



Australian Government

**Department of Agriculture
and Water Resources**

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN (AQUAVETPLAN)

Viral encephalopathy and retinopathy

Version 2.0, 2017

AQUAVETPLAN–Disease Strategy

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

National Biosecurity Committee

This disease strategy forms part of:

AQUAVETPLAN

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

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

IMPORTANT NOTE: Viral encephalopathy and retinopathy has not been listed in the OIE *Aquatic Animal Health Code* since 2005. The Aquatic Health Code is updated annually and is available on the internet at the OIE website oie.int/international-standard-setting/aquatic-code/access-online.

Further details are given in Appendix 1 of this manual.

DISEASE WATCH HOTLINE**1 800 675 888**

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

Preface

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

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

The Department of Agriculture and Water Resources provides quarantine inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture and Water Resources Biosecurity Import Conditions System (BICON) website (agriculture.gov.au/jimport/online-services/bicon).

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of viral encephalopathy and retinopathy in Australia. The strategy was scientifically reviewed by the Sub-Committee for Aquatic Animal Health of the Animal Health Committee, before being endorsed by:

- the Animal Health Committee of the National Biosecurity Committee in [May, 2017]; and the National Biosecurity Committee in [December, 2017]

Viral encephalopathy and retinopathy is listed on Australia's *National List of Reportable Diseases of Aquatic Animals* (DAWR 2016); see agriculture.gov.au/animal/aquatic/reporting.

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

Disease strategy manuals Individual strategies for each disease	Enterprise manual Includes sections on: – open systems – semi-open systems – semi-closed systems
Operational procedures manuals Destruction Disposal Decontamination	Control centre manual

[*Aquatic Animal Diseases Significant to Australia: Identification Field Guide*](#) (DA 2012) is a source of information about the aetiology, diagnosis and epidemiology of infection with viral encephalopathy and retinopathy and should be read in conjunction with this strategy.

This first edition of this manual was prepared by Dr Richard Miller, the late Dr Barry Munday and Dr Chris Baldock in 2004. This revision was prepared by Dr Paul Hick in 2015. The author was responsible for drafting the strategy, in consultation with a wide range of stakeholders from aquaculture, recreational fishing and government sectors throughout Australia. However, the text was amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not

necessarily reflect the views of the author. The author would like to thank Dr Diana Jaramillo. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual was adapted from similar manuals in AUSVETPLAN (the Australian veterinary emergency plan for terrestrial animal diseases) and from the AQUAVETPLAN Enterprise Manual. The format and content have been kept as similar as possible to these documents, in order 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 are gratefully acknowledged.

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

Government

CSIRO Australian Animal Health Laboratory

Department of Primary Industries, New South Wales

Department of Primary Industry and Fisheries, Northern Territory

Department of Agriculture and Fisheries, Queensland

Department of Primary Industries, Parks, Water and Environment, Tasmania

Department of Fisheries, Western Australia

Department of Economic Development, Jobs, Transport and Resources, Victoria

Department of Primary Industries and Regions, South Australia

Biosecurity Animal Division, Department of Agriculture and Water Resources, Australian Government

Department of the Environment, Australian Government

Industry

National Aquatic Animal Health Industry Reference Group

The complete series of AQUAVETPLAN documents is available at agriculture.gov.au/animal/aquatic/aquavetplan.

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1 Nature of the disease

Viral encephalopathy and retinopathy (VER), also referred to as viral nervous necrosis (VNN), is a disease of finfish caused by infection with nervous necrosis virus (NNV), also referred to as *Betanodavirus* (Munday et al. 2002). NNVs have a global distribution and have caused disease in more than 50 species of predominantly marine fish in aquaculture (Munday & Nakai 1997; OIE 2016b). The virus is endemic in Australia and has been the cause of mass mortality of larval and juvenile fish in aquaculture facilities since 1988 (Glazebrook et al. 1990). Outbreaks of disease are often acute episodes with 100% cumulative mortality, although mortality is variable and the disease outcome is influenced by several factors, particularly the age and species of the host fish (Munday et al. 2002). Infection with NNV does not always result in disease. Persistent subclinical infection has been reported as well as frequent detection of the agent using molecular tests in apparently healthy wild fish (Ciulli et al. 2007; Gjessing et al. 2009; Gomez et al. 2004; Johansen et al. 2004a).

For the purposes of this manual, 'VER' refers to clinical disease, and 'infection with NNV' refers to infection with the causative agent of the disease but not necessarily clinical disease.

1.1 Aetiology

NNV is classified within the genus *Betanodavirus*, family *Nodaviridae*, and is sometimes referred to as *Betanodavirus* or nodavirus. Virions have icosahedral symmetry, are approximately 30 nm in diameter and do not possess an envelope (King et al. 2011). The genome is single-stranded, positive-sense ribonucleic acid (RNA) with 4.5 kilobases of nucleotides in two segments (RNA1 and RNA2) that encode only three proteins (Mori et al. 1992). The virus replicates in the cytoplasm and has a tropism for cells of the brain and retina (Nguyen et al. 1996; Peducasse et al. 1999).

Four genotypes of NNV are recognised, corresponding to the virus species recognised by the International Committee for Taxonomy of Viruses:

- redspotted grouper NNV (RGNNV; genotype 1)
- barfin flounder NNV (BFNNV; genotype 2)
- tiger puffer NNV (TPNNV; genotype 3)
- striped jack NNV (SJNNV; genotype 4)

After sequencing of a PCR product obtained from farmed turbot, Johansen et al. (2004b) proposed a likely fifth genotype, turbot NNV (TNNV), but the virus has not been isolated to date (Oliveira et al. 2013).

Nucleotide sequence similarity of the capsid protein gene (RNA2) between genotypes is > 66% (Nishizawa et al. 1997) or > 83% for RGNNV and BFNNV, which are also the only genotypes with serologic cross-reactivity (Mori et al. 2003). Sequence analysis has enabled the many NNV isolates that are sometimes named for the host species to be classified within these genotypes, although subgroups have been described (Cutrin et al. 2007; Nylund et al. 2008; Thiery et al. 2004).

The NNV genotypes are distributed differently according to water temperature, which corresponds to the *in vitro* characteristics of the viruses (Iwamoto et al. 1999). Thus, RGNNV, which can proliferate in cell culture between 15°C and 35°C, is widely distributed in tropical waters and causes disease in temperate water fish species when the temperature increases (Ciulli et al. 2006; Hata et al. 2007). The BFNNV genotype,

which has optimal replication at 15–20°C, has caused outbreaks of VER in cool water fish species at temperatures as low as 6°C and commonly at 17°C (Grotmol et al. 1999; Johnson et al. 2002). The optimal in vitro temperature range for SJNNV and TPNNV is 20–25°C.

The different optimal replication temperatures provide some restriction on the distribution of each genotype. In addition, some restriction in host specificity exists for each genotype. However, the genotypes RGNNV, BFNNV and SJNNV infect many host fish species and the host range overlaps in some cases (Cutrin et al. 2007; Nylund et al. 2008; Thiery et al. 2004). Reassortment viruses have been described (Oliveira et al. 2009; Panzarin et al. 2012). Reassortment of the two RNA segments might alter the biological properties of NNV because the optimal replication temperature is influenced by the RNA1 genotype, and the host range is influenced by the RNA2 genotype (Ito et al. 2008; Panzarin et al. 2014).

Only the RGNNV genotype has been detected in Australia, and most isolates correspond to the subgroup 1a that is also present in many parts of Asia and Europe (Thiery et al. 2004). A second RGNNV subgroup, 1c, was identified from two different host fish species in Australia (Moody et al. 2009).

1.2 Susceptible species

NNV has a low host specificity and, consequently, VER has been reported in more than 50 species of fish from 36 families, across 10 orders (OIE 2016b). Clinical disease has been primarily reported in marine fish species (Munday et al. 2002). Clinical disease in diadromous species in freshwater environments is reported for European sea bass (*Dicentrarchus labrax*) (Athanassopoulou et al. 2003). VER has been recognised in freshwater species including *Silurus* spp. and tilapia (*Oreochromis niloticus*) (Bigarre et al. 2009). There are also reports of clinical disease in zebra fish (*Danio rerio*) and goldfish (*Carassius auratus*) (Binesh 2013). *Betanodavirus* RNA has also been detected in both brain and hepatopancreatic tissue in a small number of marine invertebrates, although their potential role as reservoirs for infection has not been demonstrated (Gomez et al. 2008; Gomez et al. 2006).

In Australia, the impact of VER has primarily been on hatchery production of barramundi (*Lates calcarifer*), also known as Asian sea bass (Hick et al. 2011a). The disease has also affected hatchery production of Australian bass (*Macquaria novemaculeata*, produced for conservation and recreational fishing) and novel aquaculture species such as yellow tail kingfish (*Seriola lalandi*), sleepy cod (*Oxyeleotris lineolatus*), gold-spotted rockcod (*Epinephelus coioides*), giant grouper (*Epinephelus lanceolatus*) and striped trumpeter (*Latris lineata*) (Agnihotri et al. 2016; Moody et al. 2009; Munday et al. 2002). VER has also been reported for cultured juvenile and sub-adult grouper (*Epinephelus* spp.) and barramundi cod (*Cromileptes altivelis*). Disease of several Australian native freshwater species was demonstrated via experimental infection with NNV (Anderson & Moody 2004).

1.3 World distribution

There are reports of VER from every continent except Antarctica, including most locations where aquaculture is practiced (Cherif et al. 2009; Munday et al. 2002; OIE 2016b; Ransangan & Manin 2010). Surveys of fish from marine environments indicate a high prevalence in apparently healthy wild fish in a wide range of locations (Anderson & Oakey 2008; Ciulli et al. 2007; Gomez et al. 2004; Kim et al. 2007; Liu et al. 2015).

The RGNNV genotype of NNV is considered endemic in Australia and outbreaks of VER have occurred in Queensland, Northern Territory, New South Wales, South Australia and

Tasmania (Moody et al. 2009). Only the RGNNV genotype has been detected in Australia. There is no evidence that genotypic subtypes within Australia have a restricted host or geographic range (Hick et al. 2013).

