CHAPTER 2.2.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

1. Scope

Infection with infectious myonecrosis virus means infection with the pathogenic agent infectious myonecrosis virus (IMNV) that is similar to members of the Family *Totiviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Phylogenetic analysis of its RNA-dependent RNA polymerase (RdRp) gene coding sequence indicates that IMNV is most closely related to *Giardia lamblia virus*, a member of the family *Totiviridae* (Fauquet *et al.,* 2005; Lightner, 2011; Nibert, 2007; Poulos *et al.,* 2006).

IMNV particles are icosahedral in shape and 40 nm in diameter, with a buoyant density of 1.366 g ml^{-1} in caesium chloride. The genome consists of a single, double-stranded (ds) RNA molecule of 8226-8230 bp (Loy *et al*., 2015; Naim *et al.,* 2015). Sequencing of the viral genome reveals two non-overlapping open reading frames (ORFs). The first ORF (ORF1, nt 470–5596) encodes a putative RNA-binding protein and a capsid protein. The coding region of the RNA-binding protein is located in the first half of ORF1 and contains a dsRNA-binding motif in the first 60 amino acids. The second half of ORF1 encodes a capsid protein, as determined by amino acid sequencing, with a molecular mass of 106 kDa. The second ORF (ORF2, nt 5884–8133) encodes a putative RdRp (Poulos *et al.,* 2006).

The complete genomes of IMNV types originating from Brazil and Indonesia have been sequenced and found to be 99.6% identical at the nucleotide level (Poulos *et al.,* 2006; Senapin *et al.,* 2007). The 99.6% full genome sequence identity (and anecdotal information on the introduction of *Penaeus vannamei* stocks from Brazil) indicate that the disease was introduced from Brazil to Indonesia in 2006.

2.1.2. Survival outside the host

Only anecdotal information is available. IMNV is apparently more difficult to inactivate with typical pond disinfection procedures (e.g. sun drying, chlorination, etc.) than are other penaeid shrimp viruses like white spot syndrome virus (WSSV), yellow head virus genotype 1 (YHV1), Taura syndrome virus (TSV) and infectious hypodermal and haematopoietic virus (IHHNV). Reservoir hosts are suspected, but none have been documented consistently.

2.1.3. Stability of the agent (effective inactivation methods)

No data.

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species (common and Latin names)

Species that fulfil the criteria for listing as susceptible to infection with IMNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: brown tiger prawn (*Penaeus esculentus*), banana prawn (*P. merguiensis*), and whiteleg shrimp (*P. vannamei*).

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 IMNV according to Chapter 1.5. of the *Aquatic Code* include: giant tiger prawn (*Penaeus monodon*) and blue shrimp (*P. stylirostris*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: southern brown shrimp (*P. subtilis*).

2.2.3. Susceptible stages of the host

Juveniles and subadults of *P. vannamei*, farmed in marine, brackish, and low salinity brackish water, appear to be most severely affected by infection with IMNV (Lightner, 2011; Lightner *et al.,* 2004; Nunes *et al.,* 2004; Poulos *et al.,* 2006).

2.2.4. Species or subpopulation predilection (probability of detection)

No data.

2.2.5. Target organs and infected tissue

The principal target tissues for IMNV include the striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, and the lymphoid organ parenchymal cells (Lightner, 2011; Lightner *et al.,* 2004; Poulos *et al.,* 2006; Tang *et al.,* 2005).

2.2.6. Persistent infection

Some members of populations of *P. vannamei* that survive IMNV infections or epizootics may carry the virus.

2.2.7. Vectors

There are no specific data on vectors. However, because of its non-enveloped particle structure, it is possible that IMNV, like TSV, will remain infectious in the gut and faeces of seabirds that feed on dead or moribund shrimp at farms with on-going infection with IMNV epizootics, and be spread within and among farms by faeces or regurgitated shrimp carcasses (Vanpatten *et al.,* 2004).

