Screening for Incidence of Microsporidian Parasite

Enterocytozoon hepatopenaei (EHP) in Litopenaeus vannamei from Aquaculture Ponds in SPSR Nellore District of Andhra Pradesh, India

M. Raveendra¹*, P. Hari Babu¹, T. Neeraja¹, D. Pamanna¹, N. Madhavan¹, A.S. Sahul Hameed² and Ch. Srilatha³

¹College of Fishery Science Muthukur, Nellore, Andhra Pradesh, India
²OIE Reference Laboratory for WTD, Department of Zoology, C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India
³College of Veterinary Science, S.V.V. U Tirupati, India

*Corresponding author

A B S T R A C T

Hepatopancreatic Microsporidiosis (HPM) caused by Enterocytozoon hepatopenaei (EHP), a microsporidian parasite to be associated with slow (retarded or stunted) growth and white feces syndrome (WFS) in cultured shrimp in many of the shrimp growing countries in Asia, also in India. In the present study, shrimp samples from various shrimp ponds from different Mandalas of SPSR Nellore district, Andhra Pradesh, India, were collected over a period of five months (February 2016 to June 2016). Important diagnosis observed were histopathological studies, molecular technique (PCR). Histologically, the infected animals showed severe degeneration of hepatopancreatic tubule, basophilic inclusions resembling the developmental stages of EHP were found in the epithelial cells and large number of spore aggregations was observed in the tubular lumen. Enlargement of haemal sinuses was also observed in some cases. From this study, out of 50 pond case studies, 31 cases were showing EHP symptoms with 62 % prevalence by using tools of detection like PCR and histology.

Introduction

Shrimp farming is one of the most profitable and fastest-growing sectors of the aquaculture industry. Over the past decade, global farmed shrimp production has grown almost threefold from 1.13 million tons in 1999 to over 3.43 million tons in 2009 (Jory, 2010). China ranked first in shrimp aquaculture with 40% of total cultured shrimp production followed by Thailand (15%), Vietnam (12%) and Indonesia (10%) (FAO, 2011). Shrimp continues to be the largest single commodity in value terms, accounting for about 15% of the total value of internationally traded fishery products in 2012. It is mainly produced in developing countries, and much of this production finds its way into the international trade (FAO, 2014). Penaeid shrimps (Litopenaeus vannamei and Peneaus monodon), which comprise around 80% of total farmed shrimp production (FAO, 2009).
According to the production statistics from Marine Products Export Development Authority (MPEDA), after the introduction of the species, export production has grown from 1,731 metric tonne (mt) to 3,53,413 mt in 2014-15.

Andhra Pradesh has the second largest brackish water area in India after West Bengal, covering an area of about 37,560 ha besides the coastline of 972 km, spreading over nine districts namely Srikakulam, Vijayanagaram, Visakhapatnam, East Godavari, West Godavari, Krishna, Guntur, Prakasam and Sri Potti Sri Ramulu Nellore. Development of coastal aquaculture in Andhra Pradesh is centered on shrimp (\textit{L. vannamei}) farming. The culture area increased from 264 Ha to 37,560 Ha during the period from 2009-10 to 2014-15. Andhra Pradesh (2,76,077 mt) is the leading farmed shrimp producer with 78% and the rest of India production was 77,336 mt (MPEDA, 2015). The shrimp (\textit{L. vannamei}) exports from Andhra Pradesh increased to all time high and valued at more than Rs. 14,000 Crores for the year 2014-15 (MPEDA, 2015).

High stocking density (maximum permissible is 60 PLs/m$^2$) and use of compounded pelleted feed in order to achieve higher production rates impose stress on the shrimps, making them susceptible to diseases (Alavandi et al., 1995). The diseases may be caused by various etiological agents such as viruses, bacteria, fungi, parasites, algal toxins, nutritional deficiency or the adverse environment. In India, the gross economic losses due to shrimp (\textit{P. monodon}) diseases were estimated at more than Rs. 1,000 crores in 2006-2008 and loss continues even now (Kalaimani et al., 2013).

Several shrimp diseases are new or newly emerged in Asia that causes serious economic losses in shrimp farms, including Acute Hepatopancreatic Necrosis Disease (AHPND) or Early Mortality Syndrome (EMS), Hepatopancreatic Microsporidiosis (HPM), Hepatopancreatic Haplosporidiosis (HPH), Aggregated Transformed Microvilli (ATM) and Covert Mortality Disease (CMD). In addition to these, White Spot Disease (WSD), Yellow Head Disease (YHD) and Infectious Myonecrosis (IMN) continued their share of losses (Thitamadee et al., 2016).