1.4 Diagnosis of viral encephalopathy and retinopathy or infection with nervous necrosis virus

There are two different purposes for laboratory tests for NNV. To diagnose the clinical disease VER, confirmation of the presence of NNV is required from histopathological observation of characteristic lesions. Surveillance involves testing for the presence of subclinical infection with NNV, for the purpose of pathogen control, because subclinical infection can occur without histopathological lesions (Jaramillo 2015; Johansen et al. 2004a).

The laboratory methods required for diagnosis of clinical disease and detection of infection are described in the Australian and New Zealand Standard Diagnostic Procedure for Betanodavirus Infections of Finfish (Moody & Crane 2014). The version approved in 2015 is available online: [Betanodavirus infections in finfish](#).

Laboratory methods and diagnostic approaches are also described in the Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.12: Viral encephalopathy and retinopathy (OIE 2016c). The OIE Aquatic Animal Health Code no longer lists VER due to the widespread distribution of NNV. The inclusion of an updated version of the VER chapter in the OIE diagnostic manual reflects the continued need for standard diagnostic approaches to regional disease management.

The case definition of VER requires demonstration of histopathological demonstration of lesions that include vacuolation in brain and retinal tissue (described in Section 1.4.2) and a test to detect NNV in the affected fish. An immunohistochemical (IHC) test that uses antibodies which bind specifically to the capsid protein of NNV can provide evidence of NNV associated with lesions (Moody et al. 2004). Alternatively, NNV infection of the affected fish can be shown using a validated, real-time, reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) assay, or virus isolation in SSN-1 cell culture (Hick et al. 2010; Hick et al. 2011b; Hick & Whittington 2010).

Subclinical infection is defined by the detection of NNV from an apparently healthy fish by virus isolation in cell culture, or by an RT-qPCR assay that is approved for the purpose and conducted in an accredited laboratory. In the case of a positive virus isolation or PCR test for NNV in a host species or location that is not previously recognised, the detection is confirmed by nucleotide sequence analysis of the RNA1 and RNA2 genes (Hick et al. 2013).

1.4.1 Field methods: clinical signs and gross pathology

VER is characterised by the occurrence of an acute-onset, high-mortality disease, most commonly in larvae or juvenile marine fish. In older fish, VER can occur with a more chronic course and the cumulative mortality is lower.

Mortality is preceded by a clinical syndrome of neurological dysfunction which is characterised by abnormal swimming and behaviour. Inappetence is one of the earliest signs of disease (Arimoto et al. 1994; Athanassopoulou et al. 2004; Azad et al. 2005; Curtis et al. 2001; Johansen et al. 2004b). The swimming patterns of affected fish are described variously as erratic, whirling, corkscrewing, spiralling or circling (Breuil et al. 1991; Munday & Nakai 1997; Yoshikoshi & Inoue 1990). The activity of fish is often

decreased (Chi et al. 1997; Curtis et al. 2001; Tanaka et al. 2004). Expected schooling behaviour ceases and the fish congregate in clusters near the water surface or are abnormally distributed in the water column (Azad et al. 2005; Breuil et al. 1991). In some cases, affected fish demonstrate hyperactivity when stimulated (Chi et al. 1997; LeBreton et al. 1997; Skliris & Richards 1999). Loss of equilibrium is evident and some fish can be upside down at rest (Tanaka et al. 1998). Abnormal colouration depends on the species of fish and the environment, for example, affected barramundi are either lighter (e.g. Glazebrook et al. (1990)) or darker in colour (e.g. Azad et al. (2005); Maeno et al. (2007)).

Although uncoordinated swimming is the most frequently reported clinical sign of NNV, it is absent in sedentary fish species and larvae of other species that die in very short and severe disease episodes (Arimoto et al. 1994; Johansen et al. 2004b).

The clinical signs are not specific and can occur with other infectious and non-infectious diseases. Similarly, there are often no gross lesions, and those that might be observed, for example hyperinflated swim bladder, are non-specific (Fukuda et al. 1996). Diagnostic tests for VER should be considered in cases of unexpected mortality of fish in which neurological dysfunction or abnormal vision is evident.

1.4.2 Laboratory methods

Sample submission

Appropriate diagnostic specimens are whole fish submitted live to a diagnostic laboratory. Alternatively, fish can be euthanased and submitted whole, some chilled (< 10°C) on wet ice and some fixed in 10% neutral buffered formalin (or seawater formalin or Bouin's fixative). Some fish should be submitted in 95% ethanol for PCR tests if transport is expected to take more than 24 hours. The size of the fish should be considered when using fixative and preservative solutions. For large fish, it is appropriate to perform a necropsy and remove nervous and eye tissue to obtain adequate preservation of the samples for histology. The cornea of larger fish is best cut open to allow rapid penetration of fixative.

The fish selected for diagnostic testing should include both clinically affected and some apparently normal fish. Dead fish should be avoided. The sample size should include at least 12 fish to enable confirmation of a case of VER (Hick et al. 2011a). Samples consistent with testing for alternative diagnoses should also be obtained.

Samples should be submitted according to the directions of the diagnostic laboratory.

When testing for freedom from NNV, the sample size should be sufficient to detect the minimum expected prevalence prescribed by the regulatory organisation, typically 2% or 10%. In this case, a sample that is representative of the population can be obtained using a random sampling strategy appropriate to the circumstance; examples are provided in Hick et al. (2011a). The diagnostic sensitivity and specificity of the laboratory detection method will influence the sample size and interpretation of the survey result.

Microscopy

Lesions consistent with VER may be observed in the retina, brain or spinal cord of affected fish using standard histological methods with haematoxylin and eosin (H&E) staining. Tissues can be decalcified using ethylenediaminetetraacetic acid (EDTA) according to routine methods before sectioning. Both the brain and retina need to be examined as lesions may be present in only one of these tissues (Dannevig et al. 2000; Jaramillo 2015; Peducasse et al. 1999).

Characteristic histopathological lesions are consistent across fish species (Munday et al. 1992; Yoshikoshi & Inoue 1990). Affected tissues are necrotic with pyknosis and karyorrhexis of individual cells (Tanaka et al. 2004). Characteristic vacuoles from 5–50 µm in diameter are considered to be intra-cytoplasmic but their origin is not always clear (Grotmol et al. 1997). Aggregates of macrophages are present in fish with persistent infection (Johansen et al. 2003). There are some reports of lesions characterised by necrotic cells and the presence of cytoplasmic vacuoles in additional tissues, including the intestine and epithelium (Grotmol et al. 1999; Nguyen et al. 1996), that were identified in conjunction with IHC staining, but such lesions are not consistently reported.

An IHC test that uses polyclonal sheep anti-serum to a recombinant NNV capsid protein was developed. This demonstrates virus antigen in association with lesions in tissue sections, using peroxidase-labelled or fluorescein-conjugated secondary antibodies (Moody et al. 2004). This test should be used according to the procedure and reagents described in the ANZSDP: *Betanodavirus* infections of finfish (Moody & Crane 2014).

Culture methods

Virus isolation tests provide the only laboratory method for showing that infectious NNV is present. NNV is readily cultured in SSN-1 cells and the clonally derived E-11 cell line (Iwamoto et al. 2000). These cells are suitable for isolation of isolates from genotypes 1 to 4 (Iwamoto et al. 1999). Both cell lines can be obtained as authenticated cell stocks from a cell culture repository (e.g. European Collection of Authenticated Cell Cultures). Multiple additional cell lines that are permissive to NNV have been described, including several derived from grouper species (Chi et al. 1999; Qin et al. 2006). A procedure for primary virus isolation of NNV from fish tissues and enumeration of the viral titre using SSN-1 has been validated and described (Hick et al. 2011b), and a procedure is detailed in the ANZSDP: *Betanodavirus* infections of finfish (Moody & Crane 2014).

Virus isolation in cell culture requires specialised expertise. It should adhere to the general principles for maintenance of cells and quality control when using virus isolation as a diagnostic test, as described in the ANZSDP: [Viruses of salmonids: Virus isolation in fish cell lines](#) (Crane & Williams 2008).

When a cytopathic agent that can be passaged is detected, characterisation and confirmatory tests include IHC, RT-qPCR and virus nucleotide sequence determination.

Molecular techniques

Molecular tests provide the most sensitive method for detecting NNV. These procedures are suitable for confirming the presence of NNV in clinical samples and are ideally suited to surveillance tests used to certify populations of fish for freedom from infection with NNV.

The ANZSDP: *Betanodavirus* infections of finfish (Moody & Crane 2014) describes a procedure to test for the NNV capsid protein gene using reverse transcription-polymerase chain reaction (RT-PCR) assays. The nested RT-PCR has lower sensitivity and specificity compared to real-time assay (RT-qPCR) but is superior to a commercial test kit (Crane et al. 2007; Hick & Whittington 2010). Preparation of an appropriate sample is a critical consideration for accurate molecular tests. Suitable methods for homogenising the target tissue by bead-beating and purifying nucleic acids have been evaluated for NNV assays (Hick et al. 2010).

Molecular tests require highly trained technicians, specialised equipment and should be undertaken only with quality control procedures consistent with those outlined by the

International Organization for Standardization in ISO 17025 (ISO 2005) and by the World Organisation for Animal Health (OIE 2012).

Confirmatory laboratory procedures, including determination of NNV sequence, are required for positive laboratory tests before NNV infection is reported in production systems, locations and fish species in which infection is not commonly detected.

1.4.3 Confirmation of infection

Confirmed detection of NNV can be achieved by detecting viral antigen using polyclonal antibodies in an IHC test, or detecting viral RNA using molecular tests (RT-qPCR). For any test, the diagnostic specificity and positive predictive value should be considered when interpreting a positive laboratory test result.

Clinical infection is indicated by a confirmed laboratory detection of NNV in conjunction with characteristic lesions observed by histopathological testing.

