Viral encephalopathy and retinopathy – disease strategy manual

Dr B Munday

December 2003

FRDC Project No. 2002/643

Australian Government
Department of Agriculture, Fisheries and Forestry

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Author: Dr B Munday

Title: Aquatic Animal Health Subprogram: viral encephalopathy and retinopathy – disease strategy manual

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NON-TECHNICAL SUMMARY

2002/643 Aquatic Animal Health Subprogram: viral encephalopathy and retinopathy – disease strategy manual

PRINCIPAL INVESTIGATOR: Dr B Munday
ADDRESS: IDEXX Laboratories
3 Overend Street
East Brisbane QLD 4169
Telephone: 07 3456 6000 Fax: 07 3891 0702

OBJECTIVES:
1. To write the disease strategy manual for VER.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

The disease strategy manual for VER has been completed following extensive consultation with industry and aquatic disease investigators. National adoption of the manual will enhance the capabilities of industry and government to quickly and effectively respond to outbreaks of VER.

The disease strategy manual for VER reviews the relevant scientific literature on an economically important aquatic viral disease. A description of the disease with special emphasis on Australian fin fish species reveals significant disease in farmed barramundi and significant potential for disease in species such as grouper and striped trumpeter with farming potential.

The general principals of control and eradication are reviewed. Various strategies outlined in the manual will enhance the capabilities of industry and government to quickly and effectively respond to outbreaks of VER.

KEYWORDS: betanodavirus, VER, aquaculture, disease strategy manual

ACKNOWLEDGMENTS

Nil

BACKGROUND

VER of barramundi has been a significant problem in the aquaculture industry since the first Australian attempts to commercialize this species. Indeed, the first hatchery failed financially principally because of this disease. Although it has not yet assumed such proportions in other aquaculture pursuits in Australia, it has potential to do so. There is no evidence that betanodavirus infection has a significant effect on wild stocks under normal conditions, probably because the density of larvae is below that at which epizootics of VER are initiated. However, in Queensland, some hatchery-raised fish are used for restocking purposes and outbreaks of VER impact adversely on such programs.

NEED

Barramundi are an important aquaculture species with total production predicted to exceed 1000 tonnes within the next few years. Grouper culture is only just being developed but these fish are greatly sought for the live fish trade and as a result, wild stocks are becoming depleted. However, outbreaks of VER in hatcheries and grow-out facilities can lead to catastrophic losses and the expected increases in production will not occur unless practicable and efficacious control programs are available and in place. Striped trumpeter culture is still at the development stage but already an outbreak of VER has resulted in significant losses of valuable juveniles. The disease control program needs to be acceptable to all stakeholders, especially State and Territory authorities so that uniformity in control procedures is ensured. At present, differing requirements between authorities reduces their abilities to adequately control VER.

OBJECTIVES

The objective was to produce a manual that will provide information that will enhance the capabilities of industry and government to quickly and effectively respond to outbreaks of Viral Encephalopathy and Retinopathy (VER).

METHODS

Format of the Disease Strategy Manual

The output from this project is a disease strategy manual for VER, with contents headings similar to that for the furunculosis manual. There are three main sections:
Section 1 - nature of the disease
Section 2 - general principles of control and eradication
Section 3 - response options
In addition, a number of appendices are required.

The format of each section is as follows:

Section 1 - The nature of the disease

- Aetiology
- Susceptible species
- World distribution and occurrence in Australia
- Diagnostic criteria
    - History
    - Clinical signs
    - Histopathology
    - Laboratory tests
- Resistance and immunity
- Epidemiology

Section 2 – Principles of control and eradication

- Introduction
- Methods to prevent spread and eliminate pathogens
    - Quarantine and movement control
    - Tracing
    - Surveillance
    - Destruction of fish
    - Treatment of fish products and by-products
    - Disposal
    - Decontamination
    - Environmental considerations
    - Vector control
- Feasibility of options for control and eradication
    - Eradication
    - Containment, control and zoning

Section 3 - Preferred control policies in Australia

- Overall policy for VER
- Problem definition
- Overview of response options
- Strategies for control and eradication
- Social and economic effects
- Criteria for proof of freedom
- Funding and compensation

Writing methodology

The Disease Strategy Manual has been written by three primary writers with 2 writers (Munday and Miller) concentrating on sections 1 and 2 and 1 writer (Baldock) concentrating on section 3. All writers were involved in the review processes.

The first two sections (Sections 1 and 2), plus some of the appendices, have been written concurrently. Much of the work for these sections has been undertaken by reviewing existing knowledge through literature searches and consultation with scientists around the world. The authors already possess an extensive collection of published and unpublished articles on diseases and pests of fish, and have ready access to an extensive network of scientists both in Australia (Departments of Agriculture, Universities and CSIRO) and

internationally (Asia, the Americas and Europe including the unit at Stirling University) who are active in the field.

Section 3, focusing on appropriate control and eradication options, has been written after the first two sections were completed to a reasonable draft stage. Based on overseas experience, and in consultation with the client-nominated steering committee, careful attention has been paid to options that are suitable for Australian conditions.

Internal and external sources with experience in fish diseases and aquaculture industries will be used to review the document on two occasions:

- Firstly, once the first three sections have been completed by the three primary writers, and
- Secondly, after the issues raised by the initial stakeholder review have been addressed by the project team.

Once this process is complete, a process of review by the client-nominated steering group will be used for further iterations of drafts.

A summary of the writing methodology is presented in the following table:

Step	Activity	Output
1	Writing by project team	Draft 1
2	Peer review	Draft 2
3	Stakeholder review	Draft 3
4	Peer review	Draft 4
5	Final report	Draft 5

RESULTS/DISCUSSION

The output of this project is attached.

