CHAPTER 2.2.3.

INFECTION WITH *HEPATOBACTER PENAELI*
(NECROTISING HEPATOPANCREATITIS)

1. Scope

Infection with *Hepatobacter penaei* means infection with the pathogenic agent *Candidatus Hepatobacter penaei*, an obligate intracellular bacterium of the Order α-Proteobacteria. The disease is commonly known as necrotising hepatopancreatitis.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

*Hepatobacter penaei* is a pleomorphic, Gram-negative, intracytoplasmic bacterium (Nunan et al., 2013). It is a member of the α-Proteobacteria (Frelier et al., 1992; Lightner & Redman, 1994; Loy et al., 1996a; Loy et al., 1996b). The predominant form is a rod-shaped rickettsial-like organism (0.25 × 0.9 µm), whereas the helical form (0.25 × 2–3.5 µm) possesses eight flagella at the basal apex (Frelier et al., 1992; Lightner & Redman, 1994; Loy et al., 1996a; Loy et al., 1996b). Genetic analysis of *H. penaei* associated with North and South American outbreaks suggest that the isolates are either identical or very closely related subspecies (Loy et al., 1996a; Loy et al., 1996b).

2.1.2. Survival outside the host

No data.

2.1.3. Stability of the agent

*Hepatobacter penaei*-infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine. *Hepatobacter penaei* frozen at –20°C –70°C and –80°C have been shown to retain infectivity in experimental transmission trials with *Penaeus vannamei* (Crabtree et al., 2006; Frelier et al., 1992).

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *H. penaei* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: whiteleg shrimp (*Penaeus 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 *H. penaei* according to Chapter 1.5. of the *Aquatic Code* include: northern white shrimp (*Penaeus setiferus*), northern pink shrimp (*Penaeus duorarum*), blue shrimp (*Penaeus stylirostris*), banana prawn (*Penaeus merguiensis*), aloha prawn (*Penaeus marginatus*), northern brown shrimp (*Penaeus aztecus*) and giant tiger prawn (*Penaeus monodon*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: American lobster (*Homarus americanus*).
2.2.3. Susceptible stages of the host

Infection with *H. penaei* has been demonstrated in juveniles, adults and broodstock of *P. vannamei*.

2.2.4. Species or sub-population predilection

See Sections 2.2.1 and 2.2.3

2.2.5. Target organs and infected tissue

The target tissue is the hepatopancreas: infection with *H. penaei* has been reported in all hepatopancreatic cell types.

2.2.6. Persistent infection

Some members of *P. vannamei* populations that survive infection with *H. penaei* may carry the intracellular bacteria for life and pass it on to other populations by horizontal transmission (Aranguren et al., 2006; Lightner, 2005; Morales-Covarrubias, 2008; Morales-Covarrubias, 2010; Vincent & Lotz, 2005).

2.2.7. Vectors

No vectors are known in natural infections.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Horizontal transmission of *H. penaei* can be through cannibalism or by contaminated water (Aranguren et al., 2006; Aranguren et al., 2010; Frelier et al., 1993; Gracia-Valenzuela et al., 2011; Morales-Covarrubias et al., 2012; Vincent et al., 2004). *Hepatobacter penaei* in faeces shed into pond water has also been suggested as a source of contamination (Aranguren et al., 2006; Brñez et al., 2003; Morales-Covarrubias et al., 2006).

2.3.2. Prevalence

Reported values for *H. penaei* prevalence in wild stocks are between 5.6 and 15% in *P. duorarum*, and between 5 and 17% in *P. aztecs* collected from Carrizal and Carbonera, Laguna Madre of Tamaulipas, Mexico (Aguirre-Guzman et al., 2010). Lightner & Redman, 1994 reported a prevalence of 0.77% in cultured *P. vannamei*, and 0.43% in cultured *P. stylirostris* from Tumbes Region, Peru.

