

Review Article

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## Hepatopancreatic Microsporidiasis (HPM) in Shrimp Culture: A Review

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### ABSTRACT

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Shrimp culture is the most lucrative food production sector in the world and it is having good commodity in international market. This review discusses about the microsporidians especially EHP their distribution, development, transmission, pathogenicity, diagnosis, treatment and prevention methods. EHP causes growth retardation in shrimp causing huge economic losses to shrimp culture. A prevalence of about 79.5% to 18.51% has been reported in many countries, majority from Southeast Asian countries. Till now there is no valid treatment available for shrimp farmers because EHP produces spores which are highly resistant and stable for long time in soil and water. It's very difficult to eradicate once spores were in shrimp pond. so we concluded SPF brood stocks, strict pathogens quarantine and Standard management strategies could be the main approach to prevent entry of unwanted pathogens especially EHP.

### Introduction

Aquaculture is the most promising food production sector, providing protein rich supplement for humans and animals worldwide. World aquaculture production continued to grow in 2013, reaching 97.2 million tons with an estimated value of 157 billion US\$. The production of farmed food fish (finfish, crustaceans, molluscs and other

aquatic animals) was 70.2 million tons in 2013, annual growth rate up by 5.6% from 66.5 million tons in 2012 (FAO, 2016). Shrimp is considered as one of the most traded food item and it commands 15% of the traded fishery products (Granjoor, 2015). Total shrimp production in the world is dominated by two penaeid species (*Penaeus vannamei* and *Penaeus monodon*) which contributes around 80% of total shrimp production (FAO, 2009). The dominance of *P. vannamei* over *P.*

*monodonis* due to its fast growth rate, low protein requirement and Availability of SPF, SPR brood stock in the worldwide.

Modernization culture techniques, high stocking densities and use of compound pelleted feeds in order to achieve higher production creates an imbalance in aquatic environment leading to diseases (Alavandi *et al.*, 1995). Diseases have had a major impact on shrimp culture and considered as one of the most serious constraints affecting production. Diseases in shrimps are caused by different agents like viruses, bacteria, parasites, fungi, nutritional deficiencies and changes in environmental parameters. Most of the microbes of shrimps are a part of natural environment of marine, brackish and fresh water system and these may become pathogenic and cause diseases in shrimp culture under stress conditions. The conditions leading to diseases in fish depends on the availability of pathogen, the quality of environment and the health status of fish. The balance of these conditions ensures the health of the fish (Inglis, 1993).

In shrimp farming the most common shrimp diseases (mostly by virus and bacteria) has been studied, and routinely observed. These pathogenic microbes can cause a significant production losses in penaeid shrimp culture i.e. White Spot Syndrome Virus (WSSV) that cause white spot disease; Yellow Head Disease (YHD) caused by yellow head virus; Covert Mortality Disease (CMD) caused by covert mortality nodavirus; *Macrobranchium rosenbergii* nodavirus cause White Tail Disease (WTD) (Thitamadee *et al.*, 2016). Infectious bacterial pathogens emerged in shrimp culture and act as threat to shrimp cultivation. Species from *Vibrio* casually cause the infection to shrimp culture called acute hepatopancreatic necrosis disease (AHPND) by *Vibrio parahaemolyticus* (Tran *et al.*, 2016). Recently the shrimp farms in the

Southeast Asia have been affected by the microsporidian parasite, *Enterocytozoon hepatopenaei* (EHP) in *P. vannamei* culture.

The parasite was first reported from *Penaeus monodon* in Thailand (Chayaburakul *et al.*, 2004), later in 2009, this was isolated from *P. monodon* in Thailand was described and named as *Enterocytozoon hepatopenaei* (EHP) (Tourtip *et al.*, 2009). Though EHP is not associated with mortality of shrimps, it will cause growth retardation in *P. vannamei* and *P. monodon* (Newman, 2015). This parasite is also been reported from China, Indonesia, Malaysia, and Vietnam. In India EHP was first reported by Rajendran *et al.*, (2016). The economic loss in shrimp production due to EHP appears to be significant.

### **Microsporidia**

Microsporidia constitute a group of spore-forming unicellular eukaryotic parasites that are ubiquitous. They were once considered protozoans or protists (Adl *et al.*, 2012), but are now known to be more closely related to fungi, or a sister group to fungi by genomic taxonomy (Newman, 2015). In general, Microsporidia are responsible for common diseases of crustaceans and fish, and most of them are opportunistic parasites. Microsporidia have been known to science for about 150 years, and 1300 to 1500 species in 187 genera have been described. These unique organisms are strictly intracellular parasites with relatively uniform life cycle (Cali and Takvorian, 1999).