BENEFITS AND ADOPTION

Sectors of the aquaculture industry included breeders and growers involved in fish farming as well as State and Territory authorities involved in disease control will benefit from this manual.

FURTHER DEVELOPMENT

The attached manual will need to be formally endorsed by industries and governments in Australia. It is recommended that when additional disease strategy manuals are developed, this and others be reviewed to further consolidate and standardise disease investigation and control strategies.

PLANNED OUTCOMES

The manual has been modeled on “AFFA (2001). Disease strategy. In: Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Agriculture, Fisheries and Forestry – Australia, Canberra, ACT for furunculosis” and, as such, will help ensure that the present

thriving commercial barramundi industry and the evolving grouper and striped trumpeter aquaculture resources are able to prosper despite the presence of endemic VER. The beneficiaries will include aquaculturists and the general public who presently enjoy access to prime marine fish products.

CONCLUSION

Preparation of the VER – disease strategy manual meets the primary objective.

REFERENCES

Nil

STAFF

Dr Barry Munday, BVSc, MVSC, DVSc, MACVSc, Specialist Veterinary Pathologist

Dr Richard Miller, BVSc, MSc, PhD, MACVSc, DACVP, Specialist Veterinary Pathologist

Dr Chris Baldock, BVSc, MACVSc, MPVM, PhD, FACVSc. Specialist Veterinary Epidemiologist

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APPENDIX 1: DISEASE STRATEGY MANUAL FOR VIRAL ENCEPHALOPATHY AND RETINOPATHY

FRDC Aquatic Animal Health Subprogram:
Viral encephalopathy and retinopathy – disease strategy manual.

Contents

1. Nature of the disease

Nodavirus infections of teleost fish, variously termed viral nervous necrosis (VNN) (Yoshikoshi and Inoue 1990), encephalomyelitis (Bloch, Gravningen and Larsen 1991) and vacuolating encephalopathy and retinopathy (VER) (Munday, Langdon, Hyatt, and Humphrey 1992) have emerged as major constraints on culture and sea-farming of a number of marine species.

VER is a disease that occurs mainly in larval and juvenile marine finfish. It is particularly a problem when stocking density is high and, therefore, has caused severe losses in hatcheries. At least one species of the virus, *Lates calcarifer encephalitis virus* (LcEV), occurs in Australia (Munday *et al.* 2002).

1.1. Aetiology

Fish nodaviruses are classified as genus Betanodavirus 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* (DlEV), *Japanese flounder nervous necrosis virus* (JFNNV), *Lates calcarifer encephalitis virus* (LcEV), *red spotted grouper nervous necrosis virus* (RGNNV), *striped jack nervous necrosis virus* (SJNNV)and *tiger puffer nervous necrosis virus* (TPNNV). Tentative species names are *Atlantic halibut nodavirus* (AHNV) and *Malabar grouper nervous necrosis virus* (MGNNV). However, there are many unnamed species and the classification of fish nodaviruses is still evolving.

Morphologically, fish nodaviruses are icosahedral, non-enveloped viruses with a commonly reported diameter of about 25 nm and a range of 20-34 nm. Some authors have described an electron-dense core of 13-21 nm, surrounded by a clear layer of about 5 nm. The virions may be membrane-bound by endoplasmic reticulum or are free in the cytoplasm and may present as paracrystalline arrays (Bloch *et al.* 1991; Boonyaratpalin *et al.*1996; Breuil *et al.* 1991, Glazebrook *et al.* 1990; Grotmol *et al.* 1997b). The virions contain two single-stranded, positive sense RNA molecules, without a poly(A) tail at the 3' end (Mori *et al.* 1992).

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

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 species involved has been cultured barramundi, which suffer 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. A. Hyatt, AAHL, Victoria, Australia (pers comm) found typical histological lesions and viral particles in the brains of Australian catfish outside cages holding barramundi in freshwater. Also, N. Moody, DPI, Queensland, Australia (pers comm) isolated a nodavirus from clinical cases of VER in sleepy cod in freshwater and not in contact with barramundi. 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, DPI, Queensland, Australia pers comm). However, it appears that the freshwater species are less susceptible to infection than barramundi (I. Anderson, DPI, Queensland, Australia pers comm).

Grotmol *et al.* (1997a) 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.L.Munday and T.Nakai unpublished).

1.3. World distribution and occurrence in Australia

It is notable that VER has been reported from all continents with the exception of Africa. Also, those regions undertaking intensive culture of marine species have reported the majority of the syndromes. The preponderance of groupers, sea bass and flatfish among the affected species is particularly apparent (Munday *et al.* 2002). Of concern is the fact that, as well as apparent spread of these viruses within the natural ranges of the affected species as a result of commerce, the diseases have also been reported in these species in other countries, where they do not naturally occur, and to which seed stock have been exported. While it is possible that they have been infected by endemic strains of nodaviruses, epidemiological evidence suggests that the seed stock may have carried exotic strains of nodaviruses with them.

In Australia, clinical VER has been recorded from sites as far apart as Queensland, the Northern Territory and Tasmania (Munday *et al.* 2002). 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 (R.Reuter, T.Nakai and B.L.Munday unpublished).

1.4. Diagnostic criteria

Presumptive diagnosis of VER can be made in endemic zones on the basis of history and clinical signs but this 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 electronmicroscopy 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 the above methods.

1.4.1. History

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, National Aquaculture Centre, Malta 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). As groupers are being developed as aquaculture species in Australia, these observations are of considerable importance.

