavirus infection is detected in a non-endemic geographic area, or voluntary, when an owner decides to establish a betanodavirus-free facility.

Eradication from a non-endemic area

Eradication of VER from a non-endemic area is only likely to be successful if infection is detected soon after larvae or juveniles have been introduced, so time is of the essence.

If there is evidence that betanodavirus infection has spread to wild fish populations, successful eradication will be most unlikely. Therefore, this discussion only covers situations in which it is believed that the infection has been contained within a farm and any escaping virus has not produced overt or covert infection in susceptible species in adjoining water bodies.

Eradication from a facility within an endemic area

Eradication from a farm within an endemic area will be based on the premise that infection is perpetuated by vertical transmission from infected broodfish. Therefore, the strategy is first to identify and eliminate infected broodfish, and then to either depopulate other stocks or to ensure adequate barrier control between possibly infected fish and presumably uninfected larvae. These measures will be supplemented by egg treatment and continual monitoring of stocks for betanodaviruses.

The following steps are required to achieve eradication.

1. *Ensure that broodfish do not produce infected eggs*

Because ovarian infection may only occur close to spawning, broodfish should be confirmed free of infection by testing blood prior to spawning and ovarian tissue close to spawning, using nested polymerase chain reaction (PCR). Any infected broodfish should be destroyed. As broodfish could be exposed to virus in influent water, they should preferably be kept in recirculated systems, with the top-up water treated with sodium hypochlorite at the rate of 50 ppm for at least 10 minutes followed by neutralisation with sodium thiosulfate.

2. *Further safeguards to minimise vertical transmission of virus*

To further guard against vertical infection if testing misses an infected broodfish, the broods should not be unduly stressed, especially by multiple spawnings, and the eggs should be ozonised at a level appropriate for the water temperature and the species (some data on ozonisation level is available for striped jack, turbot and striped trumpeter, but none is currently available for barramundi).

3. *Prevent spread of any virus not prevented from vertical spread or inadvertently introduced*

Individual batches of larvae and juvenile fish should be maintained in separate tanks/ponds and should not share water or equipment. As far as possible, only treated water should be used in the hatchery.

4. *Monitoring of larvae and juveniles*

Larvae and juveniles should be monitored monthly for betanodavirus infection from one month after hatch until at least three months after hatch. If infected fish are detected, the whole batch should be destroyed and an epidemiological investigation made to pinpoint the source of the breakdown.

2.3.2 Containment, control and zoning

It is reasonable to presume that most freshwater systems outside the range of barramundi in Australia are free of VER and should be protected from the disease. However, based on experience in other countries and detection of VER in sites as far apart as Cape York and Tasmania, it is reasonable to presume that betanodaviruses are widely distributed in the Australian marine environment. Therefore, it may be appropriate to institute different control procedures depending on geographical and environmental considerations.

Free zones

Considering the likely wide marine distribution of VER, declaration of uninfected zones in marine environments is likely to be difficult, and would depend on extensive surveillance to demonstrate freedom. However, declaration of freshwater systems as free zones may be a realistic option.

Control zones based on state/territory boundaries

Even though betanodaviruses are probably ubiquitous in marine environments, it is likely that different states/territories will try to reduce the spread of VER by requiring certification of susceptible live fish. The success of such a strategy relies on the use of a sensitive and specific test for VER applied at the right time to an adequate number of fish in any shipment. Although histology has been the main test used to date, it is of relatively low sensitivity and nested PCR should supersede it for certification purposes.

Grotmol et al (2000) reported that reverse transcriptase polymerase chain reaction (RT-PCR) was able to detect 100 to 1000 copies of in vitro transcribed ribonucleic acid (RNA). Dalla Valle et al (2000) found that a two-step nested PCR was 100 times more sensitive than the standard RT-PCR, and a one-step nested PCR increased sensitivity ten-fold. For the nested PCR, therefore, the best result is that it can detect one copy of transcribed RNA and the worst result is that it is only able to detect 100 copies. Putting these sensitivity limits in the context of infectivity, it is reasonable to consider the sensitivity of the nested PCR as 100% for certification purposes. As Arimoto et al (1993) have shown that striped jack nervous necrosis virus (SJNNV) does not spread by cohabitation when the prevalence of infected fish is 1% or less, it is suggested that certification testing should be at an intensity sufficient to detect a 1% prevalence; that is, 300 fish should be tested to provide 95% confidence of detecting at least one infected fish (Simon and Schill 1984). Obviously, such a large sample would require pooling of fish to keep the procedure affordable. In deciding on a pooling strategy, it must be remembered that 'the confidence intervals bounding estimates were generally smaller when larger numbers of groups were used and samples had few fish per pool' (Williams and Moffit 2001).

Control zones based on geography

Although Moody (N Moody, Virologist, Queensland Department of Primary Industries and Fisheries, November 2003, pers comm) has reported VER in sleepy cod in fresh water, it can generally be assumed that freshwater bodies outside the migratory limits of barramundi are not endemic zones for VER. Therefore, barramundi and other species known to be regularly infected with betanodaviruses should be subjected to the same testing regime as recommended

for movement of these fish over state/territory boundaries when they are translocated to such zones.

Endemic zones

Within endemic zones, regulatory controls would be minimal. Mitigation of disease is likely to be the preferred option for producers in these zones, unless individual operators decide to eradicate the disease in their own facilities.

Available mitigation strategies include:

- ozonation of eggs, which will require research to determine the optimal combination of concentration of ozone, water temperature and length of exposure for each species;
- separation of batches of larvae/juveniles with strict barrier controls;
- use of 'green water' culture, with the aim of maintaining larval density at less than 1 per 10 L (Munday and Nakai 1997); and
- prompt removal of all dead and moribund fish (it is better to dispose of entire batches of fish showing evidence of VER than to try to salvage them).

