**Annex 28. Item 8.1.3. – Chapter 2.2.6. Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)**

CHAPTER 2.2.6.

infection with
*MACROBRACHIUM ROSENBERGII* NODAVIRUS (white tail disease)

1. Scope

Infection with *Macrobrachium rosenbergii* nodavirus means infection with the pathogenic agent *Macrobrachium rosenbergii* nodavirus (*Mr*NV) in the Family *Nodaviridae*. The disease is commonly known as white tail disease (WTD).

Extra small virus (XSV) is associated with disease but its role has not been determined.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Two viruses are associated with WTD, namely *Mr*NV (primary) and extra small virus (XSV) (associate) (Qian *et al.,* 2003; Romestand & Bonami, 2003). MrNV is a necessary cause of WTD in prawns, however, the role of XSV in pathogenicity remains unclear.

*Mr*NV belongs in the family *Nodaviridae* (Bonami *et al.,* 2005). While the physico-chemical properties of *Mr*NV are consistent with those of other members of the *Nodaviridae*, it differs structurally and genetically from other nodaviruses within the two recognised genera, *Alphanodavirus* and *Betanodavirus* (Ho *et al*., 2017, 2018; Naveenkumar *et al*., 2013). Consequently, a third genus, *Gammanodavirus*, has been proposed for nodaviruses that infect crustaceans, including *Mr*NV and *Penaeus vannamei* nodavirus (*Pv*NV) (Naveenkumar *et al*., 2013).

XSV is the first sequenced satellite virus in aquatic animals and it is also the first record of a satellite-nodavirus association (Bonami *et al.,* 2005). XSV has been classified by the ICTV as *Macrobrachium* satellite virus 1 of the family *Sarthroviridae.*

2.1.2. Survival and stability in processed or stored samples

Both viral pathogens (*Mr*NV and XSV) are stable in processed or stored samples stored at –20 or –80°C. Storing the samples at –80°C is recommended for long-time storage and maintenance of pathogen virulence (Sahul Hameed & Bonami, 2012). The infected samples should be processed at low temperature to maintain the stability of the viruses. Viral inoculum prepared from infected prawn stored at –20°C caused 100% mortality in postlarvae (PL) of *M. rosenbergii* by immersion challenge (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a). Ravi & Sahul Hameed (2016) found that MrNV in tissue suspensions was inactivated after exposure to 50°C for at least 5 min.

2.1.3. Survival and stability outside the host

Survival outside the host is not known.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Mr*NV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: giant river prawn (*Macrobrachium rosenbergii*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with MrNV according to Chapter 1.5. of the *Aquatic Code* are: white leg shrimp (*Penaeus vannamei*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results (but not active infection) have been reported in the following species:

| *Family* | *Scientific name* | *Common name* |
| --- | --- | --- |
| *~~Aeshnidae~~* | *~~Aeshna~~* ~~sp.~~ | ~~dragonfly~~ |
| *Artemiidae* | *Artemia* sp. | brine shrimps  |
| *~~Belostomatidae~~* | *~~Belostoma~~* ~~sp.~~ | ~~giant water bug~~ |
| *~~Dytiscidae~~* | *~~Cybister~~* ~~sp~~*~~.~~* | ~~beetle~~  |
| *~~Notonectidae~~* | *~~Notonecta~~* ~~sp.~~ | ~~backswimmer~~ |
| *Palaemonidae* | *Macrobrachium rude* | hairy river prawn |
| *Macrobrachium malcolmsonii* | monsoon river prawn  |
| *Parastacidae* | *Cherax quadricarinatus* | red claw crayfish |
| *Penaeidae* | *Penaeus japonicus* | kuruma prawn |
| *Penaeus indicus* | Indian white prawn |
| *Penaeus monodon* | giant tiger prawn |

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Experimental pathogenicity studies revealed that larvae, PL and early juveniles of *M. rosenbergii* are susceptible to *Mr*NV/XSV, whereas adults are resistant (Gangnonngiwa *et al.,* 2020; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a).