1.4.4 Differential diagnosis

The pattern of characteristic clinical signs described in Section 1.4.1 is highly suggestive of VER. Acute episodes of mortality and abnormal behaviour are not specific for VER, and need to be differentiated from other infectious and non-infectious causes of neurological disease and mortality, including inadequate water quality and inappropriate nutrition. Disease in older fish is less characteristic and clinical signs might not be obvious throughout the population; mortality is typically between 10% and 40% (Bovo et al. 1999). Secondary or concurrent disease might be present. Systematic disease investigation considering the clinical signs, patterns of disease at the level of the affected aquaculture facility, and gross necropsy findings, is required to direct the laboratory testing for diagnosis of disease outbreaks.

1.5 Resistance and immunity

Resistance to disease caused by NNV infection occurs with increasing age of some fish species. For example, VER has not been confirmed in sub-adult or adult barramundi. However, outbreaks of VER have occurred in sub-adult Atlantic halibut (*Hippoglossus hippoglossus*) (Aspehaug et al. 1999), Atlantic cod (*Gadus morhua*) (Patel et al. 2007), grouper species (Fukuda et al. 1996; Nopadon et al. 2009) and sea bass (*Dicentrarchus labrax*) (Bovo et al. 1999; LeBreton et al. 1997).

Development of a specific antibody response to infection has been confirmed in barramundi and Australian bass after experimental challenge with NNV at 3 weeks of age and older (Jaramillo 2015). Numerous vaccines have been designed using DNA as well as peptides, recombinant protein, inactivated NNV and virus-like particles as antigen (Coourdacier et al. 2003; Liu et al. 2006; Lu et al. 2003; Sommerset et al. 2003; Thiery et al. 2006; Vimal et al. 2014a; Vimal et al. 2014b; Yamashita et al. 2005). Various methods have been used to evaluate the efficacy of these vaccines, although in many cases, the relative percentage survival was increased significantly by administration of the vaccine. Novel oral delivery methods are under investigation to assist with delivery to small fish (Lin et al. 2007). The use of synthetic double-stranded RNA (poly I:C) and non-pathogenic viruses to stimulate a protective response to NNV indicates the important role of innate immune mechanisms (Nishizawa et al. 2009; Pakingking et al. 2005).

Currently (2017), there are no practicable vaccines for NNV available in Australia. A patented injectable NNV vaccine is available, but as the disease is mainly a problem in fry, injection is impractical (Andrew Barnes, Associate Professor, School of Biological Sciences, University of Queensland, 2017, Personal Communication).

WO2004074467-A1; AU2004213566-A1; NO200504219-A; EP1597359-A1;
TW200425899-A; JP2006517943-W; US2006257425-A1; AU2004213566-B2

New inactivated piscine nodavirus preparation, useful as a vaccine for treating or preventing nodavirus infection in fish, preferably Viral Nervous Necrosis (VNN)

Inventor: Dos Santos, N., Ireland, J., Barnes, A. C., Horne, M.

Assignee: Novartis Ag; Novartis Pharma GmbH; Dos Santos N; Ireland J; Barnes A C; Horne M

Date: 2004

1.6 Epidemiology

Although NNV is the causative agent for VER, it is also frequently the cause of persistent subclinical infection. Several factors influence the expression of disease. The age of susceptible species of fish at the time of exposure is critical to determining the disease outcome. In some fish species, clinical disease does not occur beyond a given developmental stage but, in other species, the expression of the disease is attenuated with increasing age. The occurrence and severity of VER is influenced by environmental factors, including sub-optimal husbandry resulting in stress.

Disease control is difficult because reservoirs of infection exist in fish with persistent infection and in other aquatic organisms. NNV can also persist in the environment and is relatively resistant to disinfection. Susceptible fish can be infected by exposure to NNV by immersion, enabling many pathways for horizontal transmission, including vectors and fomites, as well as vertical transmission of infection.

1.6.1 Incubation period

The incubation period is variable and influenced by factors including the dose of virus, route of exposure, host fish and water temperature. An incubation period as short as one day has been observed in grouper (*Epinephelus coioides*) at 28°C degrees, and up to 22 days in Atlantic halibut at 6°C (Chi et al. 1999; Grotmol et al. 1999). The importance of temperature on incubation period for the same virus and host species has been shown (Skirris & Richards 1999).

Detailed epidemiological investigation of field outbreaks in barramundi implied an incubation period of less than seven days (Hick et al. 2011a). Experimental infection by immersion identified an incubation period of six days (Jaramillo 2015).

1.6.2 Persistence of the pathogen

NNV is relatively stable in the environment, retaining infectivity for one year in seawater at 15°C (Frerichs et al. 2000), and is relatively resistant to commonly used physical and chemical disinfection treatments (Table 1). Some caution is required in extrapolating the effective disinfection treatments from laboratory experiments, because the quantification of viral infectivity using the SSN-1 cell line has poor repeatability (Grove et al. 2006; Iwamoto et al. 1999). In addition, different methods were used to quantify the disinfection treatments such as ozone (Buchan et al. 2005). The effectiveness of disinfection procedures also depends on the environment surrounding the virus. For example, it has been found that chlorine disinfection is ineffective in the presence of fetal bovine serum and UV light is less effective in the presence of organic and inorganic particles (Frerichs et al. 2000; Hess-Erga et al. 2008). Effective decontamination procedures should be developed that are specific for an

individual circumstance using these data, and the guidelines in AQUAVETPLAN operational procedures manual – Decontamination, Section 2.2.8 (DAFF 2008).

Infection of wild fish and other aquatic organisms creates reservoirs of NNV in the environment. Surveys of apparently healthy wild fish indicate that NNV infection occurs at high prevalence in many species and locations (Ciulli et al. 2007; Gomez et al. 2004; Kim et al. 2007). As discussed in Section 1.2, *Betanodavirus* RNA has been detected in both marine molluscs and crustaceans, but the potential role of marine invertebrates as reservoirs for infection has not been established (Gomez et al. 2008; Gomez et al. 2006; Gomez et al. 2010).

1.6.3 Modes of transmission

It is generally accepted that both horizontal and vertical transmission of NNV infection can cause VER in larvae and juvenile fish (Mushiake et al. 1992).

Vertical transmission of infection leading to disease was assumed, due to its occurrence in very young larvae (Breuil et al. 1991) and detection of NNV in reproductive fluids and early-stage embryos and larvae before a disease outbreak (Arimoto et al. 1992; Azad et al. 2006; Dalla Valle et al. 2000; Mori et al. 1998). Selection of broodstock that test negative for NNV did not prevent cases of VER (Anderson & Oakey 2008). The need for non-destructive sampling is a limitation for detection of carrier fish. Ozone treatment of eggs was associated with reduced incidence of VER in striped jack and Atlantic halibut (Grotmol & Totland 2000). Consequently ozone treatment of fertilised eggs has become a frequently used management practice to reduce transmission of betanodaviruses via surface contamination of eggs (Schipf et al. 2007).

Alternative transmission pathways, particularly via incoming water, appear to be very important. The potential for horizontal transmission without direct contact between fish has been shown in numerous experimental trials using cohabitation or immersion challenge (Grove et al. 2003; Maeno et al. 2007; Parameswaran et al. 2008; Peducasse et al. 1999; Tanaka et al. 1998). This mode of transmission has also been indicated by epidemiological investigation of natural disease outbreaks (Hick et al. 2013). Complicated and potentially expensive disease-control measures are required, based on consideration of the many pathways through which waterborne transmission of NNV might occur.

1.6.4 Factors influencing transmission and expression of disease

Expression of VER is dependent on characteristics of the host fish. Mortality typically reaches 100% within a few days for susceptible species that are less than three weeks of age at the time of the disease outbreak. The mortality is progressively lower when VER occurs in barramundi between three and eight weeks of age, and might be as low as 10% (Hick et al. 2011a). In species that are susceptible to disease, the progression of VER is slower in sub-adult fish, to a total cumulative mortality around 30% (Bovo et al. 1999). The age of fish at the time when they are first exposed to NNV is a critical factor in development of VER in barramundi and Australian bass under experimental conditions (Jaramillo 2015). Under the trial conditions, challenge with NNV resulted in VER only in fish less than four weeks after hatching, although subclinical infection occurred in older fish.

The role of husbandry stress in expression of VER has been shown in an experimental system (Varsamos et al. 2006). The role of stress is also evident in the field where sub-optimal husbandry and nutrition (for example high stocking density, crowding and handling) has been associated with outbreaks of VER (Athanasopoulou et al. 2003;

Johansen et al. 2004b; Tanaka et al. 1998). Stress, in the form of repeated spawning of broodstock, has been reported as a risk factor for occurrence of VER following vertical transmission (Mushiake et al. 1994). The temperature of the water can affect the expression of disease in the field and in experimental systems (Aranguren et al. 2002; Bovo et al. 1999; Grotmol et al. 1999; LeBreton et al. 1997; Tanaka et al. 2004).

A dose response is seen in experimental NNV infections, suggesting that the amount of virus present influences disease expression (Arimoto et al. 1993; Maeno et al. 2007). There is also some evidence that different virus isolates of the RGNNV genotype vary in virulence in experimentally infected European sea bass (Vendramin et al. 2014).

1.7 Impact

An outbreak of VER in a batch of fish in a hatchery typically results in termination of the production run on the assumption that mortality will be very high. In addition to mortality occurring during disease outbreaks, subclinical infection limits the value of juvenile fish, because regulation prevents release into the wild and transfer to some disease control zones, as detailed in *A zoning strategy for disease control in the Northern Territory (Fisheries policy document: trans-boundary movements of living aquatic animals*, Northern Territory Department of Primary Industry, Fisheries and Mines, Darwin, NT, Australia, 2002). Disease in market-size fish is relevant in Australia for grouper species. Despite the continued occurrence of VER in Australian hatcheries, the impact is limited because the demand from grow-out farms is below the production capacity. Uncertainty about the effect of VER on fish species and production systems that are new to aquaculture may affect the expansion of these industries, as illustrated in the case of tropical grouper production. Uncertainty in the reliability of supply of juvenile fish might also be considered in assessment of the economic viability of new grow-out farms.