3 Policy and rationale

3.1 Overall policy

Viral encephalopathy and retinopathy (VER) is an endemic disease of marine finfish in Australia and can cause devastating losses in larvae and juveniles, especially in barramundi.

The policy for response to an outbreak of VER in Australia depends both on the nature of the outbreak and on the control management strategy to be adopted. The response option will be decided, following epidemiological investigation, by the director of fisheries and/or the chief veterinary officer (CVO) of the state/territory in which the outbreak occurs.

There are two possible response options for VER in Australia:

- ☞ *Option 1 – eradication of betanodaviruses; and*
- ☞ *Option 2 – containment, control and zoning of the viruses to areas with endemic infection to prevent further spread and protect uninfected areas.*

Both these options involve a combination of strategies, which may include:

- ☞ *quarantine and movement controls on fish, fish products, equipment and other things in declared areas to prevent spread of infection;*
- ☞ *destruction of all clinically diseased or dead fish as soon as possible to prevent further virus shedding;*
- ☞ *decontamination of facilities, products, equipment and other things to eliminate the viruses on infected premises and to prevent spread of infection;*
- ☞ *surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and*
- ☞ *zoning to define and maintain infected and disease-free zones.*

Betanodaviruses are known to be widely dispersed in the Australian marine environment (apparent absence from a particular area is probably because the area lacks intensive aquaculture of susceptible species, not because the viruses do not occur in the area). Therefore, a rapid and vigorous response by regulatory authorities is probably only justified if an outbreak occurs in freshwater zones outside the normal distribution of freshwater-phase barramundi. In all other instances, it will be most important to undertake a thorough epidemiological investigation before instituting eradication or control procedures. Of course, the owner of the fish may decide on immediate destocking for commercial reasons, and such action should not be impeded.

The director of fisheries and/or the CVO in the state or territory in which the outbreak occurs will be responsible for developing an emergency animal disease response plan (EAD Response Plan). This plan will be approved for technical soundness and consistency with AQUAVETPLAN by governments on the aquatic Consultative Committee on Emergency Animal Diseases (aqCCEAD).

Directors of fisheries and/or CVOs will implement the disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing decisions on follow-up disease control measures in consultation with aqCCEAD. The detailed control measures adopted will be determined using the principles of control and eradication (see Section 2) and epidemiological information about the outbreak.

For information on the responsibilities of the state or territory disease control headquarters and local disease control centres, see the **Control Centres Management Manual, Part 1**.

3.2 Problem definition

The initial phase of a response to detection of VER infection in an area or species previously thought to be free of infection will be containment, while additional information is collected to determine the extent of the problem and decide on an appropriate response.

The components of this phase are described in Sections 3.2.1 to 3.2.4.

3.2.1 Rapid confirmation of infection

Betanodavirus infection can only be confirmed by laboratory examination. At the very minimum, typical histological lesions should be present, but supplementary immunocytochemical or molecular tests should be carried out. In future, it is likely that diagnosis will be by the nested polymerase chain reaction (PCR) being developed by Oonoonba Veterinary Laboratory (FRDC project 2001/626; see Section 1.4.3).

For the purpose of initiating a response, VER is deemed to be confirmed if either of the following conditions are met:

- typical histological lesions present in fish tissues, supported by positive results from immunocytochemical or molecular tests; or
- detection of VER virus in fish tissues by culture or molecular means.

3.2.2 Epidemiological investigation and definition of the nature and extent of the problem

In non-endemic areas, epidemiological investigations must be conducted immediately upon suspicion of an outbreak of VER to determine the actual and potential spread of infection. This knowledge is required to determine the most scientifically and economically feasible response option. Thorough epidemiological investigation and tracing is fundamental to the success of an eradication or zoning program.

Some measure of the extent of the problem may be obtained by clinical examination of fish stocks. However, for barramundi in particular, older fish may be covertly infected and diagnosis must be made by laboratory examination, preferably by nested PCR.

Surveillance of fish stocks and facilities in the affected region must be undertaken immediately to determine the extent of the outbreak. Surveillance should comprise both clinical evaluation and laboratory screening of an appropriate sample of fish. Because of the likelihood of low-prevalence subclinical infection, particularly in older fish, sample sizes for surveillance should be calculated to provide 95% confidence of detecting 1% prevalence in the sampled population. The precise size of the sample should be calculated using the known specificity and sensitivity of the diagnostic tests to be employed and the known population size. This is most easily achieved with a software package such as FreeCalc.⁴ Samples may be pooled to reduce testing costs.

3.2.3 Interim measures to minimise further spread

Movement controls and other measures should be implemented immediately on infected premises (IPs) or areas, pending confirmation of VER and definition of the extent of the outbreak (see Section 3.4.1 and the **Enterprise Manual** for details). Measures may include:

- controls over the movement of live fish and fish products;
- water treatment and/or diversion; and
- isolation and/or destruction of suspected infected fish.

3.2.4 Determination of appropriate response

As soon as adequate information becomes available, a decision should be made as to the appropriate response, based on the flow chart shown in Figure 2. Eradication will only be attempted if the infection appears to be limited to farmed fish in one or a small number of facilities, and if eradication is deemed to be achievable. If infection occurs in a known endemic area, or in wild fish in an area previously regarded as free, control may still be attempted, depending on circumstances.

⁴ http://www.ausvet.com.au/content.php?page=res_software#freecalc (Accessed 4 November 2004).