2.3. Disease pattern

In early juvenile, juvenile, or adult *P. vannamei* in regions where infection with IMNV is enzootic, outbreaks of infection with IMNV associated with sudden high mortalities may follow stressful events such as capture by cast-netting, feeding, and sudden changes in water salinity or temperature. Shrimp in the acute phase of infection with IMNV will present focal to extensive white necrotic areas in striated (skeletal) muscles, especially in the distal abdominal segments and tail fan, which can become necrotic and reddened in some shrimp. Severely affected shrimp become moribund and mortalities can be high immediately following a “stress” event and continue for several days (Lightner, 2011; Lightner *et al.,* 2004; Nunes *et al.,* 2004; Poulos *et al.,* 2006). Feed conversion ratios (FCR) of affected populations can increase from a normal value of ~ 1.5 up to 4.0 or higher (Andrade *et al.,* 2007).

2.3.1. Transmission mechanisms

IMNV has been demonstrated to be transmitted horizontally by cannibalism (Lightner, 2011; Poulos *et al.,* 2006). Transmission via water probably occurs. Although vertical transmission is suspected from anecdotal evidence, it is not known whether this occurs via transovarial mechanism or by surface contamination of newly spawned eggs.

2.3.2. Prevalence

In regions where infection with IMNV is enzootic in farmed stocks of *P. vannamei*, its prevalence may reach 100% (Andrade *et al.,* 2007; Nunes *et al.,* 2004).

2.3.3. Geographical distribution

Infection with IMNV has been reported to occur in north-eastern Brazil (Andrade *et al.,* 2007; Lightner *et al.,* 2004; Nunes *et al.,* 2004; Poulos *et al.,* 2006) and in Indonesia (Naim *at al.,* 2014).

2.3.4. Mortality and morbidity

Mortalities from infection with IMNV can range from 40% to 70% in cultivated *P. vannamei*.

2.3.5. Environmental factors

Temperature and salinity effects are considered to be likely predisposing factors to disease outbreaks, but no experimental data are available (Nunes *et al.,* 2004).

2.4. Control and prevention

2.4.1. Vaccination

No effective "vaccines" for infection with IMNV are available.

2.4.2. Chemotherapy

No effective therapeutic agents have been reported for infection with IMNV.

2.4.3. Immunostimulation

No data.

2.4.4. Breeding for resistance

There are anecdotal reports of some selected lines of *P. vannamei* having better survival and culture performance in farms where infection with IMNV is enzootic. During a 20-day controlled laboratory study in which the shrimp were challenged with IMNV, some domesticated lines of *P. vannamei* were found to survive better than other lines (White-Noble *et al.,* 2010).

2.4.5. Restocking with resistant species

While there are no published reports, some shrimp farms in Indonesia are believed to have stocked *P. monodon* and *P. stylirostris* because of data from a preliminary study suggesting that these species are more resistant to infection with IMNV than *P. vannamei* (Tang *et al.,* 2005).

2.4.6. Blocking agents

No data.

2.4.7. Disinfection of eggs and larvae

While IMNV is believed to be transmitted vertically, there are no scientific data confirming this route of transmission. Disinfection of eggs and larvae (Chen *et al.,* 1992) is a good management practice recommended to reduce the transmission potential of a number of penaeid shrimp diseases from female spawners to their eggs or larvae, and the practice may reduce IMNV contamination of spawned eggs and larvae produced from them.

2.4.8. General husbandry practices

Some husbandry practices have been applied successfully to prevent infection with IMNV and development of clinical disease at shrimp farms. Foremost among these has been the application of reverse-transcription-PCR (RT-PCR) for screening pond-reared broodstock or their spawned eggs or nauplii and discarding those that test PCR-positive (Andrade *et al.,* 2007). Fallowing and restocking of affected farms or entire culture regions with IMNV-free stocks of *P. vannamei*, and the development of specific pathogen free (SPF) shrimp stocks of *P. vannamei* most suited to local culture conditions has proven to be the most successful husbandry practice for preventing and controlling other virus diseases of shrimp, and should be applicable to control and prevent infection with IMNV (Lee & O'Bryen, 2003; Lightner, 2005; Lightner *et al.,* 2009; Moss & Moss, 2009).