The Global Aquaculture Alliance (GAA, 2013) has estimated that losses to the Asian shrimp culture sector amount to US$ 1.0 billion. World farmed shrimp production volumes decreased in 2012 and particularly in 2013, mainly as a result of disease-related problems, such as Early Mortality Syndrome (EMS) (FAO, 2014).

In Nellore district the frequent outbreaks of diseases such as White Spot Syndrome Virus (WSSV), Black Gill Disease (BDG), Running Mortality Syndrome (RMS), Loose Shell Syndrome (LSS), White Faecal Syndrome (WFS), White Muscle Disease (WMD) and Infectious Hypodermal and Haematopoietic Necrosis (IHHN) in shrimps causing economic loss to the aquaculture industry (Srinivas et al., 2016).

Recently, shrimp farms in Asia and other areas have been reporting heavy infection with a microsporidian parasite, \textit{Enterocytozoon hepatopenaei} (EHP) in cultured \textit{L. vannamei} impacting the production due to severe growth retardation (Newman, 2015).

Hepatopancreatic microsporidiosis (HPM) is caused by \textit{Enterocytozoon hepatopenaei} (EHP), it was first reported as an unnamed microsporidian from growth retarded black tiger shrimp \textit{Penaeus monodon} from Thailand in 2004 (Chayaburakul et al., 2004). It was subsequently characterized in detail and named in 2009 (Tourtip et al., 2009). During 2004, it was not statistically associated with
slow growth. Although EHP does not appear to cause mortality, recent information from shrimp farmers in Southeast Asian countries indicates that it is associated with severe growth retardation in *P. vannamei*. EHP outbreaks are occurring widely in China, Indonesia, Malaysia, Vietnam and Thailand. Very recently, EHP is also reported from slow growing shrimp in India. Thus, EHP is an emerging problem that is under urgent need of control (Sritunyalucksana *et al.*, 2014).

*L. vannamei* samples drawn from Andhra Pradesh, Tamil Nadu and Puri (Odisha) were tested positive for EHP by PCR, and some samples were found positive by histopathology (CIBA, 2015).

Stunting of *L. vannamei* in shrimp culture ponds for various reasons including EHP has created confusion among shrimp farmers and farmers are unable to harvest the crop though it is uneconomical to continue the crop with stunted shrimp.

**Materials and Methods**

**Sampling area**

The present study was carried out for a period of five months between February, 2016 to June, 2016. Shrimp (*L. vannamei*) with the sign of stunted growth were collected for this study from different shrimp farms located in Nidiguntapalem, Krishnapatnam village, Pottemadu, Bodiswamikandriga, Pantapalem of Muthukurmandal, Esuruwaka, Daruvukatta, Uttama Nellore, Konduru of Kota mandal, Dugarajapatnam, Mallam, Raghavavaripalem, Kothagunta, Thuppaguntapalem, Tupilipalem of Vakadumandal, Chintavaram, Eruru village of Chillakurumandal, Ganagapatnam village of Indukuripetmadal, Mudivarthi village of Vidavalurumandal and Jadagoula village of Bhogolumandal, SPSR Nellore district, Andhra Pradesh, India.

**Experimental shrimp**

The experimental culture shrimp of the present study was *Litopenaeus vannamei* cultured in semi-intensive and intensive farms of the above mentioned areas.

**Primers**

Published universal primers were used for the amplification of ssu rRNA gene of *Enterocytozoon hepatopenaei* isolates. The names of the primers, sequence and amplification size are given below:

**Collection of samples**

Fifty (50) ponds were selected for study which were experiencing size variation/growth retardation and white feces syndrome. On each sampling day, a minimum of 60 shrimps were examined for diseases of species as per OIE guidelines (OIE, 2013). Information on behavioral abnormalities, gross and clinical signs were recorded on the sampling sheet. From each pond 2-4 shrimps were taken for diagnosis and the hepatopancreas of each sample were dissected out and fixed in Davidson’s fixative for histopathology and along with Davidson’s fixative from the 50 samples, 15 were separately fixed in 95% alcohol for molecular diagnosis (Bell and Lightner, 1984). Whole infected shrimps were also wrapped individually in sterile polythene bags, placed in icebox and brought to the laboratory. On reaching laboratory they were transferred to refrigerator and analyzed / processed.