Microsporidia lack motile structures, such as flagella, and produce highly resistant spores capable of surviving outside their host for up to several years. Spore morphology is useful in distinguishing between different species. Spores of EHP appeared oval in shape measuring 1.7-1.0  $\mu\text{m}$  (Rajendran *et al.*, 2016) Approximately 43 microsporidian genera from

crustaceans have been described and 11 of these have been reported from shrimps including *Agmasoma*, *Ameson*, *Enterocytozoon*, *Inodosporus*, *Myospora*, *Perezia*, *Pleistophora*, *Thelohania*, *Tuzetia*, *Vairimorpha* and *Vavraia*. Microsporidia have been reported from about 20 species of marine or estuarine shrimps and eight species of fresh water crayfish.

### **Hepatopancreatic microsporidiasis (HPM)**

Shrimp farms in Southeast Asia have been increasingly reporting the occurrence of severely retarded growth among shrimp populations, and shrimp from affected ponds were found to be heavily infected with the microsporidian, *Enterocytozoon hepatopenaei* (EHP) (Tourtip *et al.*, 2009; Sritunyalucksana *et al.*, 2014). It is a parasite of penaeid shrimp, EHP infections have been found in both black tiger prawns *P. monodon* and Pacific white shrimp *P. vannamei*. EHP is Obligatory, spore forming parasite, known to infect hepatopancreatic epithelial cell by changing their biochemical parameters such as total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase were measured in the haemolymph of naturally and experimentally EHP-infected shrimp (Santhoshkumar *et al.*, 2016). Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase are considered generally indicated the tissue damage (Pan *et al.*, 2003ab), and these parameters would help to understand the functional status of hepatopancreas in EHP infected shrimp.

### **Distribution and transmission**

Microsporidians are ubiquitous parasites, has been reported from different countries (Table 1). The microsporidian parasite, EHP was described as an obligatory intracellular parasite, replicates within the cytoplasm of the

tubular epithelial cells of the hepatopancreas and reported to be transmitted horizontally by cannibalism, only in shrimp (Chiyansuvata *et al.*, 2015). Microsporidian life histories vary in response to selective pressures for replication and opportunities for transmission. Although horizontal transmission is the main transmission via water (Salachan *et al.*, 2017) route among the aquatic microsporidia, many species utilize both horizontal and vertical transmission (Dunn *et al.*, 2001; Smith, 2009). The usage of live feeds possesses significant threat to shrimp culture. The infected live feeds can infect and spread EHP through faeces (Newman, 2015) and EHP can be directly transmitted to other shrimps via water (Salachan *et al.*, 2017). Along with water and feed, Artemia and Grapsidae family crabs are also recognised as the potential carriers of EHP Chiyansuvata *et al.*, (2015).

### **Infestation**

The disease caused by *Enterocytozoon hepatopenaei* (EHP) is referred as hepatopancreatic microsporidiosis (HPM), since the parasite is confined to the shrimp hepatopancreas (CIBA, 2016). Microsporidians have been shown to infect only the tubule epithelial cells of the crustacean hepatopancreas (Anderson *et al.*, 1989; Hudson *et al.*, 2001). This parasite will take about 14 days to commence infection (Salachan *et al.*, 2017).

### **Clinical signs**

The clinical signs of EHP infection are not specific and associated with growth retardation (Fig. 1), White Faeces Syndrome (WFS) and size variation are the major clinical signs of EHP (Rajendran *et al.*, 2016; Salachan *et al.*, 2017). It infects only the tubules of the hepatopancreas in shrimp, and damages the ability of this critical organ to get nutrition from feed. It is widely understood

that EHP does not cause mortality but heavily limits growth of the shrimp. Severe infections by EHP can alter the susceptibility to other bacterial infections like *Vibrio* spp. in shrimp farms leads to mortalities.

**Pathological changes**

Histologically, severe necrotic changes can be seen in the shrimp hepatopancreas. The common pathological changes associated with *Enterocytozoon hepatopenaei* is seen in hepatopancreas of shrimp (Fig. 2). Large

eosinophilic to basophilic inclusions indicating the presumptive developmental stages of EHP in the tubular epithelium. These changes were predominantly seen in distal end of hepatopancreatic tubules and showing the Detachment of tubular epithelium from basal membrane of hepatopancreas (Fig. 2A). Basal part of the tubular epithelium showed granular material and spore, sloughing of the tubular epithelial cell was pronounced due to heavily infected Hepatopancreas (Fig. 2C) and followed by heavy cellular damage (Fig. 2D).