The impact of VER on wild fish populations has not been determined, but there are examples of the disease in wild fish in other countries (Vendramin et al. 2013). The improbability of detecting disease, especially in wild fish larvae, necessitates a conservative approach to assessing these impacts. Hatchery production has the potential to produce localised pockets of extremely high NNV infection intensity in the immediate environment.

NNV is present in all parts of the world where aquaculture is practised. VER is primarily a disease of larval and juvenile marine fish. It is also infrequently described as the cause of disease in adult fish and freshwater species. Global impacts of VER include the following:

- Hatchery production of many tropical species in South East Asia is severely limited by VER where limited hatchery biosecurity measures are used. The genotype detected in this region is RGNNV. The disease is also a cause of production loss in grow-out age grouper, causing more severe economic losses.
- VER continues to be a production-limiting disease in Japan where SJNNV, BFNNV and TPNNV genotypes are present, in addition to RGNNV.
- VER occurs throughout Mediterranean countries in species including the European sea bass. The genotypes reported from warm and temperate regions are RGNNV and SJNNV and viruses that represent recombination between these genotypes.
- Aquaculture in Norway and neighbouring countries where cold water species such as halibut are produced are affected by the BFNNV genotype.

Production losses are difficult to quantify and estimates of the economic impact are not available.

Biosecurity regulations within Australia require certification of freedom from NNV infection and restrict movement of populations of hatchery-produced fish that test positive for NNV. Regulation imposes costs irrespective of the occurrence and direct costs of disease. Regulations requiring freedom of fish from NNV before transport elsewhere are designed to prevent the dissemination of NNV from high-prevalence areas (areas where it is routinely detected), to low-prevalence areas (areas where it is rarely or never detected).

Table 1 Summary of disinfection treatment data available for NNV

Treatment	Dose	Time	Starting quantity	Infectivity after treatment	Ref.
Temperature	15°C	1 year	10 ^{7.0} TCID ₅₀ .mL ⁻¹	4.0 TCID ₅₀ .mL ⁻¹	4
	37°C	4 days	10 ^{8.8} TCID ₅₀ .mL ⁻¹	0	4
	40°C and 50°C	60 min	10 ^{7.5} TCID ₅₀ .mL ⁻¹	Yes (n/m)	3
	60°C	10 min	0.02 µg	0	1
		30 min	10 ^{8.8} TCID ₅₀ .mL ⁻¹	0	4
		60 min	10 ^{7.5} TCID ₅₀ .mL ⁻¹	0	3
pH	2	42 days	10 ^{6.5} TCID ₅₀ .mL ⁻¹	0	4
	3	30 min	10 ^{8.0} TCID ₅₀ .mL ⁻¹	3.0 TCID ₅₀ .mL ⁻¹	3
	10–12	10 min	0.02 µg	0	1
		30 min	10 ^{8.0} TCID ₅₀ .mL ⁻¹	3.0 TCID ₅₀ .mL ⁻¹	3
Salinity	Freshwater	6 months	10 ^{6.5} TCID ₅₀ .mL ⁻¹	0	4
		6 months	10 ^{7.0} TCID ₅₀ .mL ⁻¹	4.5 TCID ₅₀ .mL ⁻¹	4
	Seawater	6 months	10 ^{7.0} TCID ₅₀ .mL ⁻¹	4.5 TCID ₅₀ .mL ⁻¹	4
Ultra violet radiation	440 µW.cm ²	10 min	10 µg	0	1
		10 min	10 ^{9.0} TCID ₅₀ .mL ⁻¹	0	4
	104 mJ.cm ²	–	n/a	99.9% decrease	6
Formalin	n/m	n/m	10 µg	Yes	1
	2%	6 h	10 ^{8.8} TCID ₅₀ .mL ⁻¹	4.5	
Chlorine	50 ppm	5 min	10 ^{7.5} TCID ₅₀ .mL ⁻¹	0	4
	100 ppm	30 min	10 ^{7.3} TCID ₅₀ .mL ⁻¹ (with 5% FBS) ^a	5.0	
Iodine	> 50 ppm	10 min	10 µg	0	1
	100 ppm	30 min	10 ^{6.1} TCID ₅₀ .mL ⁻¹	1.5	4
Peroxygen	1:125	5 min	10 ^{6.4} TCID ₅₀ .mL ⁻¹	3	4
Sodium hypochlorite	> 50 ppm	10 min	10 µg	0	1
Benzalkonium chloride	> 50 ppm	10 min	10 µg	0	1
Ozone	0.1 µg.mL ⁻¹	2.5 min	10 µg	0	1
(Idometric titration)	0.5 µg.mL ⁻¹	0.5 min	10 µg	0	1
	4 µg.mL ⁻¹	0.5 min	n/a	0	5
Ozone (DPD) ^b	2 mg.Cl ₂ .L ⁻¹	6.3 min	n/a	96% decrease	
	1.6 mg.Cl ₂ .L ⁻¹	31.5 min	n/a	98% decrease	6
	3.0 mg.Cl ₂ .L ⁻¹	6.7 min	10 ^{4.5} TCID ₅₀ .mL ⁻¹	0	2

a Fetal bovine serum. b Using N,N-diethyl-p-phenylenediamine colourmetric method. n/m: not measured. n/a: not available. References: 1. Arimoto et al. (1996) 2. Buchan et al. (2006) 3. Chi et al. (2001) 4. Frerichs et al. (2000) 5. Grotmol & Totland (2000) 6. Liltved et al. (2006).

2 Principles of control and eradication

2.1 Introduction

This section provides background information to enable the choice of the most appropriate response option following detection of VER disease or exotic NNV infection in Australia.

NNV is endemic in Australia and outbreaks of VER are not an uncommon cause of production losses, particularly in northern barramundi hatcheries. The economic and biological impact of the disease on reliable supply of juvenile fish to grow-out facilities could be a limiting factor to expansion of barramundi farming. It is also an uncertainty that restricts expansion of production enterprises considering novel aquaculture species, such as yellow tail kingfish (*Seriola lalandi*) and tropical grouper species (*Epinephelus* spp.).

The basic principles of eradication and other response options are described in the AQUAVETPLAN Enterprise Manual (DA 2015) and the AQUAVETPLAN Control Centres Management Manual (DAFF 2001). The AQUAVETPLAN Enterprise Manual, Appendix 1 lists State and Territory legislation relating to disease control and eradication.

Eradication is not a possibility for this endemic disease. However, RGNNV is the single genotype of NNV that is present in Australia. Several other genotypes are exotic to Australia and pose a risk to fish production if they enter the country. In circumstances where a risk analysis indicates that eradication may be feasible, it may be attempted for these exotic genotypes of NNV.

Most important for reducing the impact of VER in Australia are the zoning procedures designed to prevent dissemination of NNV from high-prevalence areas to low-prevalence areas. The key actions required are:

- correct diagnosis in all suspect cases, including in endemic areas
- certification of freedom from NNV infection in all hatchery-reared batches of fish
- ongoing surveillance of different epidemiological units to gain a greater understanding of the distribution and prevalence of NNV.

Each of these actions informs policy and regulations to identify, minimise and remove contamination with NNV and dissemination of the virus.

On-farm disease control and mitigation efforts are required in endemic areas. Enterprises manage disease control based on cost:benefit ratio for the biological and economic impacts of the disease. Impacts include reduced production and the need to comply with regulatory controls for export of the juvenile fish to different zones in Australia and access to international markets.

In Australia, VER most commonly occurs in barramundi hatcheries that are semi-open but potentially adaptable to a closed system. Disease response options including eradication, or containment and control, may be reasonable in these circumstances. Recirculation systems are amenable to many control measures, as there is a high degree of control over water and stock. Grow-out of barramundi in semi-open systems such as earth ponds, and other species in similar systems or net-cages, will limit the likely success of an attempted eradication response, because fewer effective disease control

measures can be applied to the large volumes of water typical of these systems. The most effective measures in such systems are to ensure that all stock used are NNV-free and to minimise, where possible, exposure to potential sources of NNV such as wild fish.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

Certification of freedom from infection of all batches of hatchery-reared fish is required before translocation between disease control zones or state government jurisdictions. The certification of freedom from infection for any atypical or exotic genotype of NNV requires RT-qPCR tests on a sample that is correctly selected and of sufficient size to detect a minimum expected prevalence of 2% with 95% confidence. An assumption that the laboratory testing procedures have 100% sensitivity and 100% specificity is often made, but validation should be considered where it is available. Surveys for VER based on histopathological examination are not suitable for the control of disease due to atypical or exotic genotypes of NNV, because this method does not detect fish with subclinical NNV infection (Jaramillo 2015).

A risk analysis is required before movement of NNV-positive fish populations. This will consider the nature of the system in which they will be kept and the conditions in the local environment. The aim is to ensure that the movement of fish is restricted, so the infection risk is not increased by introducing NNV into zones where NNV is absent or the infection status is inconclusive.