3. Sampling

3.1. Selection of individual specimens

Specimens suitable for testing for infection with IMNV using molecular methods (e.g. RT-PCR, nested RT-PCR, real-time RT-PCR, etc.) include postlarvae (PL), juveniles, subadults and adults. While IMNV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in

larval stages, so these life stages may not be suitable for detecting IMNV or for certification for freedom of infection with IMNV.

3.2. Preservation of samples for submission

For routine histology or molecular assays, and guidance on preservation of samples for the intended test method see Chapter 2.2.0 *General information* (on diseases of crustaceans).

3.3. Pooling of samples

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore larger shrimp should be processed and tested individually. However, samples, especially PL or specimens up to 0.5 g, can be pooled to obtain enough material for molecular testing.

3.4. Best organs or tissues

IMNV infects tissues of mesodermal origin. The principal target tissues in the acute phase of infection with IMNV are the striated muscles (skeletal and less commonly cardiac muscle), connective tissues, haemocytes, and the lymphoid organ tubule parenchymal cells. In chronic infections, the lymphoid organ may be the principal target tissue.

Haemolymph or excised pleopods may be collected and used when non-lethal testing of valuable broodstock is necessary.

3.5. Samples or tissues that are not suitable

IMNV replicates systemically but does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of IMNV infection.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Affected shrimp present with visibly white tails. Such severely affected shrimp may have been feeding just before the onset of stress and may have a full gut. Severely affected shrimp become moribund and mortalities can be instantaneously high and continue for several days. Clinical signs may have a sudden onset following stresses (e.g. capture by cast-netting, feeding, and sudden changes in temperature or salinity).

4.1.2. Behavioural changes

Only shrimp in the acute-phase of disease present behavioural changes. Typically, severely affected shrimp become lethargic during or soon after stressful events such as capture by cast-netting, feeding, sudden changes in water temperature, sudden reductions in water salinity, etc.).

4.2. Clinical methods

4.2.1. Gross pathology

Shrimp in the acute phase of disease present focal to extensive white necrotic areas in striated (skeletal) muscles, especially in the distal abdominal segments and tail fan, which can become necrotic and reddened in some individual shrimp.

Exposing the paired lymphoid organs (LO) by simple dissection will show that they are hypertrophied (3–4 times their normal size) (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Infection with IMNV in the acute and chronic phases can be presumptively diagnosed using histology (Bell & Lightner, 1988; Lightner, 2011; Lightner *et al.,* 2004; Poulos *et al.,* 2006). However, the lesions

in striated muscles and LO are not pathognomonic for infection with IMNV. White tail disease of penaeid shrimp caused by the *P. vannamei* nodavirus (PvNV) can mimic infection with IMNV (Tang *et al.,* 2007). Hence, diagnostic information from other sources (e.g. history, gross signs, morbidity, mortality, or RT-PCR findings) may be required to confirm a diagnosis of infection with IMNV.

By histology using routine haematoxylin–eosin (H&E) stained paraffin sections (Bell & Lightner, 1988), tissue sections from shrimp with acute-phase infection with IMNV present myonecrosis with characteristic coagulative necrosis of striated (skeletal) muscle fibres, often with marked oedema among affected muscle fibres. Some shrimp may present a mix of acute and older lesions. In these shrimp, the affected muscle fibres appear to progress from presenting coagulative necrosis to presenting liquefactive necrosis, which is accompanied by moderate infiltration and accumulation of haemocytes. In the most advanced lesions, haemocytes and inflamed muscle fibres are replaced by a loose matrix of fibrocytes and connective tissue fibres that are interspersed with haemocytes and foci of (presumed) regenerating muscle fibres (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

Significant hypertrophy of the LO caused by accumulations of lymphoid organ spheroids (LOS) is a highly consistent lesion in shrimp with acute or chronic-phase infection with IMNV lesions. Often, many ectopic LOS are found in other tissues not near the main body of the LO. Common locations for ectopic LOS include the haemocoelom in the gills, heart, near the antennal gland tubules, and ventral nerve cord (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

4.2.4. Wet mounts

Stained or unstained tissue squashes of affected skeletal muscle or of the LO may show abnormalities. Tissue squashes of skeletal muscle when examined with phase or reduced light microscopy may show loss of the normal striations. Fragmentation of muscle fibres may also be apparent. Squashes of the LO may show the presence of significant accumulations of spherical masses of cells (LOS) amongst normal LO tubules.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

See Section 4.2.1.