No mortality was observed either in naturally or experimentally ~~(~~*Mr*NV/XSV~~)~~ infected subadult and adult prawns. Experimental studies confirmed vertical transmission from infected broodstock to PL (Sudhakaran *et al*., 2007a).

2.2.4. Distribution of the pathogen in the host

*Mr*NV and XSV have been demonstrated in gill tissue, head muscle, heart, abdominal muscle, ovaries, pleopods and tail muscle, but not the hepatopancreas or eyestalk (Sahul Hameed *et al*., 2004a; Sri Widada *et al*., 2003).

2.2.5. Aquatic animal reservoirs of infection

~~One study has~~ Studies have indicated ~~the possibility~~ that marine shrimp may act as ~~a~~ reservoirs for *Mr*NV and XSV and that these viruses maintain virulence in the shrimp tissue system (Senapin *et al.,* 2012*;* Sudhakaran *et al*., 2006).

2.2.6. Vectors

Aquatic insects such as dragonfly (*Aeshna* sp.), giant water bug(*Belostoma* sp.), beetle (*Cybister* sp.) and backswimmer (*Notonecta* sp.) may act as mechanical carriers for *Mr*NV/XSV and are a potential transmission risk to cultivated *Macrobrachium rosenbergii* (Sudhakaran *et al.,* 2008)*.* It is recommended to remove these insects from freshwater prawn culture systems, especially at larval-rearing centres. Sudhakaran *et al*. (2008) demonstrated RT-PCR positives from insects, and infected C6/36 insect cell line with tissue homogenates from the insects. Viral replication was confirmed through EM and RT-PCR, but transmission from insects to naïve shrimp was not demonstrated.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Larvae, PL and juveniles of *M. rosenbergii* are susceptible to infection with *Mr*NV, which often causes high mortalities in these life stages. Mortality may reach a maximum in about 5 or 6 days after the appearance of the first clinical signs. Very few PL with infection with *Mr*NV survive beyond 15 days in an outbreak, but PL that survive may grow to market size. Adults are resistant to infection with *Mr*NV, but act as carriers (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a). Prevalence is variable from 10% to 100% in hatchery, nursery and grow-out systems (Arcier *et al*., 1999; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; 2004b).

2.3.2. Clinical signs, including behavioural changes

Infected PL become opaque and develop a whitish appearance, particularly in the abdominal region. The whitish discoloration appears first in the second or third abdominal segment and gradually diffuses both anteriorly and posteriorly. In severe cases, degeneration of telson and uropods may occur. Floating exuviae (moults) in the tanks appear abnormal and resemble ‘mica flakes’ (Arcier *et al*., 1999). The infected PL show progressive weakening of their feeding and swimming ability (Sahul Hameed *et al*., 2004a).

2.3.3. Gross pathology

Infection with *Mr*NV is indicated by the whitish coloration of abdominal muscle.

2.3.4. Modes of transmission and life cycle

Transmission is vertical by trans-ovum and horizontal by the waterborne route (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a).

2.3.5. Environmental factors

Not available.

2.3.6. Geographical distribution

The disease was first reported in the ~~French West Indies~~ Caribbean (Arcier *et al*., 1999), and later in Asia-Pacific (Murwantoko *et al*., 2016; Owens *et al*., 2009; Qian *et al*., 2003; Saedi *et al*., 2012; Sahul Hameed *et al*., 2004b; Wang *et al*., 2008; Yoganandhan *et al*., 2006).

See WOAH-WAHIS (<https://wahis.woah.org/#/home>) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

Preventive measures, such as screening of broodstock and PL, and good management practices may help to prevent infection with *Mr*NV in culture systems. As the life cycle of *M. rosenbergii* is completed under controlled conditions, specific pathogen-free (SPF) broodstock and PL can be produced (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

2.4.1. Vaccination

Not available

2.4.2. Chemotherapy including blocking agents

No known chemotherapeutic agents are reported to treat *Mr*NV-infected prawn.