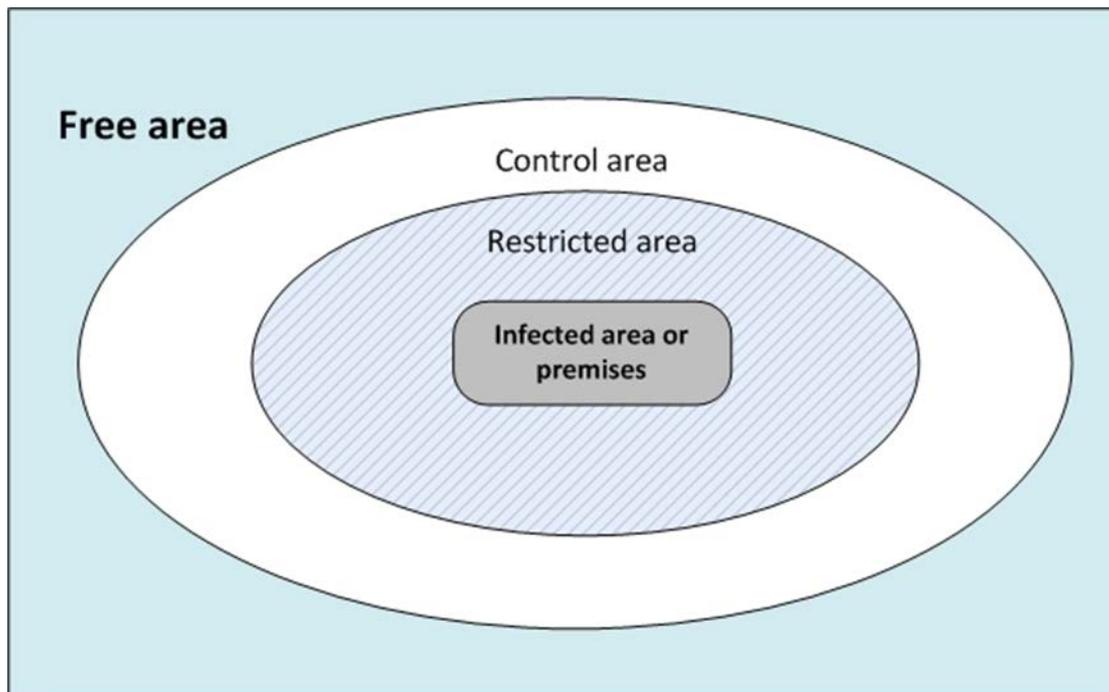
The following quarantine and movement restrictions should be implemented immediately when a case of VER caused by an exotic genotype of NNV is suspected.

Establishment of quarantine areas

Specified areas should be established (see AQUAVETPLAN Enterprise Manual, Section A, for more details)(DA 2015), including:

- declared area (infected, restricted and control areas):
 - infected area or premises — the premises (e.g. farm) or area where the infection is present, and the immediate vicinity
 - restricted area — area around infected premises or area
 - control area — a buffer between the restricted area and free areas
- free area: non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of NNV remains unassessed).

Figure 1 Establishment of specified areas to control VER disease and NNV infection



In the declaration of quarantine areas, the following factors need to be taken into account:

- co-located aquaculture facilities
- probability of release of virus
- presence of susceptible native fish species
- the local environment including any barriers to infection and spread.

The following practices must be considered when implementing response options:

- commercial or recreational fishing
- harvest activities
- discharge of untreated effluent water
- processing of fish products.

Movement controls

The nature of the controls will depend on whether the purpose is continued control of the endemic disease status or containment of an atypical or exotic outbreak of VER (e.g. a new fish species, location, production system or genotype of NNV). Movement controls include live fish and may include fish products and aquaculture equipment.

Implementation of bans and restrictions is a dynamic process, determined by the location and extent of the disease outbreak and whether the aim is to eradicate the disease agent or to control its spread. Some restrictions may be impractical or unnecessary but others will be of critical importance to eradication or control.

The feasibility of restrictions and bans and the extent to which these are able to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

2.2.2 Zoning and compartmentalisation

Zoning

As VER is endemic in many regions of Australia, a zoning policy specific for NNV infection is necessary to protect areas in which disease is not frequently reported and to reduce the incidence of disease. Zones are based on biogeographic barriers considering the hydrological characteristics of water bodies and landforms, in addition to state boundaries. A corresponding surveillance and monitoring program for NNV is required to support the zoning policy, as the infection status of many epidemiological units has to be determined. Zoning should consider the viral load, potential for spread of NNV infection and incidence of disease rather than a dichotomous positive or negative assessment.

Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines (DAFF 2000) and in the OIE *Aquatic Animal Health Code* (OIE 2016a).

Compartmentalisation

A 'compartment' means one or more aquaculture establishments under a common biosecurity management system. The compartment contains an aquatic animal population with a distinct health status relating to a specific disease or diseases for which required surveillance and control measures are applied, and basic biosecurity conditions are met, for the purpose of international trade. Such compartments must be clearly documented by the competent authority.

A compartment does not have to be contiguous facilities—it can apply to a series of farms over a large area, including over several jurisdictions. The key is that it must have in place a biosecurity management system that meets guidelines in the OIE *Aquatic Animal Health Code*, Chapters 4.1 and 4.2 (OIE 2016a), and that these systems have been documented by the competent authority (the veterinary authority of the jurisdiction).

Disease management in aquatic environments

The establishment of disease management area (DMA) boundaries during an emergency aquatic animal disease event presents particular difficulties requiring detailed consideration beyond those normally required for terrestrial animal disease control. Water movement through and around farms, within streams or rivers, and in the marine environment represent a substantial risk for spread of disease through transfer of infectious pathogens in the water column, movement of infected material (particularly on suspended organic and inorganic matter) and any infected wild organisms.

For example, although an infected area may be established around an individual land-based hatchery or farm, water bodies adjacent to the infected area as well as in the same catchment should be considered for monitoring and control measures. The establishment of DMA boundaries around marine farms or wild fisheries may need comparatively large areas that must take into consideration local currents, natural barriers and the normal range of susceptible wild species.

Establishment of the relevant DMA boundaries must also take into account dispersal of water discharged from any infected semi-closed aquaculture systems (e.g. hatcheries) or potentially infected processing facilities, and how this enters adjacent waters. Similarly, outbreaks in semi-open systems (marine farms) require the consideration of all oceanographically connected areas and distribution of wild host or vector populations.

Spread of infected material through scavenging by other species also needs to be considered.

Thus, rather than property boundaries, the geography, water flow, distance between farming areas and the range of susceptible species will define where DMA boundaries are placed.

Establishment of DMA boundaries and their classification must also take into account potential mechanisms by which disease may move beyond these boundaries. In most circumstances it is advisable to overestimate the size of DMAs and reduce their area as the response takes effect. In most cases, in the initial response, the DMA boundaries will need to include the whole of a catchment area in freshwater systems and complete bays or regions in marine environments.

With a few rare exceptions it will not be possible to establish containment of NNV because of the open nature of the aquaculture facilities and the ready source of susceptible reservoir populations in the surrounding aquatic environment. Persistent infection of wild fish provides a means for widespread dissemination of NNV.

In the case of VER, effective disease management requires a quantitative approach in which NNV infection intensity is considered, rather than the simple presence or absence of infection and disease. Rigorous decontamination measures should be applied to locations where outbreaks of VER have occurred and when fish with a high prevalence of subclinical infection are identified. Viral loads in water are positively correlated with clinical disease expression in experimentally challenged barramundi, or with the decay of infected carcasses (Jaramillo 2015), but subclinically infected barramundi can also generate large quantities of virus and act as a source of infection for susceptible cohorts (Hick et al. 2011a). The large concentrations of NNV present in these circumstances are a feasible target to reduce viral load in the affected area.

These controls are applied at the level of individual aquaculture facilities. Land-based hatcheries are amenable to thorough disinfection and isolation from the surrounding aquatic environments by treatment of discharge water. Earth ponds and sea-cages cannot be isolated but can be depopulated and subjected to disinfection measures. Dispersal of NNV by fomites can be prevented in all cases.

2.2.3 Tracing

Tracing a disease outbreak is the process of retrospectively determining the method and pattern of disease spread. Tracing investigations are crucial in determining all confirmed and potential locations of the disease, as well as defining restricted and control areas. The information gathered from tracing will assist in determining the most appropriate response action. The immediate steps required 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). Movement of the following items must be traced:

- stock
- vehicle
- aquaculture equipment (including boats)
- personnel.

Neighbouring fish farms and wild fish of many species and age classes 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 large number of susceptible fish species and vectors for NNV should be noted both upstream and downstream of the infected site. Further sources of infection may be identified if several facilities share common water.

2.2.4 Surveillance

Surveillance is necessary to:

- define the extent of the infection
- detect new outbreaks
- establish restricted and control areas to which quarantine and movement restrictions are applied
- establish infected and non-infected areas and zones for NNV for a VER zoning program
- monitor the progress and success of an eradication strategy.

Surveillance to determine spread of NNV requires detection of the virus rather than incidents of clinical disease. Disease control recommendations are based on the presence of the virus and not the occurrence of clinical disease. A positive survey result is defined as any confirmed detection of NNV in the epidemiological unit of interest, as this is the relevant information needed to make VER disease control recommendations. A negative survey result requires consideration of the sensitivity and specificity of the laboratory tests, and is interpreted based on the design prevalence and confidence level of the survey. The appropriate screening test for NNV is RT-qPCR because it is rapid and sensitive. A variety of secondary tests are available for confirmation of a positive result, as outlined by the ANZSDP: [Betanodavirus infection of finfish \(Moody & Crane 2014\)](#). Such tests require destructive sampling to obtain tissues from the retina and the brain.

Antibody enzyme-linked immunosorbent assay (ELISA) for detection of NNV-specific antibodies in fish serum may be suitable for surveillance of fish populations in the future when the test has been validated (Jaramillo 2015).

Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the OIE *Manual of Diagnostic Tests for Aquatic Animals*. The manual also provides specific information on surveillance for VER (OIE 2016b).

2.2.5 Treatment of infected host species

Specific treatments for VER have not been described or evaluated. Culling of affected populations is frequently indicated for economic reasons and to minimise the risk of amplification and distribution of virus. The impact of VER can be reduced with attention to host and environmental factors, such as treatment of concurrent disease and minimising stress through optimal nutrition, water quality and husbandry.

When VER occurs in nursery age or older barramundi and Australian bass, the cumulative mortality is low and there is potential for the batch of fish to be grown productively despite persistent infection. The fate of fish with persistent subclinical infection depends on the circumstances and jurisdictional policy. For example, barramundi populations that test positive by RT-qPCR can be grown in some northern Australian zones where NNV is considered endemic. Australian bass with a similar infection status are not permitted to be released in natural waterways and impoundments in NSW.

2.2.6 Treatment of host products and by-products

Clinically affected fish have a very high titre of NNV (Hick et al. 2011a). Effective decontamination procedures need to consider the resilience of NNV in the environment and the impact of organic material on efficacy of disinfection treatments (described in Section 2.2.9).