4.2.7. Electron microscopy/cytopathology

Not applicable for diagnostic purposes.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See Section 4.2.4.

4.3.1.1.2. Smears

See Section 4.2.5.

4.3.1.1.3. Fixed sections

See Sections 4.2.3 and 4.2.6.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

None reported to date.

4.3.1.2.2. Antibody-based antigen detection methods

Monoclonal antibodies (MAbs) have been developed to the capsid protein of IMNV (Kunanopparat *et al.,* 2011). Three MAbs were developed and when used in combination, they provided better sensitivity than any one of the MAbs used in isolation. However, the sensitivity was approximately tenfold lower than that of a one-step RT-PCR assay using the same sample.

4.3.1.2.3. Molecular techniques

Published methods are available for the molecular detection of IMNV by *in-situ* hybridisation (ISH), nested RT-PCR and quantitative real-time RT-PCR (Andrade *et al.,* 2007; Poulos *et al.,* 2006; Tang *et al.,* 2005). A nested RT-PCR kit for detection of the virus is available commercially. All PCR tests have proved to be specific to IMNV.

As the sensitivity of the nested and real-time RT-PCR is greater than any other diagnostic method available currently, approaching a detection limit of 10 viral genome copies, these tests are the gold standard for detection of IMNV (Andrade *et al.,* 2007; Poulos *et al.,* 2006).

DNA probe for ISH detection of IMNV

A cDNA library was generated from RNA extracted from purified IMNV. A IMNV-specific ISH DNA probe is prepared from clone IMNV-317 by PCR labelling with digoxigenin-11-dUTP (DIG). The PCR primers used for amplification of the 993 bp probe are IMNV993F (5'-AAC-ACA-AAA-TCT-GCC-AGC-AA-3') and IMNV993R (5'-CCC-AAC-CAC-CCA-AAT-TCA-TA-3'). Following PCR, the DIG-labelled DNA probe is precipitated with ethanol, re-suspended in water and stored at –20°C until used. The ISH procedure for detecting IMNV follows that outlined by Tang *et al.* (2005).

RT-PCR for detection of IMNV

A nested RT-PCR method was developed to detect IMNV that uses two PCR primer sets that produce a 328 bp one-step amplicon and 139 bp two-step amplicon. The 1-step PCR can detect as little as 100 IMNV RNA copies and the 2-step PCR can detect in the order of 10 IMNV RNA copies (Poulos & Lightner, 2006).

Viral RNA can be isolated using any commercially available RNA isolation kit. The amount of tissue required will depend on the kit selected (e.g. Qiagen RNA extraction kit, Promega and Roche RNA purification kit recommend using 25–50 mg of tissue[1]). Depending on the kit used, the elution volume for Roche and Qiagen and low elution volume RNA isolation Promega extraction kit is 100 µl. The high elution volume RNA isolation Promega extraction kit is 500 µl. Extracted RNA should be maintained at –20°C before testing, however, for long-term storage the RNA should be kept at –70°C.