2.4.3. Immunostimulation

The immunomodulatory effect of recombinant capsid protein and recombinant RNA-dependent RNA polymerase (RdRp) protein of *Mr*NV has been studied and the protection of viral challenged post-larvae from *Mr*NV infection has been demonstrated (Farook *et al*., 2014; NaveenKumar *et al*., 2021).

2.4.4. Breeding resistant strains

None reported

2.4.5. Inactivation methods

A viral suspension treated with heat at 65°C for 2 hours destroyed infectivity of *Mr*NV and XSV in challenge experiments (Qian *et al*., 2003). The viral inoculum exposed to UV irradiation for a period of 5 minutes and more was totally inactivated and failed to cause mortality in prawn PL ~~of prawn~~ (Ravi & Sahul Hameed, 2016).

2.4.6. Disinfection of eggs and larvae

Routine disinfection procedures followed for crustacean viral disease control are suggested.

2.4.7. General husbandry

*Mr*NV is transmitted both horizontally and vertically in culture systems (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a). Good husbandry practices, such as proper disinfection of tanks and water may help to prevent infection. It is recommended to remove insects from freshwater prawn culture systems, especially at larval-rearing centres. Specific pathogen-free (SPF) broodstock and PL can be obtained from disease free populations or by RT-PCR screening and selection of negative broodstock (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

PLs are most suitable for detection of *Mr*NV. PL showing clinical signs of disease can be sampled preferentially. Adults and juveniles can be sampled for MrNV however prevalence in these lifestages may be lower (see Section 2.3.1).

3.2. Selection of organs or tissues

The tissues most affected in moribund PLs/early juveniles are striated muscles of the abdomen, cephalothorax and tail. The whole PL body is preferred for detection of *Mr*NV (Sahul Hameed *et al.,* 2004b; Sri Widada *et al.,* 2003; Yoganandhan *et al*., 2005). All organs of adult *M. rosenbergii* except eyestalks and the hepatopancreas, are best for screening the viruses by RT-PCR.

3.3. Samples or tissues not suitable for pathogen detection

Eyestalks and the hepatopancreas of adult prawns are not suitable (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004a).

3.4. Non-lethal sampling

Pleopods (swimming legs) are a convenient source of RNA for non-destructive screening of *Mr*NV in adult prawn (Sahul Hameed *et al.,* 2004a).

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

Infected larvae or PL with prominent signs of whitish muscle in the abdominal region are collected from disease outbreak areas. Samples are washed in sterile saline, transferred to sterile tubes, and transported to the laboratory. For general guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

3.5.1. Samples for pathogen isolation

Moribund or frozen PL samples can be used for isolation of viral pathogens using cell lines (C6/36 mosquito cell line (Sudhakaran et al., 2007b).

3.5.2. Preservation of samples for molecular detection

Infected samples stored at –80°C or samples preserved in 80% (v/v) analytical/reagent-grade (undenatured) ethanol should be used for RT-PCR for detection of *Mr*NV (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004b; Yoganandhan *et al*., 2005).

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology, immunohistochemistry or *in-situ* hybridisation should be fixed immediately after collection in neutral-buffered formalin or modified Davidson’s fixative (Sri Widada *et al.,* 2003). The recommended ratio of fixative to tissue is 10:1. Standard sample collection, preservation and processing methods for histological techniques can be found in Section 5.3. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage.

**Ratings against purposes of use.** For each recommended assay a qualitative rating against the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, successful application by diagnostic laboratories, cost, timeliness, and sample throughput. For a specific purpose of use, assays are rated as:

+++ = Most suitable methods – desirable performance and operational characteristics;

++ = Suitable method(s) acceptable performance and operational characteristics under most circumstances;

+ = Less suitable methods – performance or operational characteristics may significantly limit application;

Shaded boxes = Not appropriate for this purpose.

