Milky Haemolymph Disease of Spiny Lobsters (*Panulirus* spp.)

PATHOGEN INFORMATION

1. Causative Agent

1.1. Pathogen type

Bacteria.

1.2. Disease name and synonyms

Milky haemolymph disease of spiny (*Panulirus* spp.) lobsters (MHD-SL).

1.3. Pathogen common name and synonyms

Rickettsail-like bacteria (RLB) of milky disease.

1.4. Taxonomic affiliation

1.4.1. Pathogen scientific name (Genus, species, sub-species or type).

Not classified..

1.4.2. Phylum, class, family, etc.

Not classified.

1.5. Description of the pathogen

From negatively stained bacteria "milky" haemolymph from infected spiny lobsters viewed by TEM, RLB are curved to slightly bend rod shaped organisms measuring 0.6 µm x 1.4 to 2.0 µm.

The bacterium has not been successfully cultured *in vitro*.

1.6. Authority (first scientific description, reference)

Lightner, D.V., Pantoja, C.P., Redman, R.M., Poulos, B.T., and Nguyen, H.D., Do, T.H., and Nguyen, T.C. 2008. Collaboration on milky disease of net-pen-reared spiny lobsters in Vietnam. OIE Bulletin 2008 (2): 46-47.

1.7. Pathogen environment (fresh, brackish, marine waters)

MHD-SL occurs in marine waters.

2. Modes of Transmission

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

Horizontal transmission by direct contact with lobsters in the same net-pens or indirectly by contaminated water from adjacent net-pens is suspected.

The disease has been experimentally transmitted among lobsters by cohabitation and by infection of unfiltered haemolymph from diseased lobsters into healthy lobsters. Filtered haemolymph from a 0.45 µm filter is not infectious.

2.2 Life cycle

Not applicable.

2.3 Associated factors (temperature, salinity)

None known.

2.4 Additional comments

Net-pen-reared spiny lobsters in Vietnam are fed a variety of fresh foods that includes fishery bycatch, various molluscs, and decapod crustaceans acquired locally from fishers. It is suspected that the RLB of MHD-SL infects one or more of the species in the lobster's fresh food diet.

3. Host Range

3.1 Host type

Tropical spiny lobsters.

3.2 Host scientific names

Natural infections: *Panulirus* spp., especially *Panulirus ornatus*, *P. homarus* and *P. stimpsoni*.

Experimental infections: No information.

3.3. Other known or suspected hosts

Fresh foods (see 2.4 above) are suspected as the source of the RLB agent of MHD-SL.

3.4. Affected life stage

3 month-old or older juveniles and adults.

3.5. Additional comments

Very similar diseases, with similar gross and histopathological lesions, primarily in connective tissues, have been reported in farmed black tiger shrimp (*Penaeus monodon*) and in captive-wild European shore crab (*Carcinus maenas*). Sequence information generated from 16 S rDNA amplified from the RLB from infected *C. maenas*, *P.*

monodon and *Panilurus* spp. show that the RLB in each of these diseases are similar, but not closely related (Nunan et al. 2003a & 2003b; Eddy et al. 2007).

4. Geographical Distribution

4.1. Region

Milky haemolymph disease of spiny (*Panulirus* spp.) lobsters (MHD-SL) has only been reported from Vietnam.

4.2. Countries

Vietnam.

DISEASE INFORMATION

5. Clinical Signs and Case Description

5.1. Host tissues and infected organs

Haemolymph and all connective tissues.

5.2. Gross observations and macroscopic lesions

Onset is relatively rapid. Affected lobsters become increasingly inactive and anorectic. Within another 3-5 days affected lobsters present milky haemolymph under swollen abdominal pleura of the exoskeleton (visible on ventral side), and die soon after clinical signs become apparent.

Haemolymph drawn with a syringe will range from slightly cloudy or turbid to milky white and will not clot.

Dissection of affected lobsters shows the presence of milky colored haemolymph in the hemocoel and tissue spaces and white hypertrophied connective tissues (especially serosa and capsules) of all major organs and tissues.

5.3. Microscopic lesions and tissue abnormality

Gram stained smears of haemolymph show the presence of very large numbers of small curved Gram negative rods. Stained and unstained haemolymph and tissue squashes show large numbers of small curved bacteria.