Table 1. Important 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	-
Red spotted grouper	14 dph (7-8 mm tl)+	9-10 mm tl	<40 mm tl	80%	Up to 100%
Brown spotted 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, -, data not available

1.4.2. Clinical signs

In general, the clinical signs relate to lesions in the brain and retina, ie 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 (Bloch *et al.* 1991; Boonyaratpalin *et al.*1996; Breuil *et al.*1991; Glazebrook *et al.* 1990; Grotmol *et al.* 1997b; Mori *et al.* 1991; Mori *et al.* 1992; Yoshikoshi and Inoue 1990). Swim bladder hyperinflation has been reported in barramundi, European sea bass and striped jack (Breuil

et al. 1991; Mori *et al.* 1992; B.L.Munday unpublished). Larval barramundi and halibut become paler (Glazebrook *et al.* 1990; Grotmol *et al.* 1997b), whereas groupers, juvenile halibut, European sea bass and turbot become darker. Some fish become thin due to anorexia but the main outcome is mass mortality, especially of larvae (Munday and Nakai 1997).

1.4.3. 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.

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 lesion is the presence of 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, shrinkage and basophilia of affected cells (Yoshikoshi and Inoue 1990, I. Anderson, DPI, Queensland, Australia pers comm), focal pyknosis and karyorrhexis of neural cells, granularity of the neuropil and the presence of mononuclear cell infiltrates (Grotmol *et al.* 1995, I. Anderson, DPI, Queensland, Australia pers comm). Cerebral vascular lesions have been described by Munday *et al.* (1992), who found eosinophilic, 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 brown spotted 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). I. Anderson, DPI, Queensland, Australia (pers comm) reported mild vacuolation and the presence of macrophage-like cells in the brains of asymptomatic, experimentally-infected Barcoo grunter.

Retinal lesions have been described in all species where the eye has been examined. 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.* (1997b) 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 I. Anderson, DPI, Queensland, Australia (pers comm) in experimentally-infected Barcoo grunter.

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

1.4.4. Laboratory tests

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 spread of infection between geographic sites, and even between farms, so it is necessary for aquaculturists to ascertain current regulations prior to relocating susceptible species. In most instances some form of assurance, in the form of laboratory tests, will be required for fish to be translocated between states or from endemic (marine) to non-endemic (freshwater) environments.

A number of laboratory procedures of varying sensitivity and specificity can be used to detect nodavirus infections in clinically and inapparently-infected fish. It is probable that the nested RT-PCR being developed by the Oonoonba Veterinary Laboratory (FRDC 2001/626) will become the standard technique for certification of species likely to be infected with nodaviruses.

(i) Light microscopy of central nervous system and eyes

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

As vacuolation of the central nervous system and/or retina does not occur in all subclinically-infected fish histopathology is of relatively low sensitivity when used for certification purposes. Also, the specificity is variable depending on the extent and severity of lesions; this can be improved by the use of IFAT or immunoperoxidase techniques.

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

(ii) Electronmicroscopy

Rapid demonstration of nodavirus virions can be made with negative staining techniques. This will demonstrate that the virus has the appropriate morphology but is not fully confirmatory of nodavirus. Positive staining for transmission electronmicroscopy provides more detail and allows even greater assurance that a nodavirus is involved but is not fully confirmatory and is more of a research tool. Electronmicroscopy is mainly useful for supporting a diagnosis of clinical VER and for research purposes.

Suitable specimens are live fish or central nervous system and/or eyes fixed in glutaraldehyde. Contact should be made with the laboratory concerning the exact method of fixation required.

(iii) Molecular techniques

Molecular techniques, particularly polymerase chain reaction amplification (PCR) have become the main diagnostic method for fish nodaviruses particularly as they can detect extremely small quantities of viral RNA in any tissue. The mainstay of this approach has been the PCR amplification of a target sequence of 430 bases of SJNNV RNA2, after reverse transcription of the viral RNA. However, the sensitivity of this assay varies depending on the strain of nodavirus. To overcome this, more specific primers have been

designed for a number of betanodaviruses including LcEV (Glazebrook 1995, R.Reuter, IDEXX/VPS, South Australia, Australia pers comm).

More recently, Dalla Valle *et al.* (2000) reported that a nested RT-PCR for DlEV was 10 to 100-fold more sensitive than the RT-PCR and permitted diagnosis by use of 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 (N. Moody, DPI, Queensland, Australia pers comm and FRDC 2001/626).

It is anticipated that the nested RT-PCR will become the main technique for diagnosing betanodavirus infections in Australia, especially for certification purposes as it has high sensitivity and specificity.

Specimens should be live whole fish or frozen fish or appropriate tissues. Based on other molecular techniques, samples preserved in absolute alcohol should be suitable for routine diagnosis.

(iv) Virus culture

A number of cell lines are now available for culture of betanodaviruses.

The striped snakehead cell line, SSN-1originally 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 due to its stable, clear CPE expression and production of high levels of virus. This cell line has been imported by AAHL.

Chi *et al.* (1999) and Lai *et al.* (2001) have developed cell lines from groupers *Epinephelus coioides* (Hamilton) and *E. awoara* (Temminck and Schlegel) which support grouper nervous necrosis viruses but 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 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 as part of a combined cell-culture-RT-PCR technique (Iwamoto *et al.* 2001).

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

(v) 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, DPI, Queensland, Australia pers comm) and serology may be used more

for epidemiological studies rather than diagnostic purposes in the future. The development of polyclonal antibodies, raised against the nodavirus coat protein, will enable serology-based confirmation of the presence of nodavirus in histological sections and infected cell cultures (N. Moody, DPI, Queensland, Australia pers comm and FRDC 2001/626).