**Level of validation**. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

***Table 4.1.*** *WOAH recommended diagnostic methods for MrNV and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

| Method | 1. Surveillance of apparently healthy animals
 | 1. Presumptive diagnosis of clinically affected animals
 | 1. Confirmatory diagnosis1 of a suspect result from surveillance or presumptive diagnosis
 |
| --- | --- | --- | --- |
| Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV |
| Wet mounts |  |  |  |  |  |  |  |  |  |  |  |  |
| Histopathology |  |  |  |  |  | ++ | ++ | NA |  |  |  |  |
| Cell culture |  |  |  |  |  |  |  |  |  |  |  |  |
| Real-time RT-PCR | +++ | +++ | +++ | 1 | +++ | +++ | +++ | 1 | +++ | +++ | +++ | 1 |
| Conventional RT-PCR  | ++ | ++ | ++ | 1 | +++ | +++ | +++ | 2 |  |  |  |  |
| Conventional RT-PCR followed by amplicon sequencing  |  |  |  |  |  |  |  |  | +++ | +++ | +++ | 2 |
| *In-situ* hybridisation |  |  |  |  |  | ++ | ++ | 1 |  | ++ | ++ | 1 |
| Bioassay |  |  |  |  |  |  |  |  |  |  |  |  |
| LAMP | ++ | ++ | ++ | 1 | ++ | ++ | ++ | 1 |  |  |  |  |
| Ab-ELISA |  |  |  |  |  |  |  |  |  |  |  |  |
| Ag-ELISA |  |  |  |  | ++ | ++ | ++ | 1 |  |  |  |  |
| Lateral flow assay |  |  |  |  | ++ | ++ | ++ | 2 |  |  |  |  |
| Other methods3  |  |  |  |  |  |  |  |  |  |  |  |  |

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification;
Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.
1For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). 2Susceptibility of early and juvenile life stages is described in Section 2.2.3.
3Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

None to date

4.2. Histopathology and cytopathology

The most affected tissue in infected PL is striated muscle of the cephalothorax, abdomen and tail. Histological features include the presence of acute Zenker’s necrosis of striated muscles, characterised by severe hyaline degeneration, necrosis and muscular lysis. Moderate oedema and abnormal open spaces among the affected muscle cells are also observed, as is the presence of large oval or irregular basophilic cytoplasmic inclusion bodies in infected muscles (Arcier *et al.,* 1999; Hsieh *et al.,* 2006).

4.3. Cell culture for isolation

*Mr*NV has been isolated in insect cell lines, but this is not a recommended method (Hernandez-Herrera *et al.,* 2007; Sudhakaran *et al.,* 2007b).

4.4. Nucleic acid amplification

PCR methods for MrNV and XSV are included in this section for completeness. However, the case definitions in Section 6 are based on detection methods for MrNV only.

PCR assays should always be run with the controls specified in Section 5.5. *Use of molecular techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

*Extraction of nucleic acids*

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time RT-PCR

Real-time RT-PCR assay can be performed using the SYBR Green dye based on the method described by Hernandez-Herrera *et al*. (2007) or the TaqMan assay described by Zhang *et al*. (2006).

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen /target gene | Primer/probe (5’–3’) | Concentration | Cycling parameters(a) |
| Method 1: Hernandez-Herrera *et al.* (2007); GenBank Accession No.: AY222839) |
| MrNv/RNA1 | Fwd: AGG-ATC-CAC-TAA-GAA-CGT-GGRev: CAC-GGT-CAC-AAT-CCT-TGC-G | 500 nM500 nM | 40 cycles of: 95°C/15 sec, 60°C/5 sec and 72°C/10 sec |
| Method 2: Zhang *et al.* (2006); GenBank Accession No.: AY231436) |
| MrNv/RNA1 | Fwd: CAA-CTC-GGT-ATG-GAA-CTC-AAG-GTRev: AGG-AAA-TAC-ACG-AGC-AAG-AAA-AGT-CProbe: FAM-ACC-CTT-CGA-CCC-CAG-CAA-TGG-TG-TAMARA | 1000 nM1000 nM400 nM | 50 cycles of: 94°C/30 sec and 58°C/30 sec  |
| Method 3: Zhang *et al.* (2006); GenBank Accession No.: DQ174318) |
| XSV  | Fwd: AGC-CAC-ACT-CTC-GCA-TCT-GARev: CTC-CAG-CAA-AGT-GCG-ATA-CGProbe: FAM-CAT-GCC-CCA-TGA-TCC-TCG-CA-TAMARA | 1000 nM1000 nM400 nM | 50 cycles of: 94°C/30 sec and 58°C/30 sec |