Routine H&E stained histological preparations show connective tissues, fixed phagocytes and hemocytes to possess large cytoplasmic masses (not distinct membrane-bound inclusion bodies) of very small basophilic bacterial cells. Some cells become enormously hypertrophied and their tissue type may not be discernable except by location. Haemolymph present in the hemocoel spaces may appear to contain large number of basophilic, very small bacterial cells that may occur in large aggregates, presumably from recently lysed cells.

5.4. OIE status

Listing proposed according to Article 1.2.2.2. (Emerging Disease) of the Aquatic Code.

6. Social and Economic Significance

Milky disease appeared in 2007 spiny lobster farms in Binh Dinh to Binh Thuan providences (800 km of coast line) of Vietnam. Losses in 2007 were estimated at US$10 million, or about 10% of the expected income from production for 2007.

7. Zoonotic Importance

None.

8. Diagnostic Methods

Three levels of examination procedures may be 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.

8.1. Screening methods

8.1.1. Level I

Onset of gross signs as described in section 5 (above).

8.1.2. Level II

By histopathology using routine H&E stained paraffin sections (Bell and Lightner, 1988), lobsters with advanced infections will present basophilic cytoplasmic masses of bacteria in hemocytes, fixed phagocytes and connective tissue cells.

8.1.3. Level III

PCR using the methods listed in Table 1.

8.2. Presumptive methods

8.2.1. Level I

See Section 5.

8.2.2. Level II

See Section 8.1.2.

8.2.3. Level III

See Section 8.1.3.

8.3. Confirmatory methods

8.3.1. Level I

See section 5 for the available diagnostic option.

8.3.2. Level II

See section 8.1.2. for the available diagnostic option.

8.3.3. Level III

See section 8.1.3 for the available diagnostic option.

Table 1. PCR methods for detection of MHD-SL from Vietnam.

Two PCR tests for detection of the RLB agent of MHD-SL have been developed. The primers for each are provided in the Table.

Geographic origin: Vietnam. Primer set designation: 137 F/R. Size of PCR product: 137 bp. Primer sequences: 137F: 5'-AAC-GAT-CTC-TTC-GGA-GAG-AGT-G-3' 137R: 5'-GCC-CAT-TCA-ATG--GCG-ATA-3'
Geographic origin: Vietnam. Primer set designation: 254F/R. Size of PCR product: 254 bp. Primer sequences: 254F: 5'-CGA-GGA-CCA-GAG-ATG-GAC-CTT-3' 254R: 5'-GCT-CAT-TGT-CAC-CGC-CAT-TGT-3'

9. CONTROL METHODS

Injection of oxytetracycline at 10 mg/kg into the abdominal muscle or hemocoel of lobsters presenting early signs of MHD-SL, or into at-risk lobsters at affected farms, has been found to be extremely effective in treatment and prevention of MHD-SL.

SELECTED REFERENCES

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EDDY, F., A. POWELL, S. GREGORY, L.M. NUNAN, D.V. LIGHTNER, P.J. DYSON, A.F. ROWLEY, AND R.J. SHIELDS. 2007. A novel bacterial disease of the European shore crab, *Carcinus maenas* - molecular pathology and epidemiology. Microbiology 153: 2839-2849.

HOLTHUIS L.B. (1980). Shrimps and prawns of the world: An annotated catalogue of species of interest to fisheries. *In* FAO Species Catalogue: FAO Fisheries Synopsis 125(1). Rome: Food and Agricultural Organization of the United Nations.

NUNAN, L.M., B.T. POULOS, R.M. REDMAN, M. LE GROUMELLEC, D.V. LIGHTNER. (2003a). Molecular detection methods developed for a systemic rickettsia-like bacterium (RLB) in *Penaeus monodon* (Decapoda: Crustacea). Diseases of Aquatic Organisms 53: 15-23.

NUNAN, L.M., B. NOBLE, M. LE GROUMELLEC, AND D.V. LIGHTNER. (2003b). Experimental infection of *Penaeus vannamei* by a rickettsia-like bacterium (RLB) originating from *P. monodon*. Diseases of Aquatic Organisms 54: 43-48.

OIE Reference Experts and Laboratories in 2008	
none	none

Other Reference Experts and Laboratories in 2008	
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