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.

(i) Innate immunity

Nonspecific immunity is comprised of constitutive physical (eg. skin) and chemical (eg. lysozyme) factors, which are always present, and inducible factors, such as 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, in fish, may have a more significant role than in mammals especially as the specific immune response is slower in fish, particularly at temperatures less than optimal for that species.

Because most outbreaks of VER occur in larval and juvenile fish, innate immunity is of great importance in relation to this disease.

(ii) Active immunity

Specific or active immunity is involved in responses to pathogens and other antigens that result in the formation of specific antibodies or cell-mediated immunity. As previously indicated this response is delayed and subject to ambient temperature (Watts *et al.* 2001). Experimentally, active immunity has been shown to protect against VER (Nakai 2000).

(iii) 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 that treated with normal fish serum.

(iv) Vaccination

Studies on vaccination against betanodaviruses have only recently commenced 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 at a relatively late age (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 also 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 understood and it is reasonable to expect that the disease behaves in a similar manner in other species such as barramundi (Munday *et al.* 2002). The disease appears to be perpetuated by a variable degree of vertical transmission, followed by lateral transmission.

(i) Vertical transmission

The potential for vertical transmission can be gauged from the fact that Arimoto *et al.* (1992) detected 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).

(ii) Lateral transmission

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

(iii) Other factors influencing transmission

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 although transmission by aerosols does not appear to occur (M. Heasman, NSW Fisheries, NSW, Australia pers comm) and there are no data relating to transmission on fomites.

(iv) 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/reported for Australian catfish (A. Hyatt, AAHL, Victoria, Australia pers comm), Macquarie perch, Murray cod, silver perch (Glazebrook 1995), golden perch, sleepy cod and Barcoo grunter (I. Anderson, DPI, Queensland, Australia pers comm). Thus, it is imperative to ensure that VER is not established in inland river systems.

2. Principles of control and eradication

2.1. Introduction

An outbreak of VER in Australia would be a serious threat to the fish farming industry. There are a number of different control measures that 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 option following detection of VER in Australia.

There are essentially two broad response options for VER in Australia:

Eradication — eradication of betanodaviruses (highest level of control measure and cost).

Containment, control and zoning — containment of the virus(es) 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 geographic area. Compulsory eradication may be imposed in non-endemic areas and, conceivably, could involve a number of premises under certain circumstances. In other situations control measures will be recommended/implemented to reduce the impact of the disease and minimise further spread. Movement of susceptible fish between states and territories and between endemic and non-endemic areas will require meaningful certification of the fish being translocated to minimise the risk of disease transmission.

The basic principles of eradication and other control responses are described in the AQUAVETPLAN (2002) **Enterprise Manual** and the AQUAVETPLAN (2002) **Control Centre Management Manual**.

Within these overall options, the general principles for the control and eradication of VER 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 hygienic standards.

The most appropriate option for control or eradication will depend on:

(i) Location and presence or absence of reservoirs of infection.

As 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 mitigate against successful eradication. In this circumstance continual monitoring of the wild fish population would be necessary to confirm eradication.

(ii) Chances of success of 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 greater the variation from this scenario the chance of successful eradication is lessened.

(iii) Level of risk accepted for future spread of infection (eg associated with grow-out of infected populations).
In the instance of an outbreak of VER infection in a non-endemic area this would not be an acceptable situation without a change in policy by the controlling authority.

(iv) 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 due to interruption of production occasioned by depopulation.

(v) Long-term costs of production with or without the presence of the pathogen; and

(vi) Long-term costs of control should the pathogen become endemic.

2.2. Methods to prevent spread and eliminate pathogens

Available methods for the control and eradication of VER include:

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 AQUAVETPLAN (2002) **Enterprise Manual, Section A** for more details)
  - *infected area* – the area or premise that is infected
  - *restricted area* — area around infected premises or area
  - *control area* — a buffer between the restricted area and free areas
  - *declared area* — includes restricted area and control area
  - *free area* — non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of VER remains unassessed);
- quarantine of the infected facilities;
- bans on the movement of live fish out of infected areas;

- restrictions or bans on the use and movement of equipment within and between river systems and fish farms.

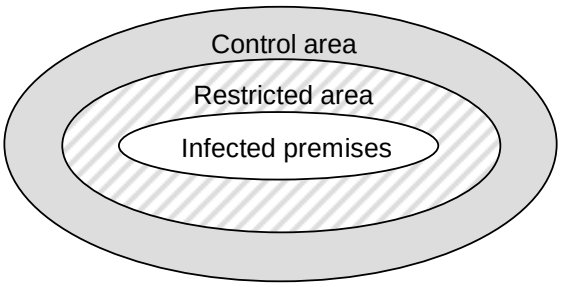


Figure 1 Establishment of specified areas to control VER

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

- live fish transportation 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 the restrictions and bans and the extent to which these are enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

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 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 AQUAVETPLAN (2002) **Zoning Policy Guidelines**. A corresponding surveillance program for VER will also be required to support a zoning policy.

Semi-open systems
There is virtually no control over the aquatic environment of semi-open systems. Fish are contained in cages moored in estuaries or sheltered areas of the sea. Impermeable liners may be placed around sea cages temporarily (eg to administer treatments for certain

diseases), however this prevents oxygen-rich water from reaching the fish and waste products from being diluted out. Therefore the use of such impermeable liners would not be a feasible method of long term water or fish control for VER. Treatment of fish in semi-open systems will impact on the surrounding environment. This must be taken into consideration before the use of disinfectant treatments.