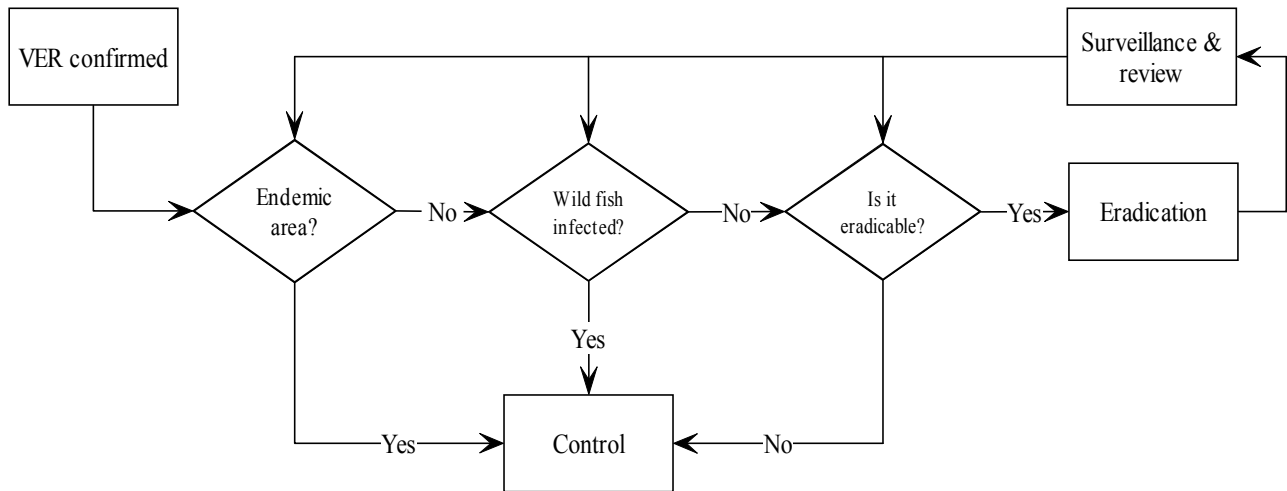


Figure 2 Decision flow chart to determine the preferred response to a VER outbreak

3.3 Overview of response options

3.3.1 Option 1 — Eradication

If epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the virus, an eradication strategy may be successful and should be attempted. Compared with the other response options, eradication has the highest short-term economic costs.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, has no point source, is unable to be contained or is present or potentially present in wild fish species, rivers or the sea.

Eradication strategies include:

- quarantine and movement controls or restrictions on fish, fish products, water and any other vectors (including fomites) in declared areas to prevent spread of infection;
- destruction and disposal of all clinically diseased fish (all fish and fish products infected and exposed to betanodaviruses must either be destroyed and disposed of, or treated/handled in such a manner as to prevent further spread of infection);
- decontamination of facilities, products, equipment, vehicles/boats etc to eliminate the virus from IPs and to prevent spread;
- tracing and surveillance to determine the source and extent of infection and to provide proof of freedom from the disease;
- zoning to define infected and disease-free areas; and
- a public awareness campaign to encourage cooperation from industry and the community.

3.3.2 Option 2 — Containment, control and zoning

If reservoirs of infection were to become established in wild fish stocks or on numerous farms, eradication would be impracticable. In such a case, the preferred response option is containment and prevention of further spread in order to protect and maintain uninfected areas. Containment, control and zoning would also apply outside any IP where eradication is being attempted.

It should be possible to maintain uninfected areas/zones free of VER, and a zoning program would both assist the Australian aquaculture industry and protect potentially susceptible wild fish species. Restrictions on the movement of fish and fish products and a surveillance program would be necessary to support zoning. Farms in endemic areas would need to implement management practices to reduce the severity and incidence of VER outbreaks.

Control procedures are similar to those for eradication. However, there would be an emphasis on managing the disease in individual facilities rather than on eradication. Strategies used for control of VER may include:

- quarantine and movement controls/restrictions on fish, fish products, water and any other vectors (including fomites) within the zone and to free zones;
- destruction and disposal of all clinically diseased fish;
- emphasis on high standards of hygiene and biosecurity;
- tracing and surveillance to determine the source and extent of infection;
- zoning to define infected and disease-free areas; and
- a public awareness campaign to encourage cooperation from industry and the community.

Within endemic zones, it may not be economically viable for some farms to institute the controls suggested above, and deregulation of VER may be appropriate. Some operators may opt for mitigation only, realising that this may restrict markets for their live fish and, possibly, for their fish products. Such mitigation practices may include ozonation of eggs, green pond culture, and separation of discrete groups of fish. Individual operators may choose to attempt eradication to minimise restrictions on trade to other zones.

3.4 Strategies for control and eradication

Strategies for the control and eradication of VER are summarised in Table 2, and described in detail in Sections 3.4.1 to 3.4.10.

Table 2 Summary of strategies used for each of the response options for VER

Strategy	Eradication	Control
Quarantine	Yes	Yes
Declared restricted/control areas	Yes	No
Zoning	Yes	Yes
Movement controls within zone	Yes	Yes
Movement controls out of zone	Yes	Yes
Destruction of clinical cases	Yes	Yes
Destruction of unexposed fish	Optional	No
Destruction of exposed or potentially exposed but clinically normal fish	Yes	Optional
Treatment of fish products and byproducts — exposed	Destroyed	Optional
Treatment of fish products and byproducts — unexposed	Yes	No
Disposal of infected fish and wastes	Yes	Yes
Decontamination	Required	Optional
Surveillance	Yes	Yes
Tracing	Yes	Optional
Disease mitigation	Yes	Optional

3.4.1 Quarantine and movement controls

Until the most appropriate control strategy is determined, quarantine and movement controls should be implemented on anything capable of transmitting infection. Once the strategy is determined, quarantine and movement controls can be altered accordingly. See the **Enterprise Manual** for details of movement controls for different enterprise systems and response options.

For eradication, quarantine and movement controls must be strictly enforced on fish, fish products, water, fomites and any vectors in declared areas capable of spreading the virus. Movement controls should be maintained until the disease is either eradicated or declared endemic.

For the other response options, movement controls are essential to maintain uninfected areas/zones. Restrictions must apply to anything capable of transmitting betanodaviruses from infected to uninfected fish populations, aquatic systems and processing plants.