**Histopathology**

Histopathology was conducted in the Department of Pathology, College of Veterinary Science, S.V.V.U. Tirupati. The hepatopancreas of infected and normal shrimps were fixed in alcoholic Davidson’s
fixative for 48-72 h for comparative study. After fixation the tissues were transferred to 70% ethyl alcohol and kept overnight. Histopathological analysis was made as described by Roberts (2001)

**Molecular diagnosis**

Molecular diagnosis has done at OIE Reference Laboratory for WTD, Department of Zoology, C. Abdul Hakeem College, Melvisharam, Tamil Nadu.

**DNA extraction**

Hepatopancreas were homogenized in NTE buffer (0.2 M NaCl, 0.02 M Tris–HCl and 0.02 M EDTA, pH 7.4), and 10% tissue suspension was made. The suspension was centrifuged at 3000 g for 15 min at 4 °C, and supernatant was collected. The tissue suspension was mixed with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris–HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulphate and 0.1 mg mL⁻¹ proteinase K) and incubated for 2 h at 65°C to extract the DNA. After incubation, the digests were deproteinized by successive phenol/chloroform/isoamyl alcohol extraction and DNA was recovered by ethanol precipitation and dried. The dried DNA pellet was suspended in TE buffer and used as a template for PCR amplification.

**Agarose gel electrophoresis**

Polymerase chain reaction products were analyzed by electrophoresis in 0.8% agarose gels stained with ethidium bromide and visualized by ultraviolet transillumination.

**DNA sequencing and analysis**

The amplified PCR product was purified using Qiagen plasmid minipreparation spin column. Sequence analysis was performed on an Auto-sequencing kit (Applied Biosystems). The nucleotide sequence of *E. hepatopenaei* (small subunit rRNA gene) has been deposited in (Gen-Bank accession no. KU198278). The sequence was aligned using bioinformatics tools such as standard nucleotide BLAST and multiple sequence analysis clustalW (Thompson *et al.*, 1994). Significant similarity with sequences available in GenBank was searched using BLAST at National Center for Biotechnology Information (NCBI).

**Results and Discussion**

**Clinical signs of infected shrimp**

Shrimp (*L. vannamei*) with the sign of slow growth were collected for this study from different shrimp farms located in SPSR Nellore district, Andhra Pradesh, India. All the samples which we collected are slow growing as well as normally growing. These animals were apparently same except for reasons of slow growth and white fecal matter.

From the selected 50 ponds a shrimp population 4-10 animals with typical clinical symptoms (white feces and slow growth) were selected for diagnosis. Test results showed 31 pond shrimp samples (Table 1) were tested positive for EHP both by histopathology and PCR. Among the 31 pond samples, 15 samples were positive in slow growth with white feces syndrome and 16 pond samples were positive in slow growth without white feces.

The shrimp samples collected from white feces syndrome affected ponds were showing floating strands of white feces and some time the fecal strand was hanging from the anal portion of the shrimp. When the problem was severe, all the floating fecal strands were coming to sides of the pond, and it become easy for the pond manager to recognize the abnormality. Associated with the white feces
syndrome is drop in daily feed consumption, slow growth and some shrimp mortality also. The freshly dead shrimp also showed loose shell condition. During the study period, the white feces syndrome first appears 50–70 days of culture (DOC). After the appearance of white feces, shrimp health will deteriorate if some management interventions are not adopted. These include treatments with medicated feed (garlic etc.) and reduction in daily feed ration. In general, the shrimp in the WFS ponds showed FCR of over 2.92–3.17 (can be considered as 3.0) as compared to the range of normal growth ponds 1.83–1.94 (can be considered as 2.0).

**Histopathology**

Histologically, severe necrotic changes were noticed in the hepatopancreas (Fig. 1) whereas, EHP spores were observed in cytoplasm (Fig. 2). Developmental stages of EHP (Fig. 3) and large eosinophilic to basophilic inclusions indicating presumptive developmental stages of the microsporidian could be noticed in the tubular epithelium (Fig. 4). These stages were predominantly seen in the distal ends of hepatopancreatic tubules and most of the tubular epithelium in this region showed detachment from the basal membrane (Fig. 5 and 6).

