

[DRAFT]

# Necrotizing Hepatopancreatitis

## Pathogen information

### 1. Causative agent

#### 1.1. Pathogen type

Obligate intracellular rickettsial-like organism

#### 1.2. Disease name and synonyms

Necrotizing Hepatopancreatitis (NHP); Texas Pond Mortality Syndrome (TPMS); Peru necrotizing hepatopancreatitis (PNHP).

#### 1.3. Pathogen common name and synonyms

NHP bacterium (NHPB); rickettsial-like organism (RLO)

#### 1.4. Taxonomic affiliation

Taxonomic classification uncertain.

##### 1.4.1. Phylum, class, family etc...

The NHP organism is classified as an  $\alpha$ -Proteobacterium based on sequence of analysis of the cloned 16S rDNA.

Phylogenetic analyses also indicate this bacterium is closely related to bacterial endosymbionts of protozoa, *Caedibacter caryophila* and *Holospora obtusa*.

#### 1.5. Description of the pathogen

The NHP bacterium is a gram-negative, dimorphic, intracellular rickettsial-like organism that occurs free within the cytoplasm of infected hepatopancreatic cells. The predominant form is a rod-shaped rickettsial-like organism (0.25 x 0.9  $\mu\text{m}$ ), whereas the helical form (0.25x 2-3.5 $\mu\text{m}$ ) possesses 8 flagella at the basal apex. The target tissue is the hepatopancreas with NHP infection reported in all hepatopancreatic cell types.

Authority (first scientific description, reference)

Frelier, P.F., Sis, R.F., Bell, T.A. and D.H.Lewis. 1992. Microscopic and ultrastructure studies of necrotizing hepatopancreatitis in Pacific white shrimp (*Penaeus vannamei*) cultured in Texas. *Vet. Pathol.* 29: 269-277.

#### 1.6. Pathogen environment (fresh, brackish, marine waters)

NHP has been reported in various penaeid species in brackish and marine water.

2. Modes of transmission

2.1. Routes of transmission (horizontal, vertical, direct, indirect)

Horizontal via contaminated water (shed in feces) and/or per os (cannibalism).

2.2. Life cycle

Not known due to inability to cultivate in vitro.

2.3. Associated factors (temperature salinity, etc...)

Elevated temperature (29-35°C) and salinity (20-40 ‰) are associated with overt clinical disease; an intermediate vector/reservoir is suspected.

2.4. Additional comments

None

3. Host range

3.1. Host type

American penaeid shrimp species

3.2. Host scientific names

Detected in *Litopenaeus vannamei*, *L. stylirostris*, *L. setiferus*, *Farfantepenaeus aztecus* and *F. californiensis*.

3.3. Other known or suspected hosts

Other penaeid species, zooplankton.

3.4. Affected life stage

Late postlarvae, juveniles and adults

3.5. Additional comments

None.

4. Geographic distribution

4.1. Region

Western hemisphere.

#### 4.2. Countries

United States, Mexico, Panama, Belize, Guatemala, Columbia, Ecuador, Nicaragua, Costa Rica, Brazil, Peru, and Venezuela.

### **Disease information**

#### 5. Clinical signs and case description

##### 5.1. Host tissues and infected organs

Reported to only infect the hepatopancreas.

##### 5.2. Gross observations and macroscopic lesions

The clinical signs displayed by infected shrimp are nonspecific in nature and characterized by lethargy, reduced feed intake, decreased growth rate, softened shell and an atrophied hepatopancreas. On pond-side exam, infected shrimp display empty midguts with increased superficial epicommensal cuticular fouling and/or opportunistic infections (i.e. black spots) also present. Pond mortality rates of up to 95% have been reported in untreated shrimp populations.

##### 5.3. Microscopic lesions and tissue abnormality

Microscopic exam of unstained tissue squashes prepared from suspect hepatopancreata may show reduced lipid and dark melanized necrotic tubules.