3.4.2 Zoning

Zoning for VER should be based on geographic and/or state/territory boundaries and the known distribution of VER and infected host species. At least initially, zoning should be limited to control (infected) and free (uninfected) zones, with strict controls on the movement of susceptible fish, fish products and equipment between zones.

Where zoning is implemented, an active surveillance program for VER is necessary in uninfected zones, and state/territory-based legislation is required to support zoning, movement controls and surveillance activities.

3.4.3 Destruction of clinically diseased fish

Immediate removal, destruction and disposal of all diseased and dead fish is essential to the success of any response strategy. These fish, along with infectious wastes, are the main source of betanodavirus in the environment. Diseased and dead fish must be removed from tanks and ponds and destroyed as a high priority. Burial sites should be chosen carefully to ensure that there is no contact with waterways or vectors.

3.4.4 Destruction of unexposed fish

Eradication

Young (pre-market sized) unexposed fish may be allowed to grow out, provided there is no risk of future infection. To ensure that the population remains unexposed throughout grow-out, harvesting and slaughter, water systems, equipment and all handling procedures must bear no risk of infection. Strict farm hygiene practices and transportation protocols are necessary to ensure that there is no transfer of infection to non-infected fish populations via handling, equipment or any husbandry practice.

Table-sized fish without any possible exposure to infection may be emergency harvested and slaughtered for human consumption. Strict hygiene practices are required at processing. The method of harvest, equipment used and location must also carry no risk of exposure to infection. Onfarm processing may be preferable, as it will prevent any potential infection during transport to off-site processing plants.

Immediate destruction should be considered for unexposed fish populations located within an infected zone, especially for larval/juvenile barramundi, which have a low unit value. Immediate destruction of such populations will decrease the chance of spread of infection to fish stocks and prevent propagation of the disease.

Containment, control and zoning

If the chosen strategy is for control, containment and zoning, grow-out and slaughter for human consumption can occur as normal. Control measures are only required to prevent transmission of infection to unexposed fish in uninfected areas/zones. The method of harvest, equipment used and choice of location should ensure that there is no exposure to infection.

3.4.5 Destruction of exposed or potentially exposed but clinically normal fish

Eradication

In facilities undergoing eradication, exposed or potentially exposed but clinically normal fish should be regarded as infected and destroyed. Healthy, covertly infected fish are safe for human consumption. However, emergency harvesting and slaughter of healthy exposed or potentially exposed fish carries a high risk of

further transfer of infection. Although it is an option, it may jeopardise the success of an eradication strategy, and destruction and disposal is preferred.

Containment, control and zoning

Grow-out of exposed or potentially exposed but clinically normal fish is possible in infected zones under current farm management practices. However, year-class separation or 'all-in, all-out' management practices may need to be implemented. Immediate destruction of these fish will decrease the infectious load on a site and should minimise not only the spread of infection but also the incidence of outbreaks. However, if infection is endemic in the area, reinfection of the newly stocked fish populations will occur via intake water and, since destocking a hatchery has significant economic impact on hatchery operations and grow-out farms, choice of this option would depend on the infectious status of the local area.

If these fish are allowed to grow-out, they must be treated and handled as infected populations. Restrictions on movements of fish and fish products, people, vehicles and boats and on market access for the final product may be necessary to protect uninfected facilities or zones.

3.4.6 Treatment of fish products and byproducts

The treatment of fish products and byproducts must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via the product. Harvested, gilled and gutted fish, potentially for human consumption, may be stored safely in a freezer until a definitive diagnosis is obtained and decisions are made regarding release of product. This will prevent the spread of infection and allow salvage of product for sale (provided the relevant authority approves release).

Eradication

All products and byproducts from facilities undergoing eradication should be destroyed.

Containment, control and zoning

Unexposed products may be marketed and disseminated without any risk of transmission of infection. However, products from exposed fish populations will require processing and/or may have their market restricted in order to maintain VER-free areas/zones.

If uninfected areas/zones are not established, there will be fewer restrictions on the treatment and release of product onto the market. Fish products and byproducts may be traded within an endemic zone without restrictions, but not from an endemic zone into an uninfected zone. Such trade in table-ready fish currently occurs, as well as translocation of dead fish caught by professional and amateur fishers. However, domestic market regulations (eg state/territory legislation) and food safety standards must be considered when determining the required treatment of products and byproducts.

3.4.7 Disposal

Eradication

Immediate, safe disposal of all infected fish, wastes, effluent and equipment (that cannot be decontaminated) is necessary for the eradication of the virus. See the **Operational Procedures Manual** for details. If processing continues in infected establishments or of infected fish, the effluent will require treatment and safe discharge/disposal to prevent spread of infection.

Containment, control and zoning

Safe disposal of all infected dead fish, wastes and effluent is important in decreasing the infectious load on a site, and will greatly assist in reducing the incidence of clinical VER outbreaks.

3.4.8 Decontamination

Eradication

All buildings, tanks, materials and equipment (including nets, boats and vehicles) that may be contaminated must be cleaned and disinfected for successful eradication. At all stages of decontamination, steps must be taken to prevent any spread of infection via water, wastes or materials, especially into natural waterways.

Containment, control and zoning

The implementation of good hygiene practices on infected sites will reduce the incidence of VER outbreaks. Thorough cleaning and disinfection of buildings, tanks, materials and equipment (including nets, boats and vehicles) that may be contaminated is especially important after a clinical outbreak, in order to reduce the infectious load on the site.

3.4.9 Surveillance

Development of a surveillance program for VER depends on the success of FRDC project 2001/626 to develop a nested PCR suitable for Australian conditions and species (see Section 1.4.3).

If a zoning program were to be implemented, targeted active surveillance for betanodaviruses would be necessary to support the declaration of VER-free zones.