The basal part of the tubular epithelium showed granular material and spore-like structures. Abnormally enlarged haemal sinuses were also noticed in the inter-tubular spaces (Fig. 7). In some of the sections, the spores were noticed in vacuolated structures. Sloughing of the tubular epithelial cells was pronounced in heavily infected HP and large spore aggregations were noticed in the tubular lumen (Fig. 8). In some of the hepatopancreas sections, large number of rod-shaped bacterial cells was also noticed in the tubular lumen indicating secondary infection.

**Molecular characterization**

The results of the targeted surveillance of EHP in *Litopenaeus vannamei* of SPSR Nellore district, Andhra Pradesh from February 2016 to June 2016 are presented in Table 1. In 0.8% agarose gel electrophoresis, samples with EHP infection show a band of PCR (510 bp) (Fig. 9).

Shrimp samples from the different villages were tested for EHP infection. Out of 50 pond shrimp samples, 31 samples were found to be EHP positive with 62% prevalence both by PCR and histologically (Table 1). Selected microsporidian isolate of *Enterocytozoon hepatopenaei* KU198278 was further characterized and identified through ssu rRNA analysis. The detailed information of the bacterial strain used, host species, clinical signs, site of infection, Gen Bank accession numbers are presented in Table 2.

Shrimp farms in Asia and other areas have been reporting heavy infection with a microsporidian parasite, *Enterocytozoon hepatopenaei* (EHP) in cultured *L. vannamei* impacting the production due to severe growth retardation (Newman, 2015). The parasite was first recorded from growth retarded tiger shrimp, *Penaeus monodon* from Thailand and reported as an undesignated microsporidian (Chayaburakul et al., 2004).

Later, this parasite was identified in *P. monodon* and named as EHP by Tourtip et al., (2009). The occurrence of this parasite was reported in pond-reared *P. monodon* from Vietnam, China, Indonesia, Malaysia and Thailand (Ha et al., 2010) and in *P. stylirostris* from Brunei (Tang et al., 2015). EHP is also reported from slow growing shrimp in India (Sritunyalucksana et al., 2014) and very recently, its occurrence was reported in farm reared *L. vannamei* in India (Rajendran et al., 2016).
**Fig. 1** Degenerative and necrotic tubules
H&E, x100

**Fig. 2** EHP spores present in the cytoplasm
H&E, x400

**Fig. 3** Development stages of EHP
H&E, x400

**Fig. 4** Basophilic inclusion (arrow; H&E, x400), EHP spores (star; H&E, x400)

**Fig. 5** EHP spores (star), basophilic inclusion (arrow) H&E, x400

**Fig. 6** Developmental stages of EHP spores (star) and detachment of tubular lumen (arrow) H&E, x400

**Fig. 7** Enlargement of haemal sinus (star) and EHP spores (arrow) (H&E, x400)

**Fig. 8** EHP infected tissue section (H&E, x400)
**Fig. 9** 0.8% Agarose gel showing PCR product of EHP of naturally infected *Litopenaeus vannamei*