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 are able to swim in and out of the cages at any time (attracted by food). 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 get out. 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 have slightly more control over water than semi-open systems, however there are differences between farms in the extent to which input and output water can be contained. These systems are not designed to be self-contained, and so preventing inflow or outflow of water may have adverse effects. These farms generally have limited facilities to hold or treat this water. Output water control and treatment are possible in theory however, under the current management practices of 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, however some 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 via intake water from the rivers.

Some inland pond systems may virtually constitute 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 assist in determining the most appropriate response action. Immediate tracing 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 with infected fish, premises and sites (to establish the current location and potential spread of infection).

The following things 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 representatives, tradespeople, veterinarians, scientists, technicians and visitors.

Neighbouring fish populations
Neighbouring fish farms and processing plants may become or may already be infected. Maps with 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, contact the relevant State/Territory fisheries or agriculture agency (see AQUAVETPLAN (2002)**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 restricted and control areas to which quarantine and movement restrictions are applied;
- establish disease-free and infected areas/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, therefore the least stressful methods should be used.

There are many different methods to anaesthetise and/or slaughter fish, all of which have limitations. Some methods used include the following:

- chemical anaesthesia — fish are herded into a liner, then exposed to an anaesthetic solution (Aqui-S in the only anaesthetic currently approved for use in Australia, it has no withholding period) in water before slaughter;
- 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 — depends on the pressure of infection and the risk of further spread;
- destination — human consumption or disposal;
- slaughter facilities — site, equipment and methods available; and
- experience and availability of personnel.

2.2.5. Treatment of fish products and by-products

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered when determining the treatment/processing and destiny of fish products and by-products. Betanodaviruses can potentially survive in dead fish (virus can survive for one year at 15ºC). Therefore, processing of fish products and by-products is necessary to prevent dissemination of infection to uninfected areas. Betanodaviruses are inactivated at a temperature of 60ºC for 30-60 min. (Arimoto *et al.* 1996, Frerichs *et al.* 2000) and heat treatment schedules should be such as to ensure that the internal tissues of processed fish meet these parameters. Betanodaviruses are transmitted vertically but viable eggs may be rendered virtually free of virus by surface disinfection with ozone – recommendations are 0.1µg mL^{-1} residual ozone for 2.5 min. for striped jack (Arimoto *et al.* 1996), 4µg mL^{-1} for 30 sec for halibut (Grotmol and Totland 2000) and 1µg mL^{-1} for 1 min. for striped trumpeter (S. Battaglene, DPIWE, Tasmania, Australia pers comm).

2.2.6. Disposal

Disposal must be immediate to decrease infection pressure on the site. See the AQUAVETPLAN (2002) **Operational Procedures Manual** for details. Burial sites must be chosen carefully to ensure 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. Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, and the State/Territory CVO and/or Director of Fisheries. The protocol should take into consideration the following factors:

- the source and location of infection;
- the type of enterprise (marine or freshwater farm or processing plant);
- the construction materials of the buildings/structures on the site;
- the 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 min at 20°C. However, the presence of organic matter requires higher concentrations for longer periods.

Since betanodaviruses are able to 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 11-12 (Arimoto et al. 1996, Frerichs et al. 2000).

2.2.8. Environmental considerations

Environmental considerations in the control of VER include the following:

- 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.
- The use of disinfectants could impact on the environment, especially if used in larger than normal quantities or concentrations as is possible in a disease control situation. The local environmental protection agency may need to be consulted — see the AQUAVETPLAN (2002) **Enterprise Manual**.
- The destruction and disposal of infected carcases/material will have an impact on the environment. This impact must be minimised while ensuring that there is no dissemination of infection.

2.2.9. 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 or ponds may attract birds and must be covered (eg using nets or tank roofs) to prevent birds from gaining access and transmitting infection.
- *Wild fish* — prevent contact, where possible, between wild fish and farmed fish (not possible in marine farming operations).

2.3. Feasibility of specific options for control

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/company 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/juveniles have been introduced. Thus, time is of the essence.

If there is evidence that betanodavirus infection has spread to wild fish populations successful eradication is most unlikely and, therefore, this discussion only realistically relates to when it is known or believed that the infection has been contained within farm environments and any virus, which has exited the farms, has not produced overt or covert infection in susceptible species in adjoining water bodies.

Eradication from a facility within an endemic area
Such an eradication will be based on the premise that infection is perpetuated by vertical transmission from infected broodfish so the strategy is to identify and eliminate infected broodfish and either depopulate other stocks or ensure adequate barrier control between possibly infected fish and presumptive uninfected larvae. These measures will be supplemented by egg treatment and continual monitoring of stocks for betanodaviruses. The steps to achieve eradication are;

(i) Ensure broodfish do not produce infected eggs.
As ovarian infection may only occur close to spawning, broodfish should be confirmed free of infection by testing blood using nested RT-PCR prior to spawning and testing ovarian tissue close to spawning. Any infected broodfish should be destroyed. As it is possible that broodfish could be exposed to virus in influent water they preferably should be kept in recirculated systems and the top-up water treated with sodium hypochlorite at the rate of 50 ppm for at least 10 minutes followed by neutralisation with sodium thiosulphate.

(ii) 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 is available for striped jack, turbot and striped trumpeter but none is presently available for barramundi).

(iii) 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.

(iv) Monitoring of larvae and juveniles

Larvae/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. Thus, 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 fresh-water 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, to date, histology has been the main test used, it is of relatively low sensitivity. The nested RT-PCR should supersede it for certification purposes.