3.4.10 Tracing

Tracing should be undertaken as described in Section 2.2.2 for all IPs identified as part of an official control or eradication program. Tracing is not required for IPs in an endemic zone unless they are suspected as the source of an outbreak in another zone.

3.5 Social and economic effects

To date, the ravages of VER have been mainly restricted to tropical and subtropical regions of Australia. VER has already caused the barramundi industry

considerable financial losses, and the move to culture grouper species in Australia will carry a similar danger unless appropriate controls are instituted.

However, temperate regions are unlikely to be spared, as instanced by an outbreak of VER in an experimental striped trumpeter hatchery. Also, initiatives to culture such susceptible species as flounder and silver trevally (striped jack) make outbreaks of VER in temperate regions more likely.

3.6 Criteria for proof of freedom

All the evidence to date supports the suggestion that betanodaviruses are only perpetuated in the marine environment. This is probably because the linchpin in the viruses' life cycle is vertical transmission by purely marine or catadromous fish. It is conceivable that endemic infection could be established in anadromous or even strictly freshwater species, but there is no evidence for this to date. Consequently, all those parts of inland Australia outside the range of catadromous species can provisionally be regarded as free of VER. To prove this would be an unnecessarily expensive task in current circumstances.

Proof of freedom could be established if the farm involved operated a completely closed system (such as a pond system using groundwater), or if the farm was in a recognised provisionally 'free' zone and any introduced fish were from a farm of equivalent status.

3.7 Funding and compensation

As VER is an endemic disease, it is not envisaged that compensation would be provided for its eradication or control.

Appendix 1 Common and scientific names of fish species mentioned in text

Common name	Scientific name
Atlantic halibut	<i>Hippoglossus hippoglossus</i>
Atlantic salmon	<i>Salmo salar</i>
Australian catfish	<i>Tandanus tandanus</i>
Barcoo grunter	<i>Scortum barcoo</i>
Barfin flounder	<i>Verasper moseri</i>
Barramundi/Asian sea bass	<i>Lates calcarifer</i>
Brownspeckled grouper	<i>Epinephelus chlorostigma</i>
European sea bass	<i>Dicentrarchus labrax</i>
Golden perch	<i>Macquaria ambigua</i>
Japanese flounder	<i>Paralichthys olivaceus</i>
Japanese parrotfish	<i>Oplegnathus fasciatus</i>
Macquarie perch	<i>Macquaria australasica</i>
Malabar grouper	<i>Epinephelus malabaricus</i>
Murray cod	<i>Maccullochella peelii</i>
Orangespeckled grouper	<i>Epinephelus coiodes</i>
Redspeckled grouper	<i>Epinephelus akaara</i>
Silver perch	<i>Bidyanus bidyanus</i>
Sleepy cod	<i>Oxyeleotris lineolatus</i>
Striped jack/silver trevally	<i>Pseudocaranx dentex</i>
Striped trumpeter	<i>Latris lineata</i>
Turbot	<i>Scophthalmus maximus</i>
Yellow grouper	<i>Epinephelus awoara</i>

Appendix 2 OIE international animal health code and manual of diagnostic tests for aquatic animals

OIE Aquatic Code

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

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

http://www.oie.int/eng/normes/fcode/a_index.htm

(Accessed on 4 November 2004)

The following chapter is relevant to this manual:

Chapter 2.1.7. Viral encephalopathy and retinopathy

OIE Aquatic Manual

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

The current edition of the OIE Aquatic Manual (3rd edition) was published in 2003 and is available on the OIE website at:

http://www.oie.int/eng/normes/fmanual/A_summry.htm

(Accessed on 4 November 2004)

The following chapter is relevant to this manual:

Chapter 2.1.7. Viral encephalopathy and retinopathy

OIE Disease Technical Cards

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

The current edition of the Disease Technical Cards was published in 2003 and is available on the OIE website at:

http://www.oie.int/aac/eng/cards/en_diseasecard.htm

(Accessed on 4 November 2004)

The following card is relevant to this manual:

Viral Encephalopathy and Retinopathy

Further information

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

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

(Accessed on 4 November 2004)

Glossary

Anadromous fish	Fish species that hatch and live initially in freshwater (as fry), undergo smolt and migrate to seawater (for 'grow-out') and then return to freshwater to spawn. <i>See also</i> Catadromous fish
Aquatic Animal Health Committee	A committee comprising representatives of the Australian government, Australian state and territory governments, the major aquaculture, wild capture, aquarium and recreational fishing industries and a CSIRO representative. The committee provides advice to PIMC on aquatic animal health matters, focusing on technical issues and regulatory policy. <i>See also</i> Primary Industries Ministerial Council (PIMC)
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. <i>See also</i> Chief veterinary officer
AQUAVETPLAN	<i>Australian Aquatic Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. <i>See also</i> AUSVETPLAN
AUSVETPLAN	<i>Australian Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.
Catadromous fish	Fish species that hatch and live initially in seawater (as fry), undergo smolt and migrate to freshwater (for 'grow-out') and then return to seawater to spawn. <i>See also</i> Anadromous fish
Chief veterinary officer (CVO)	The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. <i>See also</i> Australian Chief Veterinary Officer
Compensation	The sum of money paid by government to an owner for stock that are destroyed and property that is compulsorily destroyed because of an emergency animal disease.