A procedure for decontamination of an aquaculture facility will need to be developed that is suitable for the individual circumstance. Guidelines are provided in the AQUAVETPLAN Operational Procedures Manual – Disposal (DAFF 2009). However, the huge volumes of water with high organic loads in pond or intensive culture conditions make effective decontamination problematic.

NNV can retain infectivity in live fish, fresh fish and fish products frozen at -20°C for at least several months. Persistent subclinical infection is concentrated in the retina and brain and the titre of NNV is much lower than in a VER outbreak (Hick et al. 2011a).

NNV does not pose a food safety concern. Management of fish products with NNV infection might pose a risk to fish populations and requires a biosecurity assessment. Trade regulations, market requirements and potential spread of NNV must be considered when determining the treatment, processing and destiny of fish products and by products. Destroyed fish, for instance, must not be used as bait.

2.2.7 Destruction of hosts

Fish affected by VER or with NNV infection should be culled humanely, if required. The method of collecting and euthanasing the fish will depend on individual circumstances, including the age and size of the fish and the temperature of the water in which they were raised. Equipment used during destruction of fish, such as containment vessels and nets, requires decontamination. Destruction of the fish can be achieved through use of an overdose of anaesthetic, ice for some warm water species or practices consistent with commercial harvest.

Any chemicals used must be approved for that use by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (refer to Appendix 2).

In addition, any chemical that is used directly or indirectly for the control of an animal disease is governed in its use by relevant 'control of use' legislation in each state and territory. The relevant state or territory authority (in most cases this is the veterinary registrar within the relevant state department of primary industry or agriculture) should also be consulted for advice before use of the chemical.

2.2.8 Disposal of hosts

The most appropriate method for disposal depends on individual circumstances with the objective of ensuring containment of NNV and prevention of spread. The disposal procedure will also need to consider the accompanying decontamination methods likely to be effective for NNV present in a large volume of organic matter, and the potential for persistence of infectious NNV for prolonged periods in the environment (Section 1.6.2). It will be critical to reduce the exposure of susceptible species present in neighbouring aquatic environments to the extreme viral load generated from a population of clinically affected fish. The AQUAVETPLAN Operational Procedures Manual – Disposal should be consulted for further information on disposal (DAFF 2009).

2.2.9 Decontamination

Decontamination options for water and equipment that is contaminated with NNV can be inferred from virology studies in Table 1.

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, and the state or territory CVO and/or Director of Fisheries. The protocol should take into consideration the factors outlined in Section 1.6, in particular:

- the source and location of infection
- the type of enterprise (e.g. farm, processing plant, hatchery, grow-out ponds, water source)
- the construction materials of the buildings and structures on the site
- the design of the site and its proximity to other waterways or buildings
- current disinfection protocols
- workplace safety concerns
- the environmental impact of the disinfectant protocol
- legislative requirements (occupational health and safety, environmental protection, chemical use, local government permits)
- availability of approved, appropriate and effective disinfectants.

See the AQUAVETPLAN Operational Procedures Manual – Decontamination (DAFF 2008) for details of decontamination methods and their indicators.

2.2.10 Vaccination

None of the vaccination or immune stimulation strategies described in Section 1.5 are currently available for use in a response to VER in Australia.

2.2.11 Vector control

Vector control for prevention of VER is encompassed in hatchery biosecurity protocols. In addition to subclinical infection in many species of fish, there is PCR evidence that marine invertebrates might carry NNV, with viral RNA detected in wild-caught species of crab, shrimp and mussel collected from the Korean peninsula (Gomez et al. 2008), and from a spiny lobster collected from a commercial aquarium in Seoul, Korea (Gomez et al. 2006). Uncharacterised *Betanodavirus* species have also been detected in association with skin lesions in the common octopus *Octopus vulgaris* (Fichi et al. 2015), while a *Betanodavirus* phylogenetically related to the RGNNV genotype was detected in the Japanese common squid *Todarodes pacificus* (Gomez et al. 2010).

2.3 Environmental considerations

Adequate treatment of effluent water is required when NNV infection is known to be present at a land-based aquaculture facility. Horizontal transmission of NNV in intake and effluent water is a plausible transmission pathway, based on laboratory studies demonstrating VER in fish challenged by cohabitation and immersion (Section 1.6.3). Wild fish and neighbouring aquaculture farms are at risk of disease and there is the potential to create a reservoir of NNV infection.

The risk of damage caused by the release of chemicals associated with disinfection should be considered. See the AQUAVETPLAN Operational Procedures Manual –

Decontamination (DAFF 2008) for details of decontamination methods. Permission for discharge of any chemically treated water is essential in all jurisdictions.

2.4 Sentinel animals and restocking measures

Aquaculture facilities can be restocked after cleaning and disinfection procedures have been completed. Destruction of all aquatic species and age classes at an affected facility is required after an outbreak of VER because all are potential carriers of NNV. Hatchery hygiene decisions need to take into account the long-term stability of NNV in many environments, and the relative resistance of the virus to many common disinfection procedures. Susceptible fish, such as barramundi less than 3 weeks of age, would be required as sentinels. Disease or positive RT-qPCR tests would be expected to occur within two weeks if NNV was present.

2.4.1 Public awareness

The rationale for VER disease control policies should be effectively communicated through a community engagement program. Key messages may include information about susceptible fish species, known areas of endemic NNV infection, and the potential for disease transmission when apparently healthy fish are released into farms or the wild. It should also be emphasised that VER and NNV do not pose any human health or food safety concerns.

2.5 Feasibility of eradication or containment and control of viral encephalopathy and retinopathy in Australia

The feasibility of controlling an outbreak of VER depends on the nature and location of the outbreak and the management strategy adopted. Essentially, as outlined in Section 2.1, there are three response options:

- Option 1: eradication. This is relevant only to genotypes of NNV that are considered exotic to Australia, that is, all genotypes except RGNNV. Eradication is unlikely to be successful and would only be attempted after a risk assessment in select circumstances, such as a contained facility.
- Option 2: containment and control via zoning and compartmentalisation. Policies and procedures that limit the risk of VER affecting farmed and wild fish. These policies would be more stringent if there was an incursion of an exotic genotype of NNV that could not be eradicated.
- Option 3: control and mitigation. The bulk of efforts to reduce the impact of VER are based on measures that reduce the incidence of disease and range of fish populations and locations that are affected.

2.5.1 Response option 1: eradication

Eradication of an exotic genotype of NNV might be feasible if it is detected in a closed system with existing controls of effluent water and timely detection of the infection. The feasibility assessment should take into account the host and geographical range of the NNV genotype. Eradication would require complete destocking of the facility followed by cleaning and disinfection, as described in Section 2.2.9. Surveys of at-risk epidemiological units would inform continued efforts for eradication.

2.5.2 Response option 2: containment and control via zoning and compartmentalisation

Containment and control of VER using zoning and compartmentalisation is complicated, because although NNV infection is endemic in Australia, the detailed distribution of NNV has not been determined. The RGNNV genotype has been identified in multiple wild fish species in Queensland (Anderson & Oakey 2008), but is presumed to be widespread, an assumption based on studies conducted in other regions of the world. However, the infection status of many epidemiological units remains unknown.

Certification of freedom from infection with NNV can be used to minimise the spread of NNV and regions and types of enterprises or fish species affected by VER (see Section 3.3).

Exposed or potentially exposed clinically normal fish

An important consideration for control of VER is the potential for apparently healthy fish to be infected with a high titre of NNV. This cannot be determined without laboratory testing. A successful zoning and compartmentalisation program will rely on movement restrictions to control movement of fish from environments with a high pathogen load to epidemiological units with a lower pathogen load.

2.5.3 Response option 3: control and mitigation

Control of VER in endemic areas depends on hatchery-level biosecurity and disease control procedures. The application of control measures is at the discretion of the managers of aquaculture facilities and is dictated by economic considerations. Measures need to be applied to reduce the economic and biological impact of VER to an economically acceptable level. Considerations include the regular and reliable supply of seedstock from hatcheries to grow-out producers, and the restriction on translocation of fish with persistent infection. There are currently no regulations that take account of the need to quantitatively restrict NNV distribution, such as treatment of effluent water and destruction of fish with subclinical infection. Measures for control of VER at a hatchery are detailed in Section 2.2.

2.5.4 Trade and industry considerations

Trade regulations and market requirements apply only to live fish translocated for aquaculture. Fish and products for human consumption need not be considered as part of a response strategy. Fish purchased for stock enhancement are usually only able to be stocked if they are shown to be free from VER or NNV. There is unlikely to be any need for relevant authorities to restrict products sold for human consumption.

Export markets

Some countries may have import conditions related to VER in place, such as requiring imports to be certified free of NNV infection. The Department of Agriculture is responsible for the health certification of all exports and should be contacted for further information (exports@agriculture.gov.au).

Domestic markets

Harvesting of exposed or potentially exposed product for the domestic market requires a somewhat cautious approach, given the potential for dissemination of NNV in subclinically infected fish. The risk associated with waste products from fish processing must be effectively managed to eliminate the potential for dissemination of NNV. NNV is

not a human health consideration. Release of harvested aquatic species and their products to the domestic market generally does not require any restriction.

3 Preferred Australian response options

3.1 Overall policy for VER

Viral encephalopathy and retinopathy (VER), caused by the RGNNV genotype of nervous necrosis virus (NNV), is an endemic disease of marine finfish in Australia and can cause devastating losses in larvae and juveniles, especially in barramundi and numerous emerging aquaculture species. Additional genotypes of NNV are exotic to Australia and have the potential to increase the host and geographic range of VER. The NNV infection status of most areas in Australia is unknown, but PCR-tested NNV-positive fish are widely distributed. This may be interpreted as an indication that NNV is likely to be present in most parts of Australia and could manifest as disease if intensive aquaculture, particularly hatchery production, was undertaken in any given region. For this reason, a rapid and vigorous response by regulatory authorities may not be justified unless an outbreak with unusual characteristics occurs.