Grotmol *et al.* (2000) reported that the RT-PCR was able to detect 100 to 1000 copies of *in vitro* transcribed RNA. Dalla Valle *et al.* (2000) found that a two-step nested RT-PCR was 100 times more sensitive than the standard RT-PCR and a one-step nested RT-PCR increased sensitivity tenfold. Thus, for the nested RT-PCR 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 RT-PCR as 100% for certification purposes. As Arimoto *et al.* (1993) have shown that 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, ie 300 fish 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 within economical bounds. 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 Moffitt 2001).

Control zones based on geography
Although N. Moody, DPI, Queensland, Australia (pers comm) has reported VER in sleepy cod in freshwater, in general, it can be assumed that freshwater bodies above the migratory limits of barramundi are not endemic zones for VER. 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 being translocated to these zones.

Endemic zones

Within the 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 from their own facility. Mitigation strategies that are available include:

(i) Ozonation of eggs. This will require research to determine the optimum combination of concentration of ozone, water temperature and length of exposure for each species.

(ii) Separation of batches of larvae/juveniles with strict barrier controls.

(iii) Use of "green water" culture with the aim of maintaining larval density at less than 10 L^{-1} (Munday and Nakai 1997).

(iv) Prompt removal of all dead and moribund fish. It is desirable to dispose of entire batches of fish showing evidence of VER rather than trying to salvage them.

3. Preferred control policies in Australia

3.1. Overall policy for VER

VER is an endemic disease of marine finfish in Australia and can cause substantial losses in larvae and juveniles, especially in barramundi.

The policy for response to an outbreak of VER in Australia depends upon both the nature of the outbreak and the control management strategy adopted. The choice of response option will be decided by the Director of Fisheries and/or the CVO of the State/Territory in which the outbreak occurs, following epidemiological investigation.

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, prevention of further spread and protection of uninfected areas.**

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

quarantine and movement controls **on fish, fish products and 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 and 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.**

As betanodaviruses are already known to be widely dispersed within the Australian marine environment (apparent absence is probably because intensive aquaculture of susceptible species has not been initiated rather than the viruses being absent from a particular marine zone) a rapid and vigorous response by regulatory authorities is probably only justified when outbreaks occur 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/control procedures – of course the owner of the fish may decide on immediate destocking for commercial purposes and such action should not be impeded.

The Director of Fisheries and/or the CVO of the State/Territory in which the outbreak occurs will decide upon the appropriate response option in consultation with appropriate staff within his/her own department and other interested parties. The response will be determined mainly by whether or not the outbreak occurs within an endemic area and the geographic location of that area (see 3.2 below). The most appropriate strategy must be

chosen after epidemiological investigations have been conducted. The decision must be based on scientific effectiveness and financial feasibility.

3.2. Problem definition

The initial phase of any response to detection of VER infection in an area or species previously thought to be free of infection will be one of containment while additional information is collected to support problem definition and a decision as to the appropriate response.

The components of this phase include:

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 and these should be supplemented by immunocytochemical or molecular tests. It is likely that in future diagnosis will be by the nested RT-PCR being developed by Oonoonba Veterinary Laboratory (FRDC 2001/626).

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

- The presence of typical histological lesions in fish tissues, supported by positive results on immunocytochemical or molecular tests; or
- Detection of VER virus in fish tissues by culture or molecular means.

3.2.2. Epidemiological investigation

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 the fish stocks. However, for barramundi in particular, older fish may be covertly infected and diagnosis must be made by laboratory examination, preferably by nested RT-PCR.

Surveillance of fish stocks and other facilities in the 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 sub-clinical infection, particularly in older fish, sample sizes for surveillance should be calculated to provide 95% confidence of detecting 1% prevalence in the sampled population (300 fish). Samples may be pooled to reduce testing costs.

3.2.3. Implementation of interim measures to minimise further spread of the virus.

Movement controls and other measures should be implemented immediately on infected premises or areas, pending confirmation of VER and definition of the extent of the

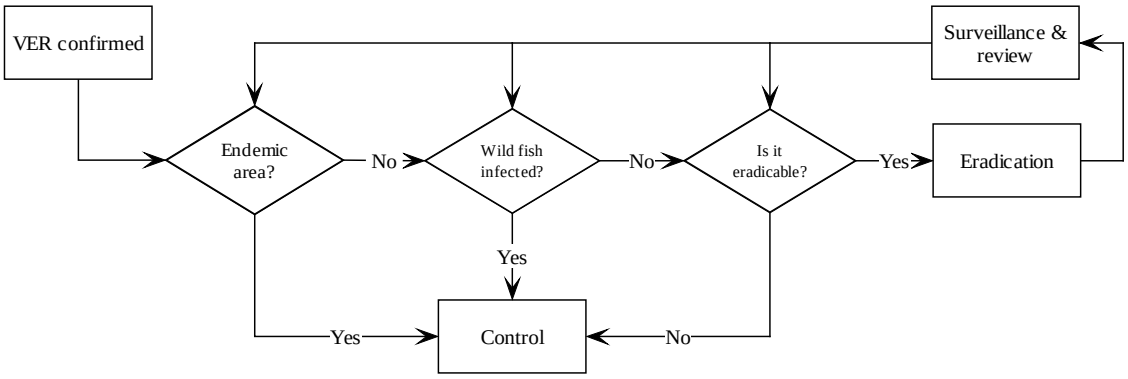
outbreak (see section 3.4.1, and **AQUAVETPLAN (2002) Enterprise Manual** for details). Measures may include:

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

3.2.4. Determination of the appropriate response

As soon as adequate information becomes available, a decision should be made as to the appropriate response, based on the flowchart 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.

