CHAPTER 2.2.3.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

1. Scope

Infection with *Hepatobacter penaei* means infection with the pathogenic agent *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 & Frelier, 1996; Loy *et al.,* 1996). 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 & Frelier, 1996; Loy *et al.,* 1996). Genetic analysis of *H. penaei* associated with North and South American outbreaks suggests that the isolates are either identical or very closely related subspecies (Loy *et al.,* 1996).

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. *H. penaei* frozen at –20°C to –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: aloha prawn (*Penaeus marginatus*), banana prawn (*P. merguiensis*), blue shrimp (*P. stylirostris*), giant tiger prawn (*P. monodon*), northern brown shrimp (*P. aztecus*), northern pink shrimp (*P. duorarum*) and northern white shrimp (*P. setiferus*).

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 transmit it to other populations by horizontal transmission (Aranguren *et al.,* 2006; Lightner, 2005; Morales-Covarrubias, 2008; 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; 2010; Frelier *et al.,* 1993; Gracia-Valenzuela *et al.*, 2011; Morales-Covarrubias *et al.*, 2012; Vincent *et al.*, 2004). *H. penaei* in faeces shed into pond water has also been suggested as a source of contamination (Aranguren *et al.,* 2006; Briñez *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. aztecus* 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 the Tumbes Region, Peru.

Reported values for *H. penaei* prevalence in shrimp farms were 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 during long periods of high temperatures (>29°C) and high salinity (20–38 ppt) (Morales-Covarrubias, 2008). In Mexico, *H. penaei* has been detected at a low prevalence (<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 prevalence and mortality (>20%) are observed (Morales-Covarrubias, 2010).

2.4. Control and prevention

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 the bottom of ponds during pond preparation before stocking can help reduce infection with *H. penaei*.

d) Preventive measures 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.

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* is detected in the initial phase (Frelier *et al.,* 1995; Morales-Covarrubias *et al.*, 2012).

2.4.1. Vaccination

No scientifically confirmed reports.

2.4.2. Chemotherapy

No scientifically confirmed reports.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Breeding for resistance

No scientifically confirmed reports.

2.4.5. Restocking with resistant species

No scientifically confirmed reports.

2.4.6. Blocking agents

No scientifically confirmed reports.

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs and larvae is a 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).

2.4.8. 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 PCR to pre-screening 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 the 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-Reantaso *et al.,* 2001; Bradley-Dunlop *et al.,* 2004; Briñez *et al.,* 2003; Frelier *et al.,* 1993; Lightner, 1996; Morales-Covarrubias *et al.*, 2012).

3.5. Samples or tissues those are not suitable

Hepatobacter penaei does 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, noticeably 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 are 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, adults and broodstock. In horizontally infected young juveniles, adult and broodstock, the incubation period and severity of the disease are somewhat size or age dependent. Gross signs are not specific, but shrimp with 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) stain 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, molecular methods are recommended for *H. penaei* detection (e.g. by PCR or application of *H. penaei* -specific DNA probes, dot-blot hybridisation tests or *in-situ* hybridisation (ISH) of histological sections).

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

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 in to the tubular lumen. 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 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 numbers of haemocyte nodules.

4.2.4. Wet mounts

Wet-mount squash examination of hepatopancreas 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 centre; pale with black stripes (melanised tubules); pale centre 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 early stages of infection with *H. penaei*.

Infection with *H. penaei* has four phases (a semiquantitative scale):

Initial phase: 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 (i.e. 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 intracellular 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 or 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.,* 1996). Gene probes and PCR methods provide greater diagnostic sensitivity than classic histological approaches to diagnose 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 of infection 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 DNA probe to H. penaei, provides a definitive diagnosis of infection with H. penaei (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 DNA probes. (See Chapter 2.2.4 Infection with infectious hypodermal and haematopoietic necrosis virus for details of the ISH method, and Chapter 2.2.0 Section B.5.3.ii 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 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 of 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. DNA extraction kits include QIAamp DNA Mini Kit (Qiagen), MagMax™ Nucelic Acid kits (Life Technologies), or Maxwell® 16 Cell LEV DNA Purification Kit (Promega)[1].

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}), magnesium 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 25 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 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) The PCR primers and TaqMan probe were selected from the 16S, rRNA gene of *H. penaei* (GenBank U65509) (Loy & Frelier*.,* 1996). The primers and TaqMan probe were designed by the Primer Express software version 2.0 (Applied Biosystems). The upstream (NHP1300F) and downstream (NHP1366R) primer sequences are: 5'-CGT-TCA-CGG-GCC-TTG-TACAC-3' and 5'-GCT-CAT-CGC-CTT-AAA-GAA-AAG-ATA-A-3', respectively. The TaqMan probe NHP: 5'-CCG-CCC-GTC-AAG-CCA-TGG-AA-3', which corresponds to the region from nucleotides 1321–1340, is synthesised and labelled with fluorescent dyes 6-carboxyfluorescein (FAM) on the 5' and N,N,N,Ntetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end.

ii) *Preparation of DNA template:* the extraction and purification of *DNA* template from hepatopancreas, is the same as that described in the section for traditional 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 plasmid DNA containing the target sequence, purified bacteria, or DNA extracted from *H. penaei*-infected hepatopancreas.

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*.

4.3.1.2.3.4. Sequencing

PCR products may be cloned and sequenced or sequenced directly when necessary to confirm infection with *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*

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	d	d	d	d
Bioassay	d	d	d	d	c	d
Direct LM (wet mount)	d	d	d	d	d	d
Histopathology	d	d	c	c	a	c
In-situ DNA probes	a	a	a	a	a	a
Transmission EM	d	d	d	d	c	c
Antibody-based assays	d	d	c	c	c	c
Real-time PCR	a	a	a	a	a	a
PCR	a	a	a	a	a	a
Sequencing	d	d	d	d	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from 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 *H. penaei*-induced lesions

in the hepatopancreas by histology (with or without confirmation by ISH with *H. penaei*-specific DNA probes) is a suitable method (Table 5.1).

7. Corroborative diagnostic criteria

7.1. Definition of a suspect case

Infection with *H. penaei* is suspected if at least one of the following criteria is met:

i) histopathology consistent with infection with *H. penaei*

or

ii) ISH positive results in target tissues

or

ii) a positive result by PCR or real-time PCR.

7.2. Definition of confirmed case

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

i) histopathology consistent with infection with *H. penaei*

ii) ISH positive result in target tissues

iii) PCR (followed by sequencing), or real-time PCR with positive results for infection with *H. penaei*.

8. References

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NB: There is 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: https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3). Please contact the OIE Reference Laboratories for any further information on infection with *Hepatobacter penaei* (necrotising hepatopancreatitis).

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