**Table 1** Targeted surveillance of *Enterocytozoon hepatopenaei* in *Litopenaeus vannamei* of SPSR Nellore district, Andhra Pradesh from February 2016 to June 2016
| Date       | Location                  | Status | Pop. | 25 | 0.8 | Test | Result  |
|------------|---------------------------|--------|------|----|-----|------|---------|
| 28.02.2016 | K.P. Village, Muthukur    | Yes    | 60   | 7  | 0.4 | 100000 | - ve    |
| 29.02.2016 | Gangapatnam, Indukuripeta| Yes    | 115  | 25 | 0.8 | 400000 | - ve    |
| 12.03.2016 | Mudivarthi, Vidalavurur  | Yes    | 20   | 16.6 | 0.8 | 400000 | - ve    |
| 16.03.2016 | Ramudupalem, Vidalavurur| Yes    | 105  | 20 | 0.8 | 400000 | - ve    |
| 20.03.2016 | Ramudupalem, Vidalavurur| Yes    | 20   | 16 | 0.8 | 400000 | - ve    |
| 26.03.2016 | Gangapatnam, Indukuripeta| Yes    | 64   | 10 | 1.0 | 600000 | - ve    |
| 12.04.2016 | Pottempadu, Muthukur     | Yes    | 50   | 8  | 0.8 | 400000 | - ve    |
| 15.04.2016 | Esuruwaka, Chittamuru    | Yes    | 55   | 9  | 0.8 | 500000 | - ve    |
| 15.04.2016 | Esuruwaka, Chittamuru    | Yes    | 50   | 8  | 0.8 | 500000 | - ve    |
| 16.04.2016 | Daruvkatta, Kota         | Yes    | 55   | 6  | 1.2 | 700000 | - ve    |
| 16.04.2016 | Uttama Nellore, Kota     | Yes    | 60   | 5  | 0.8 | 500000 | - ve    |
| 03.05.2016 | Utukuru, Vidalavurur     | Yes    | 64   | 8  | 0.6 | 150000 | - ve    |
| 03.05.2016 | Mudivarthi, Vidalavurur  | Yes    | 77   | 18 | 1.0 | 600000 | - ve    |
| 05.05.2016 | Pottempadu, Muthukur     | Yes    | 90   | 16 | 0.4 | 200000 | - ve    |
| 05.05.2016 | Jadagogula, Bhogolu      | Yes    | 44   | 4  | 0.48 | 400000 | - ve    |
| 05.05.2016 | Jadagogula, Bhogolu      | Yes    | 50   | 5  | 0.8 | 300000 | - ve    |
| 07.05.2016 | Kandriga, Muthukur       | Yes    | 60   | 10 | 0.4 | 150000 | - ve    |
| 25.05.2016 | Mallam, Vakadu           | Yes    | 105  | 14 | 1.0 | 300000 | - ve    |
| 25.05.2016 | Mallam, Vakadu           | Yes    | 95   | 10 | 1.0 | 500000 | + ve    |
| 25.05.2016 | Mallam, Vakadu           | Yes    | 78   | 9  | 0.4 | 200000 | - ve    |
| 25.05.2016 | Mallam, Vakadu           | Yes    | 85   | 10 | 1.0 | 400000 | - ve    |
| 25.05.2016 | Kothagunta, Vakadu       | Yes    | 90   | 12.5 | 1.0 | 400000 | - ve    |
| 25.05.2016 | Chintavaram              | Yes    | 90   | 12.5 | 1.0 | 500000 | + ve    |
| 26.05.2016 | Eruru                    | Yes    | 90   | 14 | 0.8 | 300000 | - ve    |
| 26.05.2016 | Eruru                    | Yes    | 84   | 12.5 | 0.8 | 200000 | - ve    |
| 26.05.2016 | Eruru                    | Yes    | 85   | 12 | 1.0 | 400000 | - ve    |
Table 2 Molecular characterization of EHP strain isolated from infected/diseased cultured shrimp

| Sample No | Date       | Location                        | Shrimp species       | Disease/Clinical sign                  | Site of infection | Length of consensus sequence (bp) | Gen Bank Accession number | Identification |
|-----------|------------|--------------------------------|---------------------|---------------------------------------|-------------------|----------------------------------|---------------------------|-----------------|
| 33)       | 27.05.2016 | Tupilipalem                     | Litopenaeus vannamei | Stunted growth/White feces Syndrome   | Hepatopancreas    | 510                              | KU198278                  | EHP            |
| 34)       | 27.05.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 35)       | 27.05.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 36)       | 06.06.2016 | Pantapalem, Muthukur            |                     |                                       |                   |                                  |                           |                 |
| 37)       | 07.06.2016 | B.S.Kandriga, Muthukur          |                     |                                       |                   |                                  |                           |                 |
| 38)       | 08.06.2016 | B.S.Kandriga, Muthukur          |                     |                                       |                   |                                  |                           |                 |
| 39)       | 14.06.2016 | Pantapalem                      |                     |                                       |                   |                                  |                           |                 |
| 40)       | 27.06.2016 | Dugarajapatnam, Vakadu          |                     |                                       |                   |                                  |                           |                 |
| 41)       | 27.06.2016 | Dugarajapatnam, Vakadu          |                     |                                       |                   |                                  |                           |                 |
| 42)       | 27.06.2016 | Dugarajapatnam, Vakadu          |                     |                                       |                   |                                  |                           |                 |
| 43)       | 27.06.2016 | Dugarajapatnam, Vakadu          |                     |                                       |                   |                                  |                           |                 |
| 44)       | 27.06.2016 | Konduru, Vakadu                 |                     |                                       |                   |                                  |                           |                 |
| 45)       | 27.06.2016 | Konduru                         |                     |                                       |                   |                                  |                           |                 |
| 46)       | 27.06.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 47)       | 27.06.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 48)       | 27.06.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 49)       | 27.06.2016 | Tupilipalem                     |                     |                                       |                   |                                  |                           |                 |
| 50)       | 28.06.2016 | Thuppaguntapalem                |                     |                                       |                   |                                  |                           |                 |
Histologically, heavily infected hepatopancreas showed severe necrotic changes as evidenced by sloughing of tubular epithelial cells, degenerated cells and spore accumulation in the tubular lumen. In the present study histological features are observed in accordance with the reports on the hepatopancreatic microsporidian infection in *P. monodon* and *L. vannamei* (Chayaburakul et al., 2004 and Toutrip et al., 2009). Further, the parasite affinity for the epithelial cells has been reported for the genus *Enterocytozoon*, including the human-infecting species *E. bieneusi* (Desportes et al., 1985). Some of the histological changes observed in the infected hepatopancreas of *P. vannamei* due to EHP also showed similarity with *Enterospora canceri* reported from crab (Stentiford et al., 2007).