Figure 2. Decision flowchart to determine the preferred response to a VER outbreak



3.3. Overview of response options

3.3.1. 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 and is present or potentially present in wild fish species, rivers or the sea.

Eradication strategies include the following:

- quarantine and movement controls/restrictions on fish, fish products, water and any other vectors (including fomites) located 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 be either 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 infected premises 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 facilitate cooperation from industry and the community.

3.3.2. Containment, control and zoning

If reservoirs of infection became established in wild fish stocks or on numerous farms, eradication would be impracticable. In this case, containment and prevention of further spread is the preferred response option in order to protect and maintain uninfected areas. Containment, control and zoning would also apply outside the affected farm, even when eradication is pursued.

It should be possible to maintain uninfected areas/zones free of VER and the implementation of a zoning program would be advantageous to the Australian aquaculture industry, as well as providing protection for potentially susceptible wild fish species. Restrictions on the movement of fish and fish products and a surveillance program will 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 eradication. Strategies used for control of VER may include the following:

- 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 facilitate cooperation from industry and the community.

Within endemic zones it may not be economically viable for some farms to institute effective 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. As previously stated, such mitigation practices may include ozonation of eggs, green pond culture, separation of discreet groups of fish, etc. 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 eradication and control of VER are summarised in Table2, and described in detail in sections 3.4.1 to 3.4.8.

Table2. Summary of strategies used for each of the response options for VER.

	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, clinically normal fish	Yes	Optional
Treatment of fish products and by-products – exposed	Destroyed	Optional
Treatment of fish products and by-products – unexposed	Yes	No
Disposal of infected fish and wastes	Yes	Yes
Decontamination	Yes	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 most appropriate control strategy for the incident is determined, quarantine and movement controls can be altered accordingly. See the **AQUAVETPLAN (2002) Enterprise Manual** for details on movement controls for different enterprise systems and response options.

For eradication, quarantine and movement controls must be stringently enforced on fish, fish products, water, fomites and any vectors located 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 are 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 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. Water system, equipment and all handling procedures must bear no risk of infection to ensure the population remains unexposed throughout grow-out, harvesting and slaughter. 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 practices.

Table-sized fish without any apparent possible exposure to infection with betanodaviruses 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 have no risk of exposure to infection. On-farm processing may be preferable, as this 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. This is particularly applicable to larval/juvenile barramundi that have a low unit value. Immediate destruction of such populations will decrease the chance of spread of infection to these and other fish stocks and prevent propagation of the disease.

Containment, control 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. Thus, the method of harvest, equipment used and the choice of location should ensure there is no exposure to infection.

3.4.5. Destruction of exposed or potentially exposed, clinically normal fish

Eradication
In facilities undergoing eradication, exposed or potentially exposed, clinically normal fish should be regarded as infected and destroyed. From a human health perspective, healthy covertly infected fish are safe for human consumption. However emergency harvesting and slaughter of healthy exposed/potentially exposed fish have 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 the preferred option.

Containment, control and zoning
Grow-out of exposed or potentially exposed, clinically normal fish within infected zones under current farm management practices is possible. 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 de-stocking a hatchery has significant economic impact on not only hatchery operations but also the grow-out farms it supplies, 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, boats and on market access for final product may be necessary to protect uninfected facilities or zones.

3.4.6. Treatment of fish products and by-products

The treatment of fish products and by-products must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product. Harvested, 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 live fish, products and by-products 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 a restricted market 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 product and by-products 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 does occur at the moment as well as translocation of dead fish caught by professional and amateur fishermen. However, domestic market regulations (eg State/Territory legislation) and food safety standards must be considered when determining the required treatment of products and by-products.

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 **AQUAVETPLAN (2002) 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. This will greatly assist in decreasing 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 decrease 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, so as to decrease the infectious load on the site.

3.4.9. Surveillance

Developing a surveillance program for VER is dependent upon the success of FRDC project 2001/626 for the development of a nested RT-PCR suitable for Australian conditions and species.

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 infected facilities identified as part of an official control or eradication program. Tracing is not required for infected facilities 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/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) makes it likely that even more outbreaks of VER may occur in temperate regions.

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 lynch pin 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 actually prove this would be an unnecessarily expensive task in the present situation. However, individual farms wishing to obtain certification of “freedom” from infection would need to have all year classes of fish tested negative by the LcEV nested RT-PCR for at least two consecutive years. The level of testing would be at the 95% confidence level for detecting a 1% prevalence of infection. This would equate to 300 fish for most year classes but would not be achievable for most populations of female broodfish, which are likely to number less than 100. In the latter case all broodstock in populations less than 100 should be tested quarterly during the two year pre-certification period.

Other requirements would be that the farm was completely closed (pond or tank system based on groundwater or treated water) or situated 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/control.