(a)A denaturation step prior to cycling has not been included.

4.4.2. Conventional RT-PCR

The protocol for the conventional RT-PCR for detection of *Mr*NV/XSV developed by Sri Widada *et al.* (2003), Sahul Hameed *et al.* (2004a; 2004b) and Sudhakaran *et al*. (2007a) is recommended. *Mr*NV and XSV can be detected by conventional RT-PCR separately using a specific set of primers or these two viruses can be detected simultaneously using a single-tube one-step multiplex RT-PCR (Yoganandhan *et al*., 2005). ~~Conventional real-time RT-PCR is recommended in situations where high sensitivity is required.~~

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen /target gene | Primer (5’–3’) | Concentration | Cycling parameters(a) |
| Method 1: One step RT-PCR (~~Sri Widada~~ *~~et al~~*~~., 2003;~~ Sahul Hameed *et al*., 2004a, b; Sudhakaran *et al*., 2007a)GenBank Accession No.: AY222840 (*Mr*NV) and AY247793 (XSV); amplicon size: 425 bp (*Mr*Nv) and 546 bp (XSV) |
| *Mr*Nv | Fwd: GCG-TTA-TAG-ATG-GCA-CAA-GGRev: AGC-TGT-GAA-ACT-TCC-ACT-GG | ~~0.02 nM~~ 400 nM~~0.02 nM~~ 400 nM | 30 cycles of: 94°C/40 sec, 55°C/40sec and 68°C/60 sec |
| XSV | Fwd: CGC-GGA-TCC-GAT-GAA-TAA-GCG-CAT-TAA-TAARev: CCG-GAA-TTC-CGT-TAC-TGT-TCG-GAG-TCC-CAA | ~~0.02 nM~~ 400 nM~~0.02 nM~~ 400 nM | 30 cycles of:94°C/40 sec, 55°C/40 sec and 68°C/60 sec |
| Method 2: nested RT-PCR using above-mentioned primers as external primers (Sudhakaran *et al*., 2007a); amplicon size: 205 bp (*Mr*Nv) and 236 bp (XSV) |
| *Mr*Nv | Internal primers:Fwd: GAT-GAC-CCC-AAC-GTT-ATC-CTRev: GTG-TAG-TCA-CTT-GCA-AGA-GG | ~~0.02 nM~~ 1000 nM~~0.02 nM~~ 1000 nM | 30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec |
| XSV | Internal primers:Fwd: ACA-TTG-GCG-GTT-GGG-TCA-TARev: GTG-CCT-GTT-GCT-GAA-ATA-CC-3 | ~~0.02 nM~~ 1000 nM~~0.02 nM~~ 1000 nM | 30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec |
| Method 3: Multiplex RT-PCR (Yoganandhan *et al*., 2005); GenBank Accession No.: AY222840 (MrNV) and AY247793 (XSV); amplicon size: 681 bp (MrNv) and 500 bp (XSV) |
| MrNV  | Fwd: GAT-ACA-GAT-CCA-CTA-GAT-GAC-CRev: GAC-GAT-AGC-TCT-GAT-AAT-CC | ~~0.02 nM~~ 400 nM~~0.02 nM~~ 400 nM | 30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec |
| XSV | Fwd: GGA-GAA-CCA-TGA-GAT-CAC-GRev: CTG-CTC-ATT-ACT-GTT-CGG-AGT-C | ~~0.02 nM~~ 400 nM~~0.02 nM~~ 400 nM | 30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec |

