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Review of *Enterocytozoon hepatopenaei* (EHP) in the Indian shrimp farming scenario: Implications, Challenges, and mitigation strategies

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Abstract
Enterocytozoon hepatopenaei (EHP) has emerged as a significant threat to the shrimp farming industry worldwide, including India, where shrimp farming plays a crucial role in the economy. This review provides an overview of the current status of EHP in the Indian shrimp farming scenario, focusing on its implications, challenges, and potential mitigation strategies. It elucidates the etiology, epidemiology, and pathogenesis of EHP infection in shrimp, highlighting its impact on growth performance, survival rates, and economic losses in aquaculture production. Furthermore, the review discusses the diagnostic methods employed for detecting EHP in shrimp populations and the challenges associated with accurate and timely diagnosis. Additionally, it addresses the management practices and biosecurity measures implemented by shrimp farmers to prevent and control EHP outbreaks. Moreover, the review explores potential treatment options, including probiotics, immunostimulants, and antiprotozoal agents, and evaluates their efficacy in managing EHP infections. Finally, it discusses future research directions and the need for collaborative efforts among stakeholders to address the challenges posed by EHP and ensure the sustainable growth of the Indian shrimp farming industry.

Keywords: Shrimp farming, India, Lifecycle, Pathogenesis, Biosecurity measures

1. Introduction

The white leg shrimp, scientifically known as *Penaeus vannamei*, holds significant economic value as it contributes to 52.9% of the global crustacean aquaculture production (9.4 million tons). Despite experiencing consistent growth in output in recent years, shrimp aquaculture remains perpetually threatened by the emergence and dissemination of novel diseases, including hepatopancreatic microsporidiosis (HPM), which is attributed to the emergent pathogen *Enterocytozoon hepatopenaei* (EHP). The initial documentation of this disease appeared in 2001, impacting the black tiger crustacean P. monodon. It was provisionally designated as monodon slow growth syndrome (MSGS). EHP was officially classified as P. monodon postlarvae/juveniles in 2009, and the resulting disease was subsequently designated HPM.

EHP has been confirmed in Thailand (Tourtip *et al*., 2009) [16], India (Rajendran *et al*., 2016) [9], Vietnam (Tang *et al*., 2015) [13], Brunei (Tang *et al*., 2015) [13], Indonesia (Tang *et al*., 2016) [12] and China (Liu *et al*., 2018) [6]. Currently documented in Thailand, Vietnam, India, China, Indonesia, Malaysia, and Venezuela, hepatopancreatic microsporidiosis has continued to expand to other nations where prawns are farmed. At this time, EHP is regarded as one of the greatest hazards to shrimp culture and is severely restricting shrimp cultivation throughout Asia. Due to the historical ineffectiveness of sanitary measures worldwide in controlling the transmission of pathogens in shrimp farming and the limited availability of functional therapeutic approaches for crustacean diseases, scholars have shifted their attention towards investigating innovative approaches, such as manipulating the host-microbiome, to prevent diseases in shrimp. As the microbiome is intimately associated with specific host functions, it can be leveraged to propel shrimp aquaculture success and potentially aid in the prevention and control of EHP infection.

Utilizing microbiome modulation to enhance the health, growth, and disease resistance of crustaceans has resulted in increased agricultural output. EHP-related economic losses in India totaled approximately $567.62 million.

2. Etiology of EHP: EHP is a disease that mostly affects prawns in aquaculture environments. *Enterocytozoon hepatopenaei*, a microsporidian parasite, is identified as the causal cause of EHP. This parasite primarily targets the hepatopancreas, a vital organ in crustaceans responsible for digestion and food absorption. EHP travels through spore stages in its life cycle, which are ingested by crustaceans. When consumed, these spores release meronts, which are infectious stages that then infiltrate the hepatopancreatic epithelial cells. The parasite undergoes reproduction and differentiation inside the host cells, leading to the formation of spores. Afterward, these particles are released into the surrounding environment, where they continue the infection cycle by infecting other crustaceans (Tourtip *et al*., 2009) [16]. The exact method of EHP transmission is yet unidentified, however it is theorized to happen through the ingestion of tainted feed or water. Adverse environmental factors, overcrowding, and low water quality might worsen the occurrence and intensity of EHP infections in shrimp communities. EHP infection presents with several clinical symptoms such as hepatopancreatic discolouration, increased growth rate, and increased susceptibility to other diseases. Examining hepatopancreatic tissues histology to observe the parasite's distinct intracellular stages is the established method for diagnosing EHP.