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

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

Appendix : ossa

Anadromous fish	Fish species that hatch and live initially in freshwater, then migrate to seawater and eventually return to freshwater to spawn.
Antibody	Specialised serum proteins produced by B lymphocytes to antigens. Usually specific for a particular antigen.
AQUAVETPLAN	A series of documents that describe the Australian response to exotic aquatic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
AUSVETPLAN	A series of documents that describe the Australian response to exotic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
Catadromous fish	Fish species that hatch and live initially in seawater, then migrate to freshwater and eventually return to seawater to spawn.
Cell-mediated immunity	Immunity produced as a result of cellular responses as distinct from the action of antibodies
Control area	A buffer between the restricted area and areas free of disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may reduce in size. The shape of the area may be modified according to circumstances, eg water flows, catchment limits etc. In most cases, permits will be required to move animals and specified product out of the control area into the free area.
Containment	To restrict the geographical distribution of a disease and/or its agent to defined bounds.
Covert infection	Clinically inapparent infection that is transmissible and that may eventually lead to clinical disease.
Dangerous contact premises or area	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	An area that has been subjected to a legal declaration and includes both a restricted area and a control area.
Decontamination	Includes all stages of cleaning and disinfection.
Disinfectant	An agent used to destroy microorganisms outside a living animal.

Disposal	Sanitary removal of fish carcases and things by burial, burning or some other process so as to prevent the spread of disease.
Electronmicroscopy	Examination of very thin sections of animal tissues (positive staining) or smears of tissues, body fluids etc (negative staining) using a beam of electrons in an electron microscope
ELISA	Enzyme linked immunosorbent assay — a serological test designed to detect and measure the presence of antibody or antigen in a sample. The test uses an enzyme reaction with a substrate to produce a colour change when antigen-antibody binding occurs.
Endemic	The constant presence of a disease or infectious agent within a given geographic area or population group. It also implies a prevalence that is usual in the area or in the population.
Fish byproducts	Products of fish origin destined for industrial use (eg fishmeal).
Fish products	Fish meat products and products of fish origin (eg eggs) for human consumption or use in animal feeding.
Fomites	Inanimate objects (eg boots, clothing, equipment, vehicles, crate, packaging) that carry the exotic agent and spread the disease through mechanical transmission.
Formal saline	10% formalin made up to normal salinity.
Free area	An area known to be free of the disease agent.
Genotype	A grouping of individual microorganisms or organisms based on their genetic make-up.
Gill pillar cells	Supporting cells of the secondary lamellae.
“Green water” culture	Culture of larval fish in water containing beneficial microalgae
Histology	Examination of thin sections of animal tissues by light microscopy
Immune serum	Serum containing antibodies against a specific pathogen. Can be homologous (from the same species) or heterologous (from a different species)
Immunocytochemistry	Specific staining of microorganisms in tissues using specific antisera that have an attached dye (eg immunoperoxidase)
Infected premises or area	The area in which the disease has been confirmed. Definition of an ‘infected area’ is more likely to apply to an open system such as an oceanic lease.

Karyorrhexis	Rupture of the nucleus of a cell leading to the production of formless granules
Lysozyme	An enzyme present in body fluids which can lyse some bacterial cell walls
Mitigation	Reduction in severity, eg mitigation of the impact of disease is to decrease the severity of the impact of the disease.
Monitoring	Collection, analysis and dissemination of information about infections or diseases that are known to occur in a specified population. (See also "Surveillance").
Movement control	Restrictions placed on movement of fish, people and things to prevent spread of disease.
Nuclear layers of the retina	Cellular layers overlaying the rods and cones.
Neuropil	Dense network of dendrites, axons and neuroglia in the central nervous system
Ozonation	Artificial exposure to ozone
Pathogen	A microorganism or organism which causes disease
PCR	A diagnostic technique involving the production of millions of copies of a specific target DNA segment *in vitro*.
Permissive cell line	A tissue culture cell line able to support the growth of a particular virus
Premises or area	Production sites that may range from an aquarium to an aquaculture lease in the open ocean.
Primers	Essential components of the PCR. They are synthetic oligonucleotides that are complementary to the sequences of interest.
Pyknosis	Shrinkage of the nucleus of a cell with increase in the optical density
Quarantine	Legal restrictions imposed on a place, fish, vehicle, or other things, limiting movement.
Recombinant coat protein	Coat protein of a virus produced by expressing the appropriate gene in bacteria or yeasts

Restricted area	The area around an infected premises (or area), likely to be subject to intense surveillance and movement controls. It is likely to be relatively small. It may include some dangerous contact premises (or area) and some suspect premises (or area), as well as enterprises that are not infected or under suspicion. Movement of potential vectors of disease out of the area will, in general, be prohibited. Movement into the restricted area would only be by permit. Multiple restricted areas may exist within one control area.
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible species	Fish that can be infected with the disease.
Suspect premises or area	Where the emergency disease is suspected but not yet confirmed. The reason for the suspicion varies with the agent, however it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, people or things that may be implicated in the spread of disease.
Transcription	The synthesis of an RNA copy from a nucleotide sequence in a limited region of DNA.
Transmission	Transfer of an infectious agent from one fish to another. May be in/on reproductive products (vertical) or via the water column (lateral).
Vector	A living organism that transmits an infection from one host to another. A *biological* vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A *mechanical* vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.
Zoning	The process of defining disease-free and infected zones.

Appendix 3: Abbreviations

AAHL	Australian Animal Health Laboratory
AQIS	Australian Quarantine and Inspection Service
CPE	cytopathic effect
DNA	deoxyribonucleic acid
DPI	Department of Primary Industries, Queensland
DPIWE	Department of Primary Industries Water and Environment, Tasmania
FRDC	Fisheries Research and Development Corporation
IDEXX/VPS	IDEXX/Veterinary Pathology Services (private veterinary laboratories)
IFAT	indirect fluorescent antibody test
ELISA	enzyme linked immunosorbent assay
OIE	Office International des Épizooties (World Organisation for Animal Health)
PAS	periodic acid Schiff
PCR	polymerase chain reaction
RNA	ribonucleic acid
RT-PCR	reverse-transcriptase polymerase chain reaction