Control area ^a	A buffer between the restricted area and areas free of disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may reduce in size. The shape of the area may be modified according to circumstances, eg water flows, catchment limits etc. In most cases, permits will be required to move animals and specified product out of the control area into the free area.
Covert infection	Clinically inapparent infection betanodavirus that is transmissible and that may eventually lead to clinical disease.
Dangerous contact animal	A susceptible animal that has been designated as being exposed to other infected animals or potentially infectious products following tracing and epidemiological investigation.
Dangerous contact premises or area ^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	A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include <i>restricted area, control area, infected premises, dangerous contact premises and suspect premises</i> .
Decontamination	Includes all stages of cleaning and disinfection.
Disease agent	A general term for a transmissible organism or other factor that causes an infectious disease.
Disinfectant	A chemical used to destroy disease agents outside a living animal.
Disinfection	The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies to premises, vehicles and different objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of fish carcasses and things by burial, burning or some other process so as to prevent the spread of disease.
Enzyme-linked immunosorbent assay (ELISA)	A serological test designed to detect and measure the presence of antibody or antigen in a sample. The test uses an enzyme reaction with a substrate to produce a colour change when antigen-antibody binding occurs.

Emergency animal disease	<p>A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications.</p> <p><i>See also</i> Endemic animal disease, Exotic animal disease</p>
Endemic animal disease	<p>A disease affecting animals (which may include humans) that is known to occur in Australia.</p> <p><i>See also</i> Emergency animal disease, Exotic animal disease</p>
Enterprise	<p><i>See</i> Risk enterprise</p>
Epidemiological investigation	<p>An investigation to identify and qualify the risk factors associated with the disease.</p>
Exotic animal disease	<p>A disease affecting animals (which may include humans) that does not normally occur in Australia.</p> <p><i>See also</i> Emergency animal disease, Endemic animal disease</p>
Fish byproducts	<p>Products of fish origin destined for industrial use (eg fishmeal).</p>
Fish products	<p>Fish meat products and products of fish origin (eg eggs) for human consumption or use in animal feeding.</p>
Fomites	<p>Inanimate objects (eg boots, clothing, equipment, instruments, vehicles, crates, packaging) that can carry an infectious disease agent and may spread the disease through mechanical transmission.</p>
Free area	<p>An area known to be free of the disease agent.</p>
Infected premises or area ^a	<p>The area in which the disease has been confirmed.</p> <p>Definition of an 'infected area' is more likely to apply to an open system, such as an oceanic lease.</p>
Local disease control centre	<p>An emergency operations centre responsible for the command and control of field operations in a defined area.</p>
Mitigation	<p>Reduction in severity of a disease to reduce its impact.</p>
Monitoring	<p>Routine collection of data for assessing the health status of a population.</p> <p><i>See also</i> Surveillance</p>
Movement control	<p>Restrictions placed on the movement of fish, people and other things to prevent the spread of disease.</p>
Nested RT-PCR	<p>A double-stage PCR process where the second round identifies a DNA sequence 'nested' within the initial sequence thus increasing the specificity.</p> <p><i>See</i> <i>Polymerase chain reaction (PCR)</i> and <i>Reverse transcriptase-PCR (RT-PCR)</i></p>

OIE Aquatic Code	<p><i>OIE International Aquatic Animal Health Code</i>. Published on the internet at: http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 4 November 2004). See Appendix 2 for further details</p>
OIE Aquatic Manual	<p><i>OIE Manual of Diagnostic Tests for Aquatic Animals</i>. Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines). The current edition is published on the internet at: http://www.oie.int/eng/normes/fmanual/A_summry.htm (Accessed 4 November 2004). See Appendix 2 for further details</p>
Operational procedures	<p>Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.</p>
Owner	<p>Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).</p>
Polymerase chain reaction (PCR)	<p>A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also <i>Reverse transcriptase-PCR (RT-PCR)</i> and <i>Nested RT-PCR</i></p>
Premises or area	<p>Production sites that may range from an aquarium to an aquaculture lease in the open ocean.</p>
Prevalence	<p>The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or positive antibody titre) at a given point in time.</p>
Primary Industries Ministerial Council	<p>The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Agriculture and Resource Management Council of Australia and New Zealand).</p>
Quarantine	<p>Legal restrictions imposed on a place, fish, vehicle, or other things, limiting movement.</p>
Restricted area ^a	<p>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.</p>

Reverse transcriptase-PCR (RT-PCR)	A highly sensitive technique for the detection and quantitation of mRNA (messenger RNA) by reverse transcription to DNA followed by PCR . <i>See Polymerase chain reaction (PCR) and Nested RT-PCR</i>
Risk enterprise	A defined livestock or related enterprise, which is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots and garbage depots.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). <i>See also Specificity</i>
Serotype	A subgroup of microorganisms identified by the antigens carried (as determined by a serology test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). <i>See also Sensitivity</i>
State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible fish	Fish that can be infected with a particular disease.
Suspect fish	Fish that may have been exposed to an emergency disease such that its quarantine and intensive surveillance, but not pre-emptive slaughter, is warranted. <i>or</i> Fish not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis.
Suspect premises or area ^a	Temporary classification of premises containing suspect fish. After rapid resolution of the status of the suspect fish contained on it, a suspect premises is reclassified either as an infected premises (and appropriate disease-control measures taken) or as free from disease. The reason for the suspicion varies with the agent; however, it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.

Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.
Vaccine	Modified strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
Vector	A living organism that transmits an infectious agent from one host to another. A <i>biological</i> vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A <i>mechanical</i> vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.
Zoning	The process of defining disease-free and infected areas.