Following RNA extraction, the method is summarised below:

RNA templates:

1. Frozen or ethanol-fixed tissue (pleopods, cephalothorax, muscle)
2. Haemolymph (less sensitive than when other tissues are used)

RT-PCR reaction mixture (Applied Biosystems rTth Enzyme and 5 × EZ Buffer #N808-0178):

Reagent	25 µl reaction	Final concentration
DD dH_2O	6.5 µl	–
5 × EZ Buffer	5.0 µl	1 ×
dNTP mix (10 mM each)	3.0 µl	300 µM each
Primer F (100 ng μl^{-1})	1.0 µl	0.62 µM
Primer R (100 ng μl^{-1})	1.0 µl	0.62 µM
$Mn(Oac)_2$ (25 mM)	2.5 µl	2.5 mM
rTth Enzyme (2.5 U μl^{-1})	1.0 µl	0.1 U μl^{-1}
Template[1]	1–5 µl	1–50 ng total RNA

[1]Template must be boiled for 3 minutes and chilled on ice just prior to adding to reaction mix.

1 Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

RT-PCR thermal cycling conditions:

PCR Primers	Temperature (°C)	Time	No. cycles	Amplicon length
4587F/4914R	60, 95	30 minutes, 2 minutes	1	328 bp
	95, 60	45 seconds, 45 seconds	39	
	60	7 minutes	1	

Nested PCR reaction (Amersham Biosciences pure Taq Ready-To-Go Beads #27-9558-01):

Reagent	25 µl reaction	Final concentration
DD H_2O	22.5 µl	–
Primer NF (100 ng μl^{-1} µM)	1.0 µl	0.465 µM
Primer NR (100 ng μl^{-1} µM)	1.0 µl	0.465 µM
Template[2]	0.5 µl	–

[2]Template for the nested reaction is the product from the first step reaction

Nested PCR thermal cycling conditions:

Primers	Temperature (°C)	Time	No. cycles	Amplicon length
4725 NF/ 4863 NR	95	2 minutes	1	139 bp
	95, 65, 72	30 seconds, 30 seconds, 30 seconds	39	
	72	2 minutes	1	

Primer sequences:

Primer	Sequence (5′ to 3′)	Amplicon Length	Ref.
4587F	CGA-CGC-TGC-TAA-CCA-TAC-AA	328 bp	Poulos & Lightner, 2006
4914R	ACT-CGG-CTG-TTC-GAT-CAA-GT		
4725 NF	GGC-ACA-TGC-TCA-GAG-ACA	139 bp	
4863 NR	AGC-GCT-GAG-TCC-AGT-CTT-G		

Quantitative (real-time) RT-PCR for detection of IMNV

A real-time qRT-PCR method was developed to detect and quantify IMNV in shrimp tissue. The method can detect as few as 10 IMNV RNA copies per µl total RNA (Andrade *et al.,* 2007). The method as published is summarised below.

The Primer Express software (Applied Biosystems) was used to aid the design of the PCR primers and TaqMan probe targeted to the ORF1 region of the IMNV genome (GenBank accession no. AY570982) (Andrade *et al.,* 2007; Poulos *et al.,* 2006). Primers IMNV412F (5'-GGA-CCT-ATC-ATA-CAT-AGC-GTT-GCA-3') and IMNV545R (5'-AAC-CCA-TAT-CTA-TTG-TCG-CTG-GAT-3') amplify a 134 bp DNA. The TaqMan probe, IMNVp1 (5'-6FAM-CCA-CCT-TTA-CTT-TCA-ATA-CTA-CAT-CAT-CCC-CGG-TAMRA-3'), which corresponds to the nucleotides 467–500, is labelled with fluorescent dyes 5-carboxyfluoroscein (FAM) at its 5'-end and N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA) at its 3'-end.

The IMNV genome fragment is amplified using StepOnePlus PCR System and the TaqMan Fast virus 1-Step Master Mix (Life Technologies). Prior to the qRT-PCR, extracted RNA is boiled at 95–100°C for 3 minutes to denature the dsRNA and chilled immediately in ice. The reaction mixture contains 1 µl RNA sample, TaqMan Master mix (2×), 300 nM each primer IMNV412F and IMNV545R, 200 nM. IMNVp1TaqMan probe in a 10–20 µl final volume. The qRT-PCR thermal cycling conditions used are 50°C for 3 minutes, 95°C for 20 seconds followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. At the end of the reaction, fluorescence intensity is measured, a threshold will be set to be above the baseline. Samples with a Ct value lower than 40 cycles are considered to be positive. It is necessary to include a 'no template' control in each reaction run. This is to rule out the presence of fluorescence contaminants in the reaction mixture. A positive control should also be included, and it can be

RNA extracted from IMNV-infected tissue, or *in-vitro* transcribed IMNV RNA containing the target sequence (see below).