Reported values for *H. penaei* prevalence in shrimp farms are between 0.6% and 1.3% in *P. vannamei* from shrimp farms in Belize, Brazil, Guatemala, Honduras, Mexico, Nicaragua and Venezuela (Morales-Covarrubias et al., 2011).

2.3.3. Geographical distribution

*Hepatobacter penaei* appears to have a Western hemisphere distribution in both wild and cultured penaeid shrimp (Aguirre-Guzman et al., 2010; Del Río-Rodríguez et al., 2006). In the Western Hemisphere, *H. penaei* is commonly found in cultured penaeid shrimp in Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru, United States of America, and Venezuela (Frelier et al., 1992; Ibarra-Gámez et al., 2007; Lightner, 1996; Morales-Covarrubias, 2010; Morales-Covarrubias et al., 2011).

2.3.4. Mortality and morbidity

In *P. vannamei*, infection with *H. penaei* results in an acute, usually catastrophic disease with mortalities approaching 100%.

2.3.5. Environmental factors

The occurrence of infection with *H. penaei* in farms may increase at long periods of high temperatures (>29°C) and high salinity (20–38 ppt) (Morales-Covarrubias, 2008). In Mexico, *H. penaei* has been detected at low prevalences (<7%) in shrimp farms in the months of April, May, July and August. However, in the months of
September and October when temperatures are high during the day and low at night, high prevalences and mortality (>20%) are observed (Morales-Covarrubias, 2010).

2.4. Control and prevention

2.4.1. Prevention

a) Early detection (initial phase) of clinical infection with *H. penaei* is important for successful treatment because of the potential for cannibalism to amplify and transmit the disease.

b) Shrimp starvation and cannibalism of infected shrimps, and positive conditions for *H. penaei* multiplication, are important factors for the spread of *H. penaei* in *P. vannamei*.

c) The use of hydrated lime (Ca(OH)₂) to treat pond bottoms during pond preparation before stocking can help reduce infection with *H. penaei*.

d) Preventive measures can include raking, tilling and removing sediments from the bottom of the ponds, prolonged drying (through exposure to sunlight) of ponds and water distribution canals for several weeks, disinfection of fishing gear and other farm equipment using calcium hypochlorite, and extensive liming of ponds.

e) The use of specific pathogen-free (SPF) broodstock is an effective preventive measure.

2.4.2. Control

The use of the antibiotics, oxytetracycline and florfenicol, in medicated feeds every 8 hours for 10 days is probably the best treatment currently available, particularly if infection with *H. penaei* disease is detected in the initial phase (Frelier et al., 1995; Morales-Covarrubias et al., 2012).

2.4.3. Vaccination

No scientifically confirmed reports.

2.4.4. Chemotherapy

No scientifically confirmed reports.

2.4.5. Immunostimulation

No scientifically confirmed reports.

2.4.6. Breeding for resistance

No scientifically confirmed reports.

2.4.7. Restocking with resistant species

No scientifically confirmed reports.

2.4.8. Blocking agents

No scientifically confirmed reports.

2.4.9. Disinfection of eggs and larvae

Disinfection of eggs and larvae is good management practice (Lee & O’Bryen, 2003) and is recommended for its potential to reduce *H. penaei* contamination of spawned eggs and larvae (and contamination by other disease agents).

2.4.10. General husbandry practices

The prevalence and severity of infection with *H. penaei* may be increased by rearing shrimp in relatively crowded or stressful conditions. Some husbandry practices have been successfully applied to the prevention of infection with *H. penaei*. Among these has been the application of polymerase chain reaction (PCR) to prescreening of wild or pond-reared broodstock.
3. Sampling

3.1. Selection of individual specimens

Suitable specimens for testing for infection with *H. penaei* are the following life stages (postlarvae [PL], juveniles and adults).

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.