^aDue 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
AHNV	Atlantic halibut nodavirus
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
aqCCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
AUSVETPLAN	Australian Veterinary Emergency Plan
BFNNV	barfin flounder nervous necrosis virus
CA	control area
CCEAD	Consultative Committee on Emergency Animal Diseases
CPE	cytopathic effect
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Department of Agriculture, Fisheries and Forestry (Australian Government)
DIEV	Dicentrarchus labrax encephalitis virus
EAD	emergency animal disease
ELISA	enzyme-linked immunosorbent assay
FRDC	Fisheries Research and Development Corporation
H&E	haematoxylin and eosin
IFAT	indirect fluorescent antibody test
IP	infected premises
JFNNV	Japanese flounder nervous necrosis virus
LcEV	<i>Lates calcarifer</i> encephalitis virus
MGNNV	Malabar grouper nervous necrosis virus
OIE	World Organisation for Animal Health (Office International des Epizooties)

PAS	periodic acid Schiff
PCR	polymerase chain reaction
RA	restricted area
RGNNV	redspotted grouper nervous necrosis virus
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SDCHQ	state or territory disease control headquarters
SJNNV	striped jack nervous necrosis virus
SP	suspect premises
TPNNV	tiger puffer nervous necrosis virus
VER	viral encephalopathy and retinopathy

References

- AFFA (Agriculture, Fisheries and Forestry – Australia) (2000). *AQUAPLAN Zoning Policy Guidelines*, AFFA, Canberra.
- Arimoto M, Mushiaki K, Mizuta Y, Nakai T, Muroga K and Furusawa I (1992). Detection of striped jack nervous necrosis virus (SJNNV) by enzyme-linked immunosorbent assay (ELISA). *Fish Pathology* 27:191–195.
- Arimoto M, Mori K, Nakai T, Muroga K and Furusawa I (1993). Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack, *Pseudocaranx dentex* (Bloch and Schneider). *Journal of Fish Diseases* 16:461–469.
- Arimoto M, Sato J, Maruyama K, Mimura G and Furusawa I (1996). Effect of chemical and physical treatments on the inactivation of striped jack nervous necrosis virus (SJNNV). *Aquaculture* 143:15–22.
- Aspehaug V, Devold M and Nylund A (1999). The phylogenetic relationship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). *Bulletin of the European Association of Fish Pathologists* 19:196–202.
- Ball LA, Hendry DA, Johnson JE, Ruechert RR and Scotti PD (2000). Family Nodaviridae. In: *Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses*, Van Regenmortel MHV, Fauquet CM, Bishop DHZ, Carstens EB, Estes MK, Lemon SM, Miniloff J, Mayo MA, McGeoch DJ, Pringle CR and Wickner RB, eds, Academic Press, New York, 747–755.
- Bloch B, Gravningen K and Larsen JL (1991). Encephalomyelitis among turbot associated with a picornavirus-like agent. *Diseases of Aquatic Organisms* 10:65–70.
- Boonyaratpalin S, Supamattaya K, Kasornchandra J and Hoffman RW (1996). Picorna-like virus associated with mortality and a spongiosis encephalopathy in grouper *Epinephelus malabaricus*. *Diseases of Aquatic Organisms* 26:75–80.
- Breuil G, Bonami JR, Pepin JF and Pichot Y (1991). Viral infection (picorna-like virus) associated with mass mortalities in hatchery-reared sea-bass (*Dicentrarchus labrax*) larvae and juveniles. *Aquaculture* 97:109–116.
- Breuil G, Pepin JF, Castric J, Fauvel C and Thiery R (2000). Detection of serum antibodies against nodavirus in wild and farmed adult sea bass: Application to the screening of broodstock in sea bass hatcheries. *Bulletin of the European Association of Fish Pathologists* 20:95–100.
- Chang SF, Ngoh GH, Kueh LFS, Qin QW, Chen CL, Lam TJ and Sin YM (2001). Development of a tropical marine fish cell line from Asian seabass (*Lates calcarifer*) for virus isolation. *Aquaculture* 192:133–145.
- Chi SC, Hu WW and Lo BL (1999). Establishment and characterization of a continuous cell line (GF-1) derived from grouper, *Epinephelus coioides* (Hamilton): a cell line susceptible to grouper nervous necrosis virus (GNNV). *Journal of Fish Diseases* 22:173–182.

- Chong SY, Ngoh GH and Chew-Lim M (1990). Study of three tissue culture viral isolates from marine foodfish. *Singapore Journal of Primary Industry* 18:54-57.
- Dalla Valle L, Zanella L, Patarnello P, Paolucci L, Belvedere P and Colombo L (2000). Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. *Journal of Fish Diseases* 23:321-327.
- Frerichs GN, Rodger HD and Peric Z (1996). Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, *Dicentrarchus labrax*. *Journal of General Virology* 77:2067-2071.
- Frerichs GN, Tweedie A, Starkey WG and Richards RH (2000). Temperature, pH and electrolyte sensitivity, and heat, UV and disinfection inactivation of sea bass (*Dicentrarchus labrax*) neuropathy virus. *Aquaculture* 185:13-24.
- Fukuda Y, Nguyen HD, Furuhashi M and Nakai T (1996). Mass mortality of cultured seven band grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. *Fish Pathology* 31:165-170.
- Glazebrook J (1995). Disease risks associated with the translocation of a virus lethal for barramundi (*Lates calcarifer*) Bloch. Master of Environmental Management thesis, Griffith University, Queensland, Australia.
- Glazebrook JS, Heasman MP and de Beer SW (1990). Picorna-like viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* (Bloch). *Journal of Fish Diseases* 13:245-249.
- Grotmol S and Totland GK (2000). Surface disinfection of Atlantic halibut *Hippoglossus hippoglossus* eggs with ozonated sea-water inactivates nodavirus and increases survival of the larvae. *Diseases of Aquatic Organisms* 39:89-96.
- Grotmol S, Totland GK, Kvellestad A, Fjell K and Olsen AB (1995). Mass mortality of larval and juvenile hatchery-reared halibut (*Hippoglossus hippoglossus* L.) associated with the presence or virus-like particles in the central nervous system and retina. *Bulletin of the European Association of Fish Pathologists* 15:176-180.
- Grotmol S, Totland GK, Torud K and Hjeltnes BK (1997a). Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms* 29:85-97.
- Grotmol S, Totland GK and Kryvi H (1997b). Detection of a nodavirus-like agent in heart tissue from reared Atlantic salmon *Salmo salar* suffering from cardiac myopathy syndrome (CMS). *Diseases of Aquatic Organisms* 29:79-84.
- Grotmol S, Nerland AH, Biering E, Totland GK and Nishizawa T (2000). Characterisation of the capsid protein gene from a nodavirus strain affecting the Atlantic halibut *Hippoglossus hippoglossus* and design of an optimal reverse-transcriptase polymerase chain reaction (RT-PCR) detection assay. *Diseases of Aquatic Organisms* 39:79-88.
- Herfort, A (2004). *Aquatic Animal Diseases Significant to Australia: Identification Field Guide*. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.