The hepatopancreas is the target tissue for this organism. Histologically, infected tubular epithelial cells will initially be hypertrophied with a generalized basophilic intracytoplasmic granularity due to the presence of numerous pleomorphic intracytoplasmic rickettsial-like organisms. Three stages of infection have been described and defined in histologic studies. In early NHP infection (stage I), intracytoplasmic rickettsial-like organisms can be detected free within the cytoplasm of resorptive, fibrillar and/or B cells of infrequent scattered tubules with increasing levels of tubular epithelial cell hypertrophy/ desquamation/attenuation, tubular necrosis, interstitial hemocytic infiltrates and lipid depletion occurring in stages II & III.

#### 6. OIE status

Listed by the OIE as “under study”

#### 7. Social and economic significance

Outbreaks can result in significant economic losses due to high pond mortalities associated with no or delayed treatment.

## 8. Zoonotic importance

None

## 9. Diagnostic methods

Three levels of examination procedures are used: screening methods for surveillance, presumptive diagnostic methods when abnormal mortalities occur, and confirmatory methods if available when a pathogen is encountered during screening or mortality outbreaks.

### 9.1. Screening methods

#### 9.1.1. Level I

During periods of elevated water temperature and/or salinity, pond-side exams of statistically significant numbers of shrimp can be conducted to detect and select suspect shrimp (e.g. demonstrating reduced feed intake, empty midguts, soft shells and atrophy of the hepatopancreas) for further diagnostic testing.

#### 9.1.2. Level II:

Histopathologic examination of routine H&E stained paraffin sections (Bell and Lightner 1988) is generally conducted to identify NHP-infected shrimp. Varying degrees of hepatopancreatic inflammation/necrosis will be present in affected shrimp with the presence of intracytoplasmic organisms confirmed with special stains (e.g. Steiner's & Steiner's stain) and/or molecular-based assays (e.g. *In situ* hybridization).

#### 9.1.3. Level III:

PCR using the published methods described in Loy & Frelief (1996c) and Loy et al. 1996b).

ISH using specific cDNA probes to NHP according to the methods described in Loy & Frelief (1996c).

### 9.2. Presumptive methods

9.2.1. Level II: see section 9.1.2.

9.2.2. Level III: see section 9.1.3.

### 9.3. Confirmatory methods

9.3.1. Level III: see section 9.1.3 for available diagnostic options.

## 10. Control methods

The use of specific pathogen-free (SPF) stocks (Wyban et al. 1992) of *P. vanamei* under biosecure culture conditions (Lee & O'Byren 2003; Lightner 2005) is the recommended method for prevention of NHP infection.

Treatment with oxytetracycline-medicated feeds has been demonstrated to be efficacious with early diagnosis critical to successful control.

## Selected references

- Bell, T. A. & Lightner, D. V. (1988). A Handbook of Normal Penaeid Shrimp Histology. Baton Rouge, LA: World Aquaculture Society.
- Bondad-Reantasco, M.G., McGladdery, S.E., East, I. and R.P. Subasinghe (eds.) Necrotizing Hepatopancreatitis (C.10) in Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fisheries Technical Paper No. 402. Supplement 2. Rome. FAO. 2001. p 207.
- Bradley-Dunlop, D.J., Pantoja, C. and D.V. Lightner. 2004. Development of monoclonal antibodies for detection of necrotizing Hepatopancreatitis in penaeid shrimp. Diseases of Aquatic Organisms. 60: 233-240.
- Brinez, B., Aranguren, F. and M. Salazar. 2003. Fecal samples as DNA source for the diagnosis of Necrotizing Hepatopancreatitis (NHP) in *Penaeus vannamei* broodstock. Diseases of Aquatic Organisms 55:69-72.
- Brock, J.A. and F. Main. 1994. A Guide to the Common Problems and Disease of Cultured *Penaeus vannamei*. Oceanic Institute. Makapuu Point, Honolulu, Hawaii. 241p.
- Frelier, P.F., Sis, R.F., Bell, T.A. and D.H. Lewis. 1992. Microscopic and ultrastructural studies of necrotizing Hepatopancreatitis in Pacific white shrimp (*Penaeus vannamei*) cultured in Texas. Veterinary Pathology 29: 269-277.
- Frelier, P.F., Loy, J.K. and B. Kruppenbach. 1993. Transmission of necrotizing Hepatopancreatitis in *Penaeus vannamei*. Journal of Invertebrate Pathology 61: 44-48.
- Frelier, P.F., Loy, J.K., Varner, P., Thompson, J.A. Lawrence, A.L. and W.A. Bray. 1995. Management Procedures for the treatment of necrotizing hepatopancreatitis in farmed shrimp. In: Swimming Through Troubled Waters. Proceedings of the