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

*Hepatobacter penaei* infects most enteric tissue. The principal target tissue for *H. penaei* is hepatopancreas. Faeces may be collected and used for testing (usually by PCR, or dot-blot hybridisation with specific probes) when non-lethal testing of valuable broodstock is necessary (Bondad-Reantasco et al., 2001; Bradley-Dunlop et al., 2004; Bríñez et al., 2003; Frelier et al., 1993; Lightner, 1996; Morales-Covarrubias et al., 2012).

3.5. Samples/tissues those are not suitable

*Hepatobacter penaei* do not replicate in the midgut, caeca, connective tissue cells, the gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

A wide range of gross signs can be used to indicate the possible presence of infection with *H. penaei*. These include: lethargy, reduced food intake, atrophied hepatopancreas, anorexia and empty guts, noticeable reduced growth and poor length weight ratios (‘thin tails’); soft shells and flaccid bodies; black or darkened gills; heavy surface fouling by epicommensal organisms; bacterial shell disease, including ulcerative cuticle lesions or melanised appendage erosion; and expanded chromatophores resulting in the appearance of darkened edges in uropods and pleopods. None of these signs is pathognomonic.

4.1.2. Behavioural changes

In acute disease, *P. vannamei* may present behavioural changes including lethargy and reduced feeding activity.

4.2. Clinical methods

4.2.1. Gross pathology

Infection with *H. penaei* often causes an acute disease with very high mortalities in young juveniles, adult and broodstock. In horizontally infected in young juveniles, adult and broodstock, the incubation period and severity of the disease is somewhat size and/or age dependent. Gross signs are not specific, but acute infection with *H. penaei* show a marked reduction in food consumption, followed by changes in behaviour and appearance (see Section 4.1.1).

4.2.2. Clinical chemistry

Not applicable.
4.2.3. Microscopic pathology

Acute and chronic infection with *H. penaei* in *P. vannamei* can be readily diagnosed using routine haematoxylin and eosin (H&E) stained histological methods (see Section 4.2.6).

4.2.3.1. Initial phase of infection with *H. penaei*

Initial infection with *H. penaei* is difficult to diagnose using routine H&E histological methods. For diagnosis of initial infections, the use of molecular methods are recommended for *H. penaei* detection (e.g. by PCR or application of *H. penaei*-specific DNA probes to dot-blot hybridisation tests or in-situ hybridisation [ISH] of histological sections).

4.2.3.2. The acute phase of infection with *H. penaei*

The acute infection with *H. penaei* is characterised by atrophied hepatopancreas with moderate atrophy of the tubule epithelia, presence of bacterial cells and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations). Hypertrophic cells, individual epithelial cells appeared to be separated from adjacent cells, undergo necrosis and desquamation into tubular lumen and the tubular epithelial cell lipid content is variable.

4.2.3.3. Transition phase of infection with *H. penaei*

The transitional phase of infection with *H. penaei* is characterised by haemocytic inflammation of the intertubular spaces in response to necrosis, cytolysis, and sloughing of hepatopancreas tubule epithelial cells. The hepatopancreas tubule epithelium is markedly atrophied, resulting in the formation of large oedematous (fluid filled or 'watery') areas in the hepatopancreas. Tubule epithelial cells within multifocal encapsulation are typically atrophied and reduced from simple columnar to cuboidal in morphology. They contain little or no stored lipid vacuoles, markedly reduced or no secretory vacuoles and masses of bacteria. At this phase haemocyte nodules were observed in the presence of masses of bacteria in the centre of the nodule.

4.2.3.4. Chronic phase of infection with *H. penaei*

In the chronic phase of infection with *H. penaei*, tubular lesions, multifocal encapsulation and oedematous areas decline in abundance and severity and are replaced by infiltration and accumulation of haemocytes at the sites of necrosis. There are areas with fibrosis, few melanised and necrotic tubules and very low presence of hypertrophied cells with masses of bacteria in the cytoplasm and low haemocyte nodules.