- Huang B, Tan C, Chang SF, Munday B, Mathew J, Nghoh GH and Kwang J (2001). Detection of nodavirus infection in experimentally infected and commercial barramundi (*Lates calcarifer* Bloch) using recombinant coat protein-based ELISA and RT-PCR. *Journal of Fish Diseases* 24:135–142.
- Iwamoto T, Mori K, Arimoto M and Nakai T (1999). High permissivity of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* 39:37–47.
- Iwamoto T, Nakai T, Mori K, Arimoto M and Furusawa I (2000). Cloning of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* 43:81–89.
- Iwamoto T, Mori K, Arimoto M and Nakai T (2001). A combined cell-culture and RT-PCR method for rapid detection of piscine nodaviruses. *Journal of Fish Diseases* 24:231–236.
- Lai YS, Murali S, Chiu HC, Ju HY, Lin YS, Chen SC, Guo IC, Fang K and Chang CY (2001). Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus-susceptible cell line from yellow grouper, *Epinephelus awoara* (Temminck and Schlegel), brain tissue. *Journal of Fish Diseases* 24:299–309.
- Le Breton A, Grisez L, Sweetman J and Ollevier F (1997). Viral nervous necrosis (VNN) associated with mass mortalities in caged-reared sea bass *Dicentrarchus labrax* L. *Journal of Fish Diseases* 20:145–151.
- Moody NJ, Horwood PF and McHardy S (2004). *Development of Diagnostic Tests for the Detection of Nodavirus*. Final report of FRDC project 2001/626. Fisheries Research and Development Corporation, Canberra, Australia.
- Mori, K Nakai T, Nagahara M, Muroga K, Mekuchi T and Kanno T (1991). A viral disease in hatchery-reared larvae and juveniles of redspotted grouper. *Fish Pathology* 26:209–210.
- Mori K, Nakai T, Muroga K, Arimoro M, Mushiake K and Furusawa I (1992). Properties of a new virus belonging to Nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* 187:368–371.
- Munday BL and Nakai T (1997). Special topic review: Nodaviruses as pathogens in larval and juvenile marine finfish. *World Journal of Microbiology and Biotechnology* 13:375–381.
- Munday BL, Langdon JS, Hyatt A and Humphrey JD (1992). Mass mortality associated with a viral-induced vacuolating encephalopathy and retinopathy of larval and juvenile barramundi, *Lates calcarifer* Bloch. *Aquaculture* 103:197–211.
- Munday BL, Kwang J and Moody N (2002). Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases* 25:127–142.
- Mushiake K, Arimoto M, Furusawa T, Furusawa I, Nakai T and Muroga K (1992). Detection of antibodies against striped jack nervous necrosis virus (SJNNV) from brood stocks of striped jack. *Nippon Suisan Gakkaishi* 58:2351–2356.
- Nakai T (2000). Recent advances in the diagnosis and control of viral nervous necrosis (VNN) in groupers. APECFWG 02/2000. Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development, Bangkok, 18–20 October 2000.

- Nguyen HD, Mushiake K, Nakai T and Muroga K (1997). Tissue distribution of striped jack nervous necrosis virus (SJNNV) in adult striped jack. *Diseases of Aquatic Organisms* 28:87-91.
- Nilsen R, Ranheim T, Hansen MK, Taksdal T and Totland GK (2001). Pathology in persistent nodavirus infected juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Abstracts of 10th International Conference of the EAFP*, 9-14 September 2001. Abstract P-117.
- Simon RC and Schill WB (1984). Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. *Journal of Fish Diseases* 7:515-520.
- Tanaka A, Aoki H and Nakai T (1998). Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathology* 33:31-36.
- Tanaka S, Mori K, Arimoto M, Iwamoto T and Nakai T (2001). Protective immunity of sevenband grouper, *Epinephelus septemfasciatus* Thunberg, against experimental viral nervous necrosis. *Journal of Fish Diseases* 24:15-22.
- Thiery R, Raymond JC and Castric J (1999). Natural outbreak of viral encephalopathy and retinopathy in juvenile sea bass, *Dicentrarchus labrax*: study by nested reverse transcriptase-polymerase chain reaction. *Virus Research* 63:11-17.
- Watanabe K, Nishizawa T and Yoshimizu M (2000). Selection of broodstock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. *Diseases of Aquatic Organisms* 41:219-223.
- Watts M, Munday BL and Burke CM (2001). Immune responses of teleost fish. *Australian Veterinary Journal* 79:570-574.
- Williams CJ and Moffit CM (2001). A critique of methods of sampling and reporting pathogens in populations of fish. *Journal of Aquatic Animal Health* 13:300-309.
- Yoshikoshi K and Inoue K (1990). Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathus fasciatus* (Temminck and Schlegel). *Journal of Fish Diseases* 13:69-77.
- Yuasa K, Koesharyani I, Roza D, Mori K, Katata M and Nakai T (2002). Immune response of humpback grouper, *Cromileptes altivelis* (Valenciennes) injected with the recombinant coat protein of betanodavirus. *Journal of Fish Diseases* 25:53-56.

AQUAVETPLAN CD

Agriculture, Fisheries and Forestry – Australia (2002). AQUAVETPLAN (CD), Canberra (due to be updated in 2005). Available upon request from aah@daff.gov.au.

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