(a)A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods: Loop-mediated isothermal amplification (LAMP)

Haridas *et al*. (2010) have applied loop-mediated isothermal amplification (LAMP) for rapid detection of *Mr*NV and XSV in the freshwater prawn. A set of four primers, two outer primers and two inner primers, have been designed separately for detection of *Mr*NV and XSV.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

The presence of *Mr*NV in infected cells can be demonstrated in histological sections using a DIG-labelled DNA *in-situ* hybridisation probe specific for *Mr*NV (Sri Widada *et al.,* 2003).

4.7. Immunohistochemistry

None developed.

4.8. Bioassay

Not used for diagnostic purposes.

4.9. Antibody~~-~~ or antigen-based detection methods

4.9.1. ELISA

Antibody-based diagnostic methods for *Mr*NV include the ELISA described by Romestand & Bonami (2003) or the triple-antibody sandwich (TAS) ELISA based on a monoclonal antibody (Qian *et al.,* 2006).

4.9.2. Lateral flow assay (LFA)

An antibody-based lateral flow assay (LFA) has been developed for the early detection of *Mr*NV in the PL stage (Jamalpure *et al*., 2021).

4.10. Other methods

None

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time RT-PCR is recommended for targeted surveillance to declare freedom from infection with *Mr*NV.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[[1]](#footnote-1)

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *Mr*NV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR

ii) Positive result by conventional RT-PCR

iii) Positive result by LAMP

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *Mr*NV is considered to be confirmed if the following criterion is met:

i) Positive result by real-time RT-PCR result and positive result by conventional RT-PCR and sequence analysis

6.2 Clinically affected animals

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *Mr*NV shall be suspected if at least one of the following criteria is met:

i) Clinical signs consistent with infection by *Mr*NV

ii) Histopathology consistent with infection by *Mr*NV

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result by *in situ* hybridisation

vi) Positive result by LAMP

vii) Positive result by Ag ELISA

viii) Positive result by lateral flow assay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *Mr*NV is considered to be confirmed if at least one of the following ~~criterion~~ criteria is met:

1. Positive result by real time RT-PCR and positive result by conventional RT-PCR with sequence analysis
2. Positive result by ISH followed by positive result by conventional RT-PCR with sequence analysis
3. Positive result by ISH followed by positive result by real-time RT-PCR

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Mr*NV are provided in Tables 6.3.1. and 6.3.2 (no data are currently available). This information can be used for the design of surveys for infection with *Mr*NV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test type | Test purpose | Source populations | Tissue or sample types | Species | DSe (*n*) | DSp (*n*) | Reference test | Citation |
| RT-PCR | Diagnosis  | Clinically affected PL from hatchery and nursery | Whole post-larvae  | *Macrobrachium rosenbergii*  | 100 (*n*=20) | 100 (*n*=20) | Western blot or ELISA | Sri Widada *et al*. (2003); Sahul Hameed *et al*. (2011)  |
| Lateral flow immune-assay  | Surveillance | PL from prawn hatcheries  | Whole post-larvae  | *Macrobrachium rosenbergii* | 100(*n*=80) | 90 (*n*=80) | RT-PCR  | Jamalpure *et al*. (2021) |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
RT-PCR: = reverse transcription polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test type | Test purpose | Source populations | Tissue or sample types | Species | DSe (*n*) | DSp (*n*) | Reference test | Citation |
|  |  |  |  |  |  |  |  |  |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
RT-PCR: = reverse transcription polymerase chain reaction.

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**NB:** There is a WOAH Reference Laboratory for infection with *Macrobrachium rosenbergii* nodavirus (white tail disease) (please consult the WOAH web site:
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>) any further information on infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)

**NB:** First adopted in 2009. Most recent updates adopted in 2017.

1. For example transboundary commodities. [↑](#footnote-ref-1)