4.2.4. Wet mounts

Wet-mount squash examination of hepatopancreas (HP) tissue is generally conducted to detect presumptive infection with *H. penaei*. The hepatopancreas may be atrophied and have any of the following characteristics: soft and watery; fluid filled center; paled with black stripes (melanised tubules); pale center instead of the normal orange coloration. For wet mount analysis the shrimp must be in the intermolt stage, and have not undergone a treatment that could alter the tubules. This technique uses tubular deformation or atrophy mainly of the apical region to indicate the early stages of infection with *H. penaei*.

Infection with *H. penaei* has four phases (a semi-quantitative scale):

- **Phase Initial**: Low presence of tubular deformation (1–5 field^{-1} organism^{-1}) and cell detachment.

- **Acute phase**: Infiltration of haemocytes, increased numbers of deformed tubules (6–10 field^{-1} organism^{-1}), encapsulation present in different regions of the sample, which is atrophied tubules surrounded by multiple layers of haemocytes.

- **Transition phase**: Infiltration of haemocytes, increased numbers of deformed tubules (11–15 field^{-1} organism^{-1}), melanised tubules, necrotic tubules and a high level of encapsulation present in different regions of the sample.

- **Chronic phase**: Areas with fibrosis, few melanised and necrotic tubules and very low presence of hypertrophied cells.
4.2.5. Smears
Not applicable.

4.2.6. Electron microscopy/cytopathology
Not currently 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
Not applicable.

4.3.1.1.3. Fixed sections
See section 4.2.3

4.3.1.1.4. Bioassay method

Confirmation of infection with *H. penaei* may be accomplished by bioassay of suspect animals with SPF juvenile *P. vannamei* serving as the indicator of the intra cellular bacteria (Cock et al., 2009; Johnson, 1990; Lee & O’Bryen, 2003; Lightner, 2005). Oral protocols may be used. The oral method is relatively simple to perform and is accomplished by feeding chopped hepatopancreas of suspect shrimp to SPF juvenile *P. vannamei* in small tanks. The use of a negative control tank of indicator shrimp, which receive only a normal feed, is required. When the hepatopancreas feeding (*per os*) protocol is used to bioassay for *H. penaei*, *H. penaei*-positive indicator shrimp (by gross signs and histopathology) are typically apparent within 3–4 days of initial exposure, and significant mortalities occur by 3–8 days after initial exposure. The negative control shrimp must remain negative (for at least 10–15 days) for gross or histological signs of infection with *H. penaei* and unusual mortalities.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

*Hepatobacter penaei* has not been grown *in vitro*. No crustacean cell lines exist (Morales-Covarrubias et al., 2010; Vincent & Lotz, 2007).

4.3.1.2.2. Antibody-based antigen detection methods

Immunohistochemistry (IHC) tests using monoclonal antibodies (MAbs) to *H. penaei*, according to the methods described in Bradley-Dunlop et al., 2004, are available for *H. penaei* detection.

4.3.1.2.3. Molecular techniques

ISH and PCR tests for detection of *H. penaei* have been developed, and PCR kits are commercially available (Loy & Frelier, 1996; Loy et al., 1996b). Gene probes and PCR methods provide greater diagnostic sensitivity than classic histological approaches to the diagnosis of infection with *H. penaei*. Furthermore, these methods have the added advantage of being applicable to non-lethal testing of valuable broodstock shrimp.