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 after epidemiological investigation by the director of fisheries and/or the chief veterinary officer (CVO) of the state or territory where the outbreak occurs.

The disease is familiar in barramundi hatcheries in multiple states and the Northern Territory. A minimal response is required in this circumstance. Normal decontamination and safe disposal of destroyed stock are essential to allow restocking or resumption of hatchery production.

Laboratory confirmation of a diagnosis of VER is required in all cases. This distinguishes the endemic and exotic virus species and assists with deciding on control measures for outbreaks of VER in previously unaffected species, production systems and locations.

There are three possible response options for VER in Australia:

- Option 1: eradication of exotic strains of betanodaviruses
- Option 2: containment and control of the virus to endemic infection areas to prevent further spread and protect uninfected areas
- Option 3: control and mitigation.

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

- quarantine and movement controls on fish, fish products, equipment and fomites in declared areas to prevent spread of infection
- destruction and disposal of all clinically diseased or dead fish as soon as possible to prevent further virus shedding
- decontamination of facilities, products, equipment and fomites to eliminate the virus in 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
- zoning and compartmentalisation to define and maintain infected and disease-free zones and compartments.

Identification of fish with persistent subclinical NNV infection using laboratory tests is required for all disease control measures.

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 submitted to the Aquatic Consultative Committee on Emergency Animal Disease (AqCCEAD), who will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

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 response measures in consultation with AqCCEAD. The detailed response measures adopted will be determined using the principles of containment, control, mitigation and eradication (see Section 2), epidemiological information about the outbreak, and the financial feasibility of the option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres Management Manual (DAFF 2001).

3.2 Response options

The circumstances surrounding an outbreak of VER will greatly influence selection of the most suitable response option. Recurrence of the disease in the familiar contexts described in Section 2.1 will not trigger a response. The actions outlined in Figure 2 are required in scenarios where there is suspicion that VER has occurred in an unfamiliar species or production system, or an exotic genotype of NNV is present.

Figure 3 has been developed to help identify the most appropriate response option. These decision trees are flexible, depending on the specific situations experienced.

Figure 2 Decision matrix for suspected NNV infection

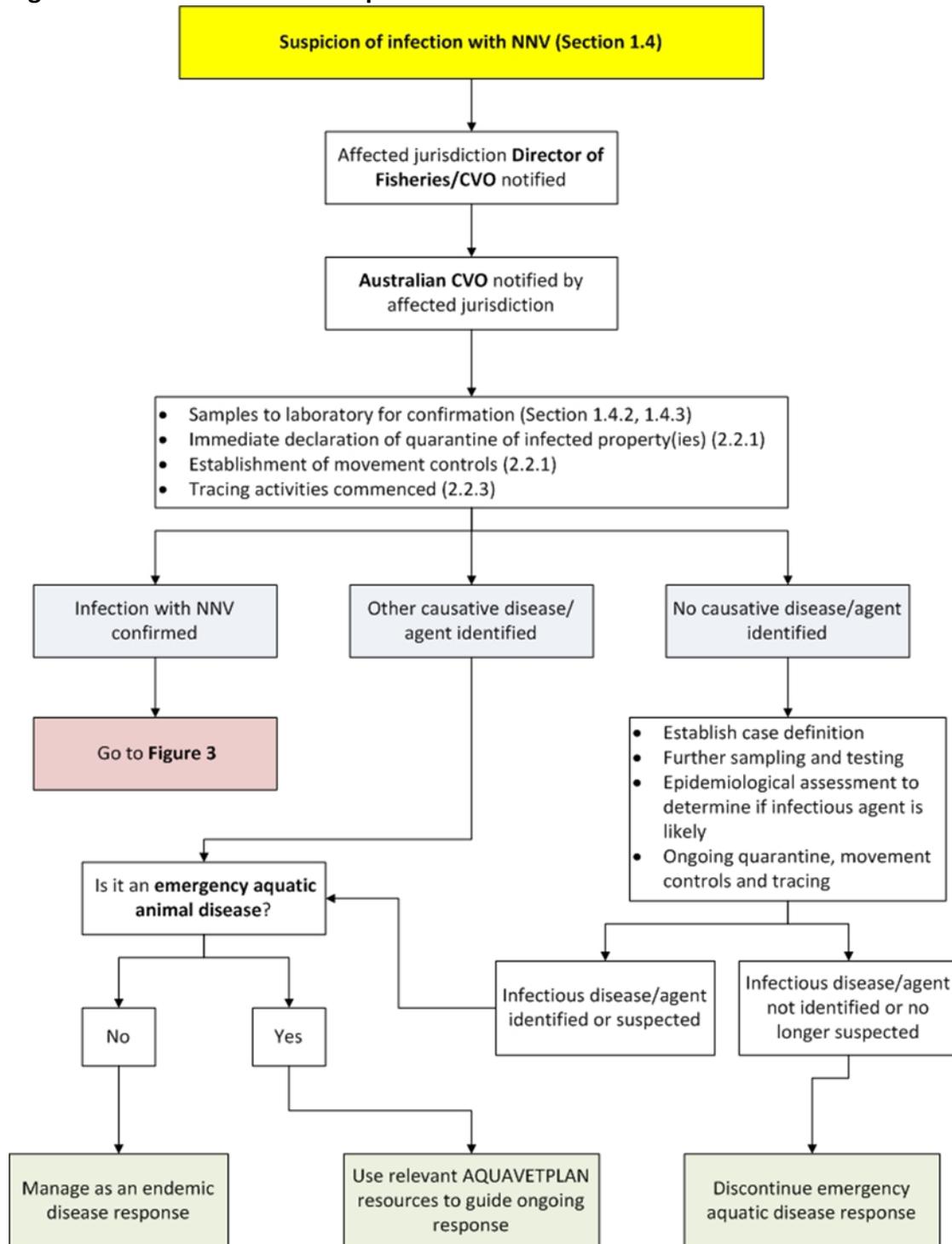
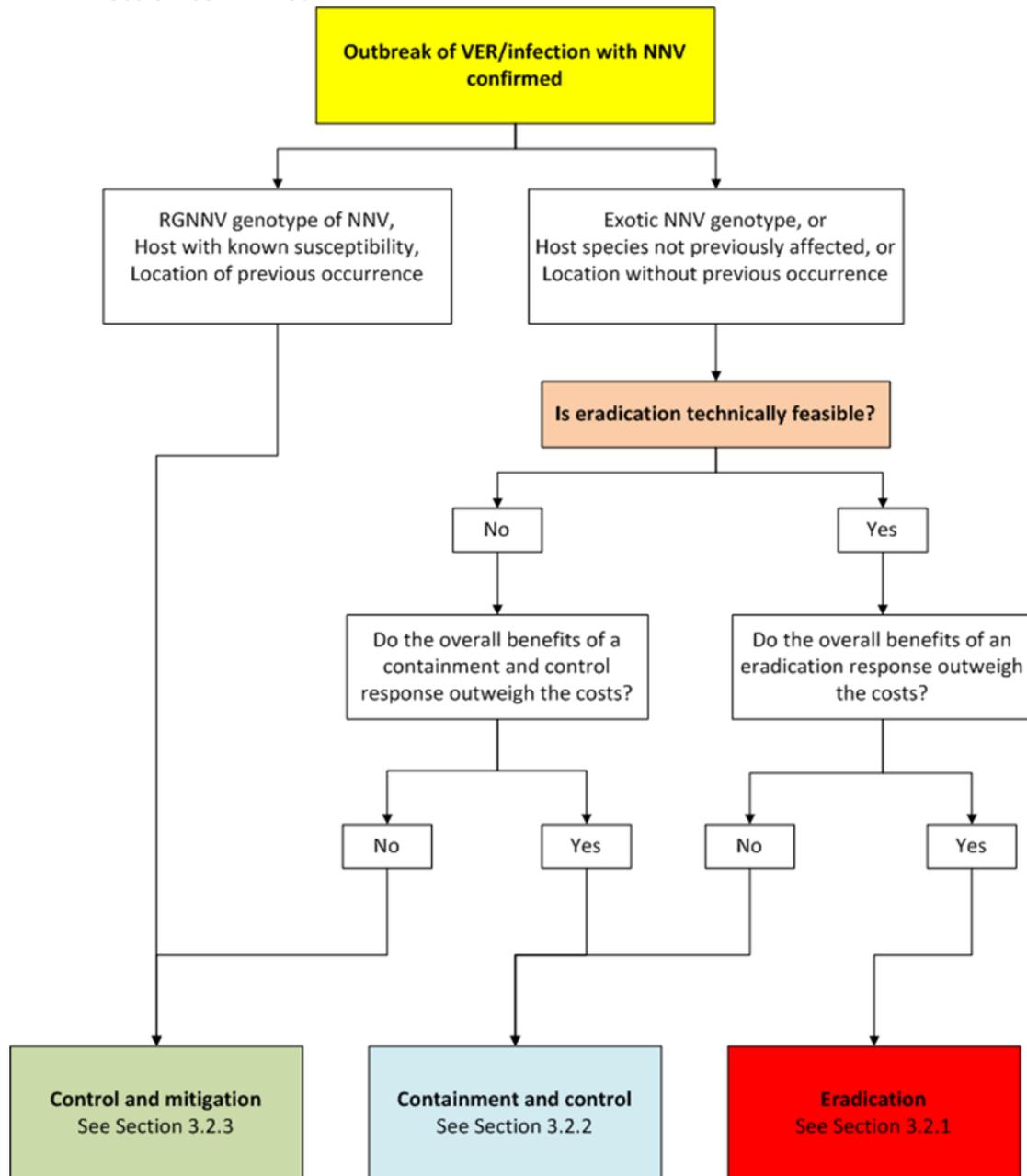


Figure 3 Determination of most appropriate response option to VER outbreak or NNV infection confirmed



3.2.1 Option 1: eradication

Eradication of VER caused by the RGNNV genotype of NNV is not possible at a national or state level in Australia. Recurrent outbreaks of VER caused by the RGNNV genotype have been recognised since 1988. There is also evidence provided by RT-PCR tests that NNV is present in apparently healthy wild fish in Queensland (Anderson & Oakey 2008). This means there is a natural reservoir from which infection cannot be eliminated.