PCR and Light microscopic observation of *L. vannamei* from 50 ponds located in SPSR Nellore district revealed the prevalence of EHP infection (16%). However, PCR screening from 15 ponds showed relatively high prevalence of EHP infection (33%) compared to histopathology results.

The prevalence of EHP was estimated to be 63.5% for 137 samples by Rajendran et al., (2016). In the present study the EHP prevalence was observed as 16% for 50 samples. If we calculate these 50 samples as 137, the prevalence was 23%. Rajendran et al., (2016) collected animals from white faeces syndrome (WFS) affected pond showed higher prevalence of EHP (96.4%) compared to those from the unaffected pond (39.7%). But in the present study, WFS affected pond showed low prevalence (12%) for 25 samples compared to those from only stunted growth ponds (20%) for 25 samples. We agree with the statement given by Rajendran et al., (2016) that the EHP could be detected from slow growing as well as WFS-affected animals.

Previously Ha et al., (2010) had been reported that the association of microsporidian *Enterocytozoon hepatopenaei* with white faeces syndrome (WFS) and Fegel (2012) has indicated that severe infection with a microsporidian morphologically similar to *E. hepatopenaei* was associated with WFS of *P. vannamei*. In the present study WFS affected ponds showed low prevalence. Ponds with stunted growth only but no WFS showed higher prevalence. These results are comparable to the statement of Tangrasittipap et al., (2013) that EHP is not the cause of WFS.

**References**

Alavandi, S. V., Vijayan, K. K. and Rajendran, K. V., 1995. Shrimp diseases, their prevention and control. *CIBA Bulletin*, 3: 1-17.

Bell, T.A. and Lightner, D.V. 1984. IHHN virus: infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. *Aquaculture*. 38: 185–194.

Chayaburakul, K., Nash, G., Pratanpipat, P., Sriurairatana, S. and Withyachumnarkul, B., 2004. Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand. *Dis. Aquat. Org.* 60: 89–96.

Chiyansuvata, P., Chantangsi, C., Chutmongkonkul, M., Tangtrongpiros, J. and Chansue, N., 2015. Molecular Biological Screening of *Enterocytozoon hepatopenaei*in Aquatic Macro Fauna in Pacific White Shrimp (*L. vannamei*) Pond. Proceedings of the 14th Chulalongkorn University Veterinary Conference (CUVC) 2015: Responsible for Lives Bangkok, Thailand. April 20-22.
CIBA, 2015. Annual report 2014-15. Central Institute of Brackish Water Aquaculture, Chennai.

Desportes, I., Le Charpentier, Y., Galian, A., Bernard, F., Coch and Priollet, B., Lavergne, A., Ravisse, P. and Modigliani, R. 1985. Occurrence of a new microsporidian: Enterocytozoon bieneusi n. g., n. sp., in the enterocytes of human patients with AIDS. *J Protozool* 32:49–60.

FAO (Food and Agriculture Organization of the United Nations), 2009. Fishmeal market report—May 2009. Food and Agriculture Organization of the United Nations—Globefish. Online: [http://www.globefish.org](http://www.globefish.org).

FAO (Food and Agriculture Organization), 2014. The state of world fisheries and aquaculture. Rome, Italy: Food and Agriculture Organization of the United Nation.

Flegel, T.W., 2012. Historic emergence, impact and current status of shrimp pathogens in Asia. *J. Invertebr. Pathol.* 110: 166-173.