4.3.1.2.3.1. DNA probes for ISH applications with non-radioactive cDNA probes

The ISH method of Loy & Frelier, 1996 and Lightner, 1996 provides greater diagnostic sensitivity than do more traditional methods for *H. penaei* detection and diagnosis that employ classical histological methods (Johnson, 1990; Lightner, 1996; Morales-Covarrubias, 2010; Morales-Covarrubias et al., 2012). The ISH assay of routine histological sections of acute-, transition- and chronic phase lesions in
hepatopancreas with a specific DIG-labelled cDNA probe to *H. penaei*, provides a definitive diagnosis of NHPB infection (Lightner, 1996; Loy & Frelier, 1996; Morales-Covarrubias et al., 2006). Pathognomonic *H. penaei*-positive lesions display prominent blue to blue-black areas in the cytoplasm of affected cells when reacted with the cDNA probes. (See Chapter 2.2.2 Infection with infectious hypodermal and haematopoietic necrosis virus for details of the ISH method, and Chapter 2.2.0 Section B.5.3.i for detailed information on the use of Davidson's AFA fixative).

### 4.3.1.2.3.2. PCR method

Hepatopancreas and faeces may be assayed for *H. penaei* using RT-PCR. Primers designated as NHPF2: 5'-CGT-TGG-AGG-TTC-GTC-CTT-CAGT-3' and NHPR2: 5'-GCC-ATG-AGG-ACC-TGA-CAT-CAT-C-3', amplify a 379 base pair (bp) fragment corresponding to the 16S rRNA of *H. penaei*. The PCR method outlined below generally follows the method described in Aranguren et al., 2010.

i) **Preparation of DNA template**: DNA can be extracted from 25–50 mg fresh, frozen and ethanol-preserved hepatopancreas. Extraction of DNA should be performed using commercially available DNA tissue extraction kits following the manufacturer's procedures for production of quality DNA templates. Other DNA extraction kits include QIAamp DNA Mini Kit (Qiagen), MagMax™ Nucleic Acid kits (Life Technologies), or Maxwell® 16 Cell LEV DNA Purification Kit (Promega).

ii) The following controls should be included when performing the PCR assay: a) known *H. penaei*-negative tissue sample; b) a known *H. penaei*-positive sample (hepatopancreas); and c) a 'no template' control.

iii) The PuReTaq™ Ready-To-Go PCR Bead (RTG beads, GE Healthcare) is used for all amplification reactions described here.

iv) The optimised PCR conditions (5–50 ng DNA) (final concentrations in 25 μl total volume) for detection of *H. penaei* in shrimp hepatopancreas samples are: primers (0.2 μM each), dNTPs (200 μM each), Taq polymerase (0.1 U μl–1), manganese chloride (1.5 mM) in 10 mM Tris-HCl, pH 9.0, 50 mM KCl.

v) If the thermal cycler does not have a heated lid, then light mineral oil (50 μl) is overlaid on the top of the 50 μl reaction mixtures to prevent condensation or evaporation during thermal cycling.

vi) The cycling parameters are: Step 1: 95°C for 5 minutes, 1 cycle; Step 2: 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds, 35 cycles; Step 3: 60°C for 1 minute, 72°C for 2 minutes, 1 cycle; 4°C infinite hold.

Note: The conditions should be optimised for each thermal cycler using known positive controls.

### 4.3.1.2.3.3. Real-time PCR method

Real-time PCR methods for the detection of *H. penaei* have the advantages of speed, specificity and sensitivity. The sensitivity of real-time PCR is ~100 copies of the target sequence from the *H. penaei* genome (Aranguren et al., 2010; Vincent & Lotz, 2005).

The real-time PCR method using TaqMan chemistry described below for *H. penaei* generally follows the method used in Aranguren et al., 2010.

i) **Preparation of DNA template**: the extraction and purification of DNA template from hepatopancreas, is the same as that described in the section for traditional real-time PCR.
iii) The real-time PCR reaction mixture contains: TaqMan One-step real-time PCR SuperMix (Quanta, Biosciences), 0.3 μM of each primer, 0.1 μM of TaqMan probe, 5–50 ng of DNA, and water in a reaction volume of 25 μl. For optimal results, the reaction mixture should be vortexed and mixed well.

iv) Amplification is performed with the master cycler Realplex 2.0 (Eppendorf). The cycling consists of initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. After each cycle, the levels of fluorescence are measured.

v) 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 and also to rule out reagent contamination with the specific target of the assay. A positive control should also be included, and this can be a plasmid DNA containing the target sequence, purified bacteria, or DNA extracted from *H. penaei*-infected hepatopancreas.