- Special Session on Shrimp Farming (eds. Browdy, C.L. & J.S. Hopkins). World Aquaculture Society '95. San Diego, CA. pg 240.
- Humason, G.L., 1972. "Animal Tissue Techniques", (3rd Ed.), W. H. Freeman and Company, San Francisco.
- Johnson, S.K. 1990. Handbook of shrimp diseases. Texas A&M University Sea Grant College Program. Galveston. TX. TAMU-SG-90-601. 25 pg.
- Jory, DE. 1997. Necrotizing hepatopancreatitis and its management in shrimp ponds. Aquaculture Magazine 23 (5): 98-101.
- Lee C.S., & O'Bryen, P.J. . (Eds.). 2003. Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables. World Aquaculture Society, Baton Rouge, LA, 293 p.
- Lightner, D.V. Redman, R.M. and J.R. Bonami. 1992. Morphological evidence for a single bacterial aetiology in Texas necrotizing hepatopancreatitis in *Penaeus vannamei* (Crustacea:Decapoda) Dis. Aquat. Org. 13: 235-239.
- Lightner, D.V. and R.M. Redman. 1994. An epizootic of necrotizing hepatopancreatitis in cultured penaeid shrimp (Crustacea: Decapoda) in northwestern Peru. Aquaculture 122: 9-18.
- Lightner, D. V (ed.). 1996. A handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society. Baton Rouge, LA. 304p.
- Lightner D.V. 2005. Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. J. World Aquacult. Soc. 36: 229-248.
- Loy, J.K., Dewhirst, F.E., Weber, W., Frelier, P.F., Garbar, T.L., Serban, I.T. and J.W. Templeton. 1996a. Molecular Phylogeny and In Situ Detection of the Etiologic Agent of Necrotizing Hepatopancreatitis in Shrimp. Appl. Environ. Microbiol. 62 (9): 3439-3445.
- Loy, J.K., Frelier, P.F., Varner, P. and J.W. Templeton. 1996b. Detection of the etiologic agent of necrotizing Hepatopancreatitis in cultured *Penaeus vannamei* from Texas and Peru by polymerase chain reaction. Diseases of Aquatic Organisms. 25: 117-122.
- Loy, J.K. and P.F. Frelier. 1996c. Specific, nonradioactive detection of the NHP bacterium in *Penaeus vannamei* by in situ hybridization. Journal of Veterinary Diagnostic Investigations 8: 324-331.

- Mc Vey, J. (Ed.). Bacterial, rickettsial and chlamydial diseases. In CRC Handbook of Mariculture (2<sup>nd</sup> ed.)\_ Crustacean Aquaculture. CRC Press. 1993. pg: 414-417.
- Vincent, A.G. and J.M. Lotz. 2005. Time course of necrotizing Hepatopancreatitis (NHP) in experimentally infected *Litopenaeus vannamei* and quantification of NHP-bacterium using real-time PCR. Disease of Aquatic Organisms 67: 163-169.
- Vincent, A.G., Breland, V.M. and J.M. Lotz. 2004. Experimental infection of Pacific white shrimp *Litopenaeus vannamei* with Necrotizing Hepatopancreatitis (NHP) bacterium by per os exposure. Disease of Aquatic Organisms 61: 227-233.
- Wyban, J. A., Swingle, J. S., Sweeney, J. N. & Pruder, G. D. (1992). Development and commercial performance of high health shrimp using specific pathogen free (SPF) broodstock *Penaeus vannamei*. In Proceedings of the Special Session on Shrimp Farming, pp. 254–259. Edited by J. Wyban. Baton Rouge, LA: World Aquaculture Society.