Genotypes of NNV other than RGNNV are exotic to Australia and have a different spectrum of host range and potential geographic distribution. There are circumstances in which eradication of an exotic NNV genotype might be considered both feasible and of sufficient long-term value (e.g. from a cost-benefit analysis) to warrant this response. Eradication of an exotic genotype would protect some species of farmed and wild fish.

For example, the BFNNV and TNNV genotypes cause disease at lower water temperatures and in different species of fish (e.g. pleuronectids) compared to RGNNV.

Eradication is only likely to be possible if the agent is detected in a closed aquaculture system, is associated with a point-source introduction, and the potential for viral spread is limited. In these circumstances, the affected premises would need to be placed under quarantine. Destocking would require destruction and disposal of all age classes and species of aquatic animals within the facility. Epidemiological investigation would begin immediately on detection of incursion of the agent. Information from field investigations and laboratory tests would inform and update the extent of the outbreak and determine if eradication remains the best approach. Investigations would include trace-forward and trace-back activities to identify at-risk farmed and wild fish populations.

The most likely aim of delimiting surveys will be to detect NNV rather than map the distribution of clinical cases of VER, because the pathogen is likely to be present in fish with subclinical infection. Surveillance for infection should be conducted according to the principles of testing for freedom from infection in each epidemiological unit at risk of exposure during the outbreak. The sampling strategy requires selection to maximise the probability of detecting NNV if it is present. Laboratory tests require the use of RT-qPCR as the most rapid and sensitive test for NNV. Determination of the nucleotide sequence of the capsid protein gene (RNA2) is the most rapid method of confirming the result and determining the genotype of NNV.

3.2.2 Option 2: containment and control via zoning and compartmentalisation

A zone or compartment is a hydrodynamic region that might be isolated for the purpose of disease control during a response to VER. This might include individual aquaculture facilities, or water bodies such as river catchments and bays, that form an isolated hydrodynamic unit. Expert consultation and/or hydrological modelling will be required to identify delimiting boundaries in the specific circumstance. Certification of freedom from infection with NNV will enable control and zoning. The relative quantity of NNV (viral load and prevalence) in defined populations of fish and locations should be minimised to reduce the prevalence of NNV infection and potentially reduce the incidence of VER. Tests for freedom from NNV and prevalence surveys will assist disease control by identifying the NNV infection status of different epidemiological units.

Rapid identification of VER outbreaks with subsequent destruction of infected and exposed populations of fish, and appropriate disinfection of facilities and equipment, is required to reduce the overall load of NNV in the environment.

3.2.3 Option 3: control and mitigation

Control and mitigation of disease is the most suitable approach for VER caused by RGNNV in Australia. The incidence of disease can be reduced through efforts to reduce the incursion of NNV from environmental reservoirs into populations of susceptible young fish and larvae in hatcheries. This requires a suite of stringent biosecurity and hygiene measures.

Rapid, laboratory-confirmed diagnosis of VER and surveillance for subclinical infection will enable the burden of NNV to be reduced through timely application of disease and infection control measures. Disinfection procedures appropriate for NNV in the specific facility should be applied when NNV is detected. Most important is an effective method to disinfect incoming seawater.

The primary cause of VER is a viral infection for which no practical and effective treatments have been described. Efforts to reduce stress on broodstock and provision of optimal husbandry for larvae and juvenile fish will reduce the impact and severity of disease (Section 1.6.4). Guidelines for barramundi culture have been provided by Schipp et al. (2007). Similarly, treatments for correctly diagnosed secondary disease can reduce the mortality, although the surviving fish will be persistently infected (Hick et al. 2011a) and further grow-out or stocking options will be restricted.

Measures can be implemented at each stage of the production cycle to reduce the risk of VER, using methods to detect NNV before disease occurs. Screening of broodstock is difficult because definitive exclusion of NNV infection is not possible without destructive testing. Options include repeated PCR testing of gonad fluids, application of an antibody ELISA and sourcing stock from known NNV-free populations. Fertilised eggs can be treated with an appropriate dose of ozone or an iodophore, with consideration of the susceptibility of eggs of the fish species to chemical damage. A concentration \times time (CT) dose of ozone of between 0.8 and 0.9 has been used in some cases (Schipp et al. 2007). This dose is calculated as the average concentration of ozone (starting at 0.5 mg/L) multiplied by the time (in minutes) of exposure.

Larvae can be reared in conditions with very high biosecurity measures in place, including disinfection of rotifer culture water and use of dedicated staff and equipment. The most common methods for disinfection of intake water are sodium hypochlorite treatment, exposure of filtered water to large doses of ultraviolet irradiation, or ozone treatment. Adequate treatment of water can be impractical in many production systems, but it may be possible to target water treatment efforts at younger fish cohorts that are most susceptible to infection. For example, VER could be prevented in barramundi and Australian bass by eliminating exposure to NNV in the first 4 weeks after hatching (Jaramillo 2015). However, these species are still susceptible to subclinical infection up to 9 weeks of age and possibly at older ages. An 'all-in, all-out' production system with routine cleaning and disinfection is desirable. Fish for grow-out and translocation should be sourced from populations with laboratory confirmation of freedom from NNV infection.

3.3 Criteria for proof of freedom

Proof of freedom from VER may be important for international trade and movement of fish between different zones and compartments in Australia. Freedom from disease can be established at the aquaculture establishment and zone or compartment level but, in the case of RGNNV in Australia, not at the state or country level. Certification of freedom from disease requires demonstration that NNV infection is not present at a minimum expected prevalence (often 2%) with 95% confidence, irrespective of evidence of pathology and disease. The general criteria for proof of freedom at each level are given in the OIE Aquatic Animal Health Code, Chapter 1.4 Aquatic Animal Health Surveillance, available online at oie.int/en/international-standard-setting/aquatic-code/access-online.

Certification of batches of fish for routine translocation between jurisdictions or disease control zones within Australia varies depending on the jurisdiction and the species of fish. For example, routine health clearance for VER in barramundi being translocated from Queensland to South Australia or Victoria typically uses histopathological examination of 150 formalin-fixed 21-day old larvae. The same movement of barramundi from Queensland to the Northern Territory would require RT-PCR testing of 150 barramundi in pools of five brains (or heads) per pool. Some jurisdictions may also require an additional 21-day quarantine period in barramundi fry cleared of infection at 21 days of age (this quarantine period may be carried out at the source

facility before translocation, or at the receiving facility after translocation). This further reduces the risk of translocating infected fish, by recognising that barramundi may occasionally manifest mild clinical disease up to 42 days of age (Ian Anderson, Principle Veterinary Pathologist, Biosecurity Sciences Laboratory, 2015, personal communication).

Histopathological examination of fish may be considered a suitable diagnostic test for determining the disease status at the zone or compartment level during an emergency disease response to VER, but this will vary with the species and age-class of the fish being tested. During an emergency disease response to VER, when it may be particularly important to detect subclinical carriers, it is suggested that RT-qPCR be used as the diagnostic test of choice, due to its greater sensitivity, specificity and short laboratory turnaround (two to five days, assuming that testing has been prioritised by the relevant diagnostic laboratory during an emergency disease response (Nick Moody, Team Leader, Aquatic Diagnostic Capability, Australian Animal Health Laboratory, 2015 and Ian Anderson, 2015, personal communication). For the purposes of establishing freedom of disease at the zone or compartment level, or before approved translocation of fish between disease control zones, negative RT-qPCR tests on a sample of 150 fish selected randomly from the population is recommended.

3.4 Funding and compensation

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

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

OIE Aquatic Code

The objective of the OIE *Aquatic Animal Health Code* (OIE 2016a) is to prevent the spread of aquatic animal diseases, while facilitating international trade in aquatic animals and aquatic animal products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade of aquatic animals and their products.

The current edition of the OIE Aquatic Code (19th edition) was published in 2016 and is available on the OIE website at oie.int/international-standard-setting/aquatic-code/access-online.

Viral encephalopathy and retinopathy has not been listed by the OIE since 2005, because it does not meet the criteria for listing described in chapter 1.2: oie.int/index.php?id=171&L=0&htmfile=chapitre_criteria_diseases.htm.

OIE Aquatic Manual

The purpose of the OIE *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2016c) 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 world.

The current edition of the OIE Aquatic Manual was published in 2016 and is available at oie.int/international-standard-setting/aquatic-manual/access-online.

The chapter 2.3.12 Viral encephalopathy and retinopathy is relevant to this manual: oie.int/index.php?id=2439&L=0&htmfile=chapitre_viral_encephalopathy_retinopathy.htm.

Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at oie.int/international-standard-setting/overview

Appendix 2 Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. In addition, an import permit is required from the Department of Agriculture and Water Resources if a product containing biological material is to be sourced from overseas.

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permits. Since the assessment process is so detailed, the evaluation may take some time to complete.

Minor use permits

The minor use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. The system was devised by the APVMA for Australia, and allows the restricted use of a limited amount of a drug or chemical in a specified species when inadequate data are available to satisfy APVMA requirements for registration. Conditions are applied to the permit, which often include the collection of data related to the use of the product. The MUP system aims to enable restricted use of a drug or chemical until sufficient data are available to enable full registration.

For example, the APVMA may set a temporary withholding period with a wide margin of safety for an MUP. This withholding period may have been extrapolated from data relating to the use of the product in other species. In such cases, a condition of the MUP will be the collection of residue testing data. Results from the data are assessed by the APVMA (usually after 12 months, which is the duration of most permits) and used to more accurately set a withholding period for the product.

Emergency use permits

The APVMA has a permit system for the emergency use of a product that is either unregistered in Australia or registered for use in a different species or for a different use pattern. The APVMA will verify with the appropriate state and territory coordinators that the emergency is genuine.

For further details or permit application forms, visit the APVMA website (apvma.gov.au/node/10886).

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