4.3.1.2.3.4. Sequencing

PCR products may be cloned and sequenced or sequenced directly when necessary to confirm infection by *H. penaei* or to identify false positives or nonspecific amplification (Aranguren et al., 2010; Bustin et al., 2009; Vincent & Lotz, 2005).

4.3.1.2.4. Agent purification

Methods for *H. penaei* isolation and purification are available (Aranguren et al., 2010; Nunan et al., 2013; Vincent et al., 2004; Vincent & Lotz, 2005). *Hepatobacter penaei* is unculturable using traditional bacteriological methods, thus infection with *H. penaei* must be maintained through continual exposure of uninfected *P. vannamei* stock to a population undergoing an epizootic of infection with *H. penaei*.

4.3.2. Serological methods

Not applicable because shrimp are invertebrate animals that do not produce specific antibodies that could be used to demonstrate infection by or prior exposure to *H. penaei*.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of infection with *H. penaei* 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**

<table>
<thead>
<tr>
<th>Method</th>
<th>Targeted surveillance</th>
<th>Presumptive diagnosis</th>
<th>Confirmatory diagnosis</th>
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<tbody>
<tr>
<td></td>
<td>Larvae</td>
<td>PLs</td>
<td>Juveniles</td>
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<tr>
<td>Gross signs</td>
<td>d</td>
<td>d</td>
<td>d</td>
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<tr>
<td>Bioassay</td>
<td>d</td>
<td>d</td>
<td>d</td>
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<td>Direct LM (wet mount)</td>
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<td>d</td>
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<tr>
<td>Histopathology</td>
<td>d</td>
<td>d</td>
<td>c</td>
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<tr>
<td>In-situDNA probes</td>
<td>a</td>
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<tr>
<td>Transmission EM</td>
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<tr>
<td>Antibody-based assays</td>
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<td>c</td>
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<tr>
<td>Real-time PCR</td>
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<td>a</td>
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</tbody>
</table>
6. Test(s) recommended for targeted surveillance to declare freedom from \textit{infection with H. penaei}

As indicated in Table 5.1., real-time PCR (Section 4.3.1.2.3.2) 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, demonstration of pathognomonic \textit{H. penaei}-induced lesions in the hepatopancreas by histology (with or without confirmation by ISH with \textit{H. penaei}-specific DNA probes) is a suitable method (Table 5.1.).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Infection with \textit{H. penaei} is suspected if at least one of the following criteria is met:

\begin{itemize}
  \item[i)] histopathology consistent with infection with \textit{H. penaei} or
  \item[ii)] ISH positive results in target tissues or
  \item[iii)] a positive result by PCR or real-time PCR.
\end{itemize}

7.2. Definition of confirmed case

Infection with \textit{H. penaei} is considered to be confirmed if two or more of the following criteria are met:

\begin{itemize}
  \item[i)] histopathology consistent with infection with \textit{H. penaei} \\
  \item[ii)] ISH positive results in target tissues \\
  \item[iii)] PCR (followed by sequencing), or real-time PCR with positive results for infection with \textit{H. penaei}.
\end{itemize}

8. References


Chapter 2.2.3. - Infection with Hepatobacter penaei (Necrotising hepatopancreatitis)


Chapter 2.2.3. - Infection with Hepatobacter penaei (Necrotising hepatopancreatitis)


NB: At the time of publication (2017), there was not yet an OIE Reference Laboratory for infection with Hepatobacter penaei (necrotising hepatopancreatitis) (see Table at the end of this Aquatic Manual or consult the OIE web site for the most up-to-date list: http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).

NB: FIRST ADOPTED IN 2012; MOST RECENT UPDATES ADOPTED IN 2017.