Food and Agriculture Organization (FAO). 2011. The state of world fisheries and aquaculture 2008. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1-235.

Global Aquaculture Alliance (GAA). 2013. Cause of EMS shrimp disease identified. GAA News Releases. Available: [http://www.gaalliance.org/newsroom](http://www.gaalliance.org/newsroom). Accessed 29 March 2014.

Ha, N.T.H., Ha, D.T., Thuy, N.T. and Lien, V.T.K., 2010. Enterocytozoon hepatopenaei has been detected parasitizing tiger shrimp (*Penaeus monodon*) cultured in Vietnam and showing white feces syndrome (In Vietnamese with English abstract). *Agric. Rural Dev.: Sci. Technol.* 12: 45–50 (translation from Vietnamese).

Jory, D. E. 2010. GOAL production data projects tempered aquaculture growth. *Global aquaculture advocate.* 1: 8–10.

Kalaimani, N., Ravisankar, T., Chakravarthy, N., Raja, S., Santiago, T.C. and Ponniah, A.G. 2013. Economic losses due to Disease Incidences in Shrimp Farms of India. *Fish. Techn.* 50: 80-86.

MPEDA, 2015. Annual Report 2014-2015, Marine Products Export Development Authority, Cochin.

Newman, S.G. 2015. Microsporidian impacts shrimp production-Industry efforts address control, not eradication. *Global Aquaculture Advocate*, March/April 2015, 16-17.

Rajendran, K.V., Shivam, S., Praveena, P.E., Sahayakajan, J.J., Kumar, T.S., Avunje, S., Jagadeesan, V., Babu, P.S.V.A.N.V., Prande, A., Krishnan, A.N., Alavandi, S.V. and Vijayan, K.K. 2016. Emergence of Enterocytozoon hepatopenaei (EHP) in farmed *Penaeus (Litopenaeus) vannamei* in India. *Aquaculture*. 454: 272–280.

Roberts, R.J. 2001. The parasitology of teleosts, In: *Fish pathology*, Roberts, R.J., (Ed.), W. B. Saunders, London, pp. 254 - 296.

Srinivas, D. and Venkatrayulu, Ch. 2016.Current Status and Prospects of Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931) Farming in Coastal Districts of Andhra Pradesh in India. *International Journal of Science and Research.* 5(3): 1211-1214.

Sritunyalucksana, K., Sanguanrut, P., Salachan P. V., Thitamadee, S. and Flegel, T.W. 2014. Urgent appeal to control spread of the shrimp microsporidian parasite Enterocytozoon hepatopenaei (EHP).

Stentiford, G.D. and Bateman, K.S. 2007. *Enterospora canceri* sp., an intranuclear microsporidian parasite
infection of hermit crab *Eupagurus*. *Dis. Aquat. Org.* 75: 73–78.

Tang, K.F.J., Pantoja, C.R., Redman, R.M., Han, J.E., Tran, L.H. and Lightner, D.V. 2015. Development of in situ hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. *J. Invertebr. Pathol.* 130: 37–41.

Tangprasittipap, A., Srisala, J., Chouwdee, S., Somboon, M., Chuchird, N., Limsuwan, C., Srisuvan, T., Flegel, T.W. and Sritunyalucksana, K., 2013. The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in white leg shrimp *Penaeus* (Litopenaeus vannamei). *BMC Vet. Res.* 9: 139.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachanb, P.V., Sritunyalucksana, K., Flegel, T.W. and Itsathitphaisrn, O., 2016. Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture.* 452: 69-87.

Tourtip, S., Wongtripop, S., Stentiford, G.D., Bateman, K.S., Sriurairatana, S., Chavadej, J., Sritunyalucksana, K. and Withyachumnarnkul, B. 2009. *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): fine structure and phylogenetic relationships. *J. Invertebr. Pathol.* 102: 21–29.

---

**How to cite this article:**

Raveendra, M., P. Hari Babu, T. Neeraja, D. Pamanna, N. Madhavan, A.S. Sahul Hameed and Srilatha, Ch. 2018. Screening for Incidence of Microsporidian Parasite *Enterocytozoon hepatopenaei* (EHP) in *Litopenaeus vannamei* from Aquaculture Ponds in SPSR Nellore District of Andhra Pradesh, India. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 1098-1109.

doi: [https://doi.org/10.20546/ijcmas.2018.703.131](https://doi.org/10.20546/ijcmas.2018.703.131)