**Table.1** Prevalence of EHP in different countries

Country	Year	Prevalence	References
Thailand	2009	ND	Tourtip <i>et al.</i> , 2009
Thailand	2013	ND	Suebsing <i>et al.</i> , 2013
Thailand	2013	ND	Tangprasittipap <i>et al.</i> , 2013
Vietnam	2015	ND	Tang <i>et al.</i> , 2015
India	2016	With WFS (96.4%) Without WFS (39.7%)	Rajendran <i>et al.</i> , 2016
India	2016	69%	Biju <i>et al.</i> , 2016
UK	2009	ND	Tourtip <i>et al.</i> , 2009
USA	2015	ND	Tang <i>et al.</i> , 2015
India	2017	18.51%	Giridharan and Uma., 2017
China	2017	Greenhouse ponds (54.4%), Earthen ponds (79.5%)	Chen <i>et al.</i> , 2017

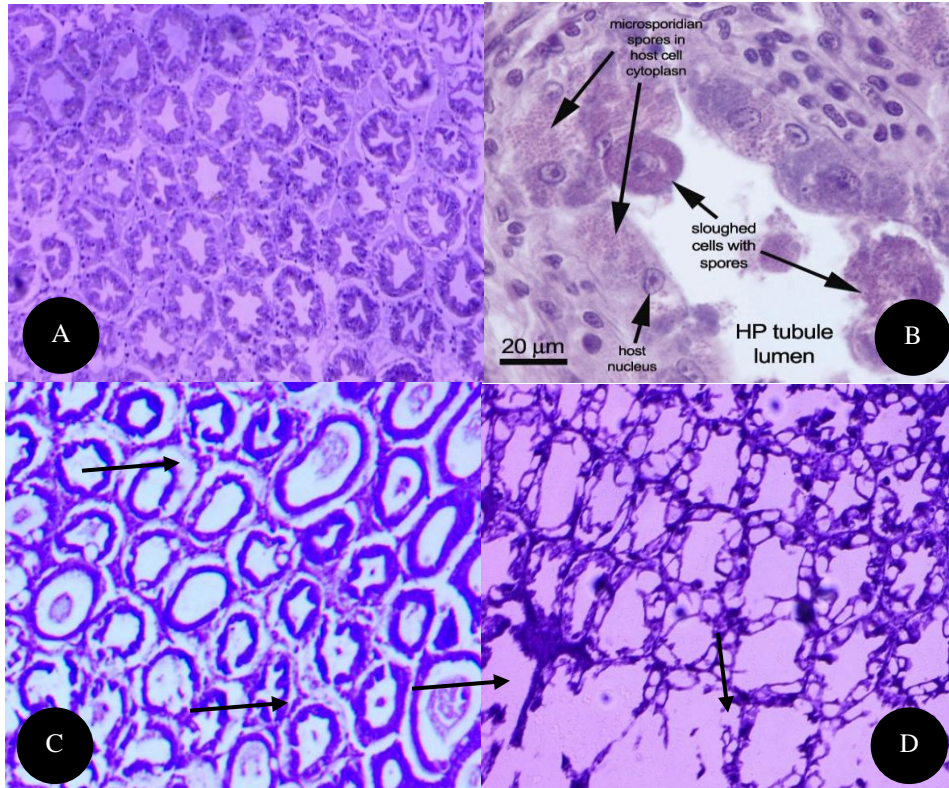
ND\*- Not Determined

**Fig.1** A. Showing the size variation in same aged shrimps in EHP infected pond. B. White faeces floating on water surface