To synthesise an RNA standard for the real-time qRT-PCR, the PCR primers IMNV218F and IMNV682R (5'-GCT-GGA-CTG-TAT-TGG-TTG-AG-3' and 5'-AAC-CAA-GTT-CTT-CTT-CTC-CAG-TT-3', respectively) are used to amplify a 464 bp DNA product from the IMNV genome. The PCR product purified using a QIAuick PCR Purification kit (QIAGEN) was cloned into pGEM-T Easy Vector. A recombinant plasmid, pIMNV-1, confirmed to contain the 464 bp insert by sequence analysis, is linearised by digestion with *Pst*I and used as the template for an *in-vitro* RNA transcription using T7 RNA polymerase and associated reagents (Promega). RNA is synthesised at 37°C for 2 hours in a 50 µl reaction containing 1 µg plasmid DNA, followed by DNase I digestion at 37°C for 30 minutes for remove DNA. The length and integrity of the synthesic ssRNA is confirmed by electrophoresis in a 1.5% agarose gel containing ethidium bromide. The RNA is purified using a Qiaquick PCR Purification kit, quantified by a spectrophotometer, and stored at –70°C.

4.3.1.2.4. Agent purification

While IMNV has been purified from infected shrimp tissue by sucrose density gradient ultracentrifugation (Poulos *et al.,* 2006), this is not recommended for diagnostic purposes.

4.3.2. Serological methods

Not applicable because shrimp are invertebrates which do not produce specific antibodies that could be used to demonstrate infection by or prior exposure to IMNV.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of Infection with IMNV are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	c	c	c	c
Direct LM (wet mount)	d	d	d	d	c	c
Histopathology	d	d	b	b	a	c
Transmission EM	d	d	d	d	d	d
Antibody-based assays	d	d	d	d	c	c
DNA probes (ISH)	d	d	b	b	a	a
Nested RT-PCR or real-time RT-PCR	a	a	a	a	a	a
Sequencing	d	d	d	d	a	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; ISH = *in-situ* hybridisation (ISH); RT-PCR = reverse-transcription polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with infectious myonecrosis virus

As indicated in Table 5.1, nested RT-PCR (Section 4.3.1.2.3) is the recommended method for targeted surveillance for reasons of availability, utility, and diagnostic specificity and sensitivity.

When investigating acute mortality episodes as part of a targeted surveillance programme, histological demonstration of characteristic IMNV-induced lesions in the striated muscles and the extreme hypertrophy of the LO caused by LOS formation (with or without confirmation by ISH with IMNV-specific DNA probes) is a suitable method (Table 5.1). The occurrence of significant mortality distinguishes infection with IMNV from penaeid white tail disease caused by PvNV, in which the gross signs and histopathology mimics infection with IMNV (Tang *et al.,* 2007).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Infection with IMNV is suspected if at least one of the following criteria is met:

i) Clinical signs consistent with infection with IMNV

or

ii) histopathology consistent with infection with IMNV

or

iii) a positive result by nested RT-PCR or real-time RT-PCR.

7.2. Definition of confirmed case

Infection with IMNV is considered to be confirmed if two or more of the following criteria are met:

i) histopathology consistent with infection with IMNV

ii) ISH positive result in target tissues .

iii) One step or nested RT-PCR (followed by sequencing), or real-time RT-PCR with positive results for IMNV.

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NB: At the time of publication (2021) there was no OIE Reference Laboratory for infection with infectious myonecrosis virus
(see Table at the end of this *Aquatic Manual* or consult the OIE web site:
https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 2009. MOST RECENT UPDATES ADOPTED IN 2017.