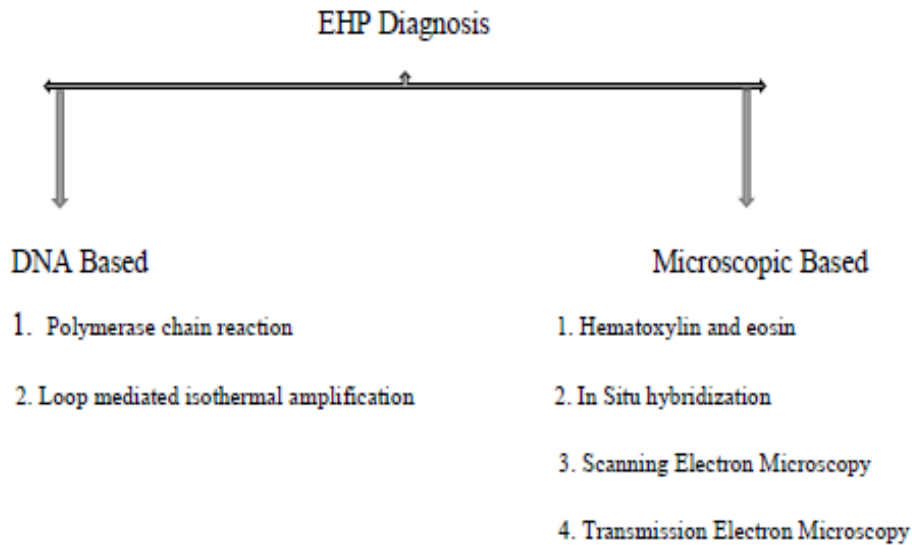


**Fig.2** Histological changes of shrimp infection with EHP



*P. vannamei* infection with EHP. A. Normal structure of hepatopancreas, B. Spores and sloughing of hepatopancreas, C. Detachment of tubules of hepatopancreas, D. Heavy cellular damage.

**Fig.3** Flow chart of EHP diagnosis



## Prevalence

There is a close connection with EHP and white faeces syndrome in shrimp culture. Without WFS (39.7%) and With WFS (96.4%) of prevalence was identified in EHP infected ponds (Rajendran *et al.*, 2016). Nevertheless the prevalence of EHP depends on the conditions of culture pond and the prevalence of EHP in Greenhouse ponds (54.4%) was lower than that of in earthen ponds (79.5%) (Shen *et al.*, 2017).

## Diagnosis

EHP can be diagnosed mainly by two methods (Fig. 3) i.e. DNA based (PCR and LAMP) and Microscopic based (Hematoxylin-eosin, In Situ hybridization, Scanning Electron Microscopy and Transmission Electron Microscopy) (Karhikeyan *et al.*, 2016). All these methods can be performed by careful observation of spores in faecal samples and hepatopancreatic tissue by microscopic examination of wet smears and histology. Further, EHP can be demonstrated with techniques like PCR and LAMP are used for rapid diagnosis in shrimp farms and hatcheries. Live feeds, PL and brood stock can be tested before rearing into culture ponds to keep EHP at bay.

## Treatment

Known treatment is not available till to date for EHP. But some of shrimp farmers employing their own methods to keep the parasite away. We have found discussions through the local farmers or producers in Andhra Pradesh, India, by applying garlic and bitter guard paste (30-40 g/ kg of feed).

In Indonesia, local producers applying Probiotics, Garlic paste (10-20g/kg feed) and vitamin C (2g/kg feed) to avoid EHP and WFS in shrimp culture (Tang *et al.*, 2016)

## Prevention and control

Good management practices and biosecurity measures in cultured farms are only way to keep pathogen away. Moreover, once spores are present in ponds it is difficult to eradicate the disease. Hence it is recommended to follow strict biosecurity and good pond management protocols like drying, ploughing, chlorination and de-chlorination of water and liming can be done to prevent EHP infection through water and aquatic carriers. EHP tested seed can be stocked to ensure absence of EHP (CIBA, 2016). Avoid live animals like polychaetes for feeding brood stock. If this advice is ignored, such feeds should have to be frozen and Pasteurized (heating at 70°C for 10 minutes) before it to use since it would kill AHPND, Bacteria, Viruses and EHP (Sritunyalucksana *et al.*, 2014). Shen *et al.*, 2017 stated that construction of greenhouse ponds could in a certain extent control spread of pathogens by the avoiding the entry of carriers in cultured ponds and concluded that use of SPF brood stocks, strict pathogens quarantine and Standard management strategies could be the main approach to prevent entry of unwanted pathogens.

The current review is addresses about microsporidian parasite, EHP and its effects on shrimp culture. EHP is an emerging disease in shrimp culture causing significant production losses to farmers. Diseases have had a great impact on shrimp culture and the durability of the production is dependent on the equilibrium between (i) The environment (ii) The prevention of diseases by diagnosis and epidemiological surveys of the pathogens, and (iii) The health condition of the shrimp. Shrimp culture is also dependent on the selection of animals resistant to diseases. Therefore, the prevention and the control of shrimp diseases need an integrated approach in which knowledge of shrimp immunity must be improved, consideration given to other

research areas related to pathology and shrimp physiology and in close connection to research in genetics. It is essential to focus efforts on producing high quality seed, follow improved better management practices, routine farm biosecurity measures and responsible trade practices to prevent epizootics in aquaculture. The establishment of regular health checks would permit the detection of shrimp immune deficiencies and consequently, reduce disease vulnerability, but at the same time, it could also contribute to the control and improvement of environment quality.

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