2.1 Taxonomy

EHP is a member of the Enterocytozoonidae family within the Microsporidia phylum. This family consists of very simplified and specialized unicellular parasites that generate spores, infecting various species such as humans, insects, and crustaceans (Vávra and Lukeš, 2013).

2.2 Morphology

2.2.1 Spores: EHP spores are small and oval-shaped. They typically measure around 1.0 to 1.5 micrometers in length and 0.5 to 1.0 micrometers in width. These spores are the infective stage of the parasite.

2.2.2 Meronts: Inside the host's hepatopancreatic cells, EHP undergoes a series of developmental stages. Meronts are one of the stages observed during intracellular replication. They are characterized by their size and structure within host cells.

2.2.3 Life Cycle: The life cycle of EHP includes shrimp ingesting spores. Upon ingestion, the spores release infective stages known as meronts. Meronts infiltrate the epithelial cells of the hepatopancreas, where they multiply and mature. This leads to the creation of fully developed spores, which are later discharged into the surroundings.

2.2.4 Intracellular Development: EHP primarily infects and replicates within the epithelial cells of the hepatopancreas. Within host cells, the parasite forms characteristic structures such as meronts and mature spores.

2.2.5 Histological Features: Histological examination of infected hepatopancreatic tissues reveals characteristic intracellular stages of EHP. These include the presence of meronts and mature spores within host cells. Spores of EHP are freely discharged into the HP tubule lumens from lysed or sloughed epithelial cells. These characteristics can be identified by the use of conventional hematoxylin and eosin (H&E) stained tissue samples (Tourtip *et al*., 2009; Tangprasittipap *et al*., 2013; Tang *et al*., 2015) [16, 14, 13].

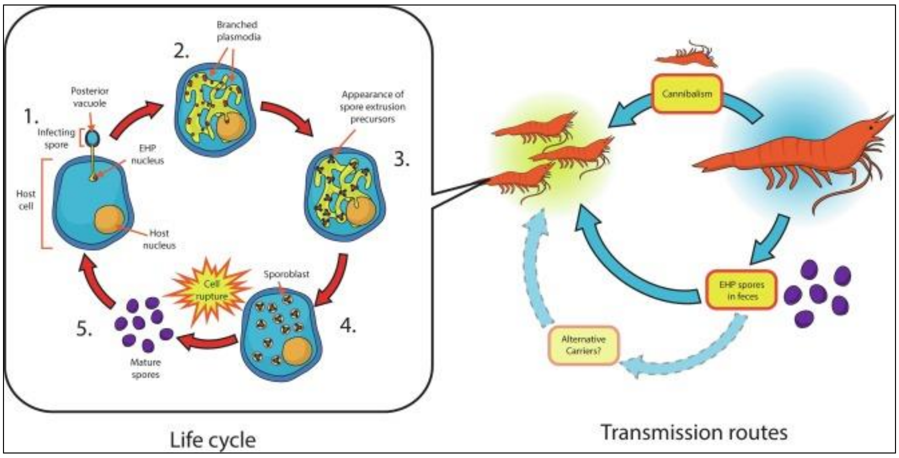

Fig 1: Life Cycle Transmission routes

Schematics illustrating the life cycle and transmission routes of EHP. Stages in the life cycle include: (1) Spore germination, in which the spore punctures the target host cell membrane with the polar tube and transfers the content into the cytoplasm. (2) The sporoplasm undergoes nuclear division to generate a branched plasmodium. (3) Spore extrusion precursors are formed inside the plasmodium. (4) Plasmodium is cleaved to generate sporoblasts. (5) The host cell ruptures to release mature spores. Transmission of EHP can occur via feces and cannibalism. Alternative hosts that serve as EHP carriers may also exist, but the possibility has not been experimentally confirmed. This transmission route, is faintly colored to connote this fact (Source: Chaijarasphong *et al*., 2021) [21].

2.3 Transmission Routes

2.3.1 Horizontal Transmission: EHP can horizontally spread among shrimp populations through many pathways. Contaminated water sources are considered a primary route of transmission. Shrimp infected with EHP discharge spores into the environment via excrement or by cannibalism of infected shrimp potentially contaminating water and sediments. Other possible ways of horizontal transmission include consuming contaminated feed and coming into touch with diseased individuals (Tangprasittipap *et al*., 2013) [14].

2.3.2 Vertical Transmission: Evidence suggests that EHP can be transferred vertically from sick broodstock to their progeny. This transmission can occur from one generation to the next. It has been reported that the parasite can be transmitted from mother to offspring through the use of eggs; it has been proved in lab conditions by Karthikeyan *et al*., 2019 [5] however, further research is required to determine the frequency and relevance of this route of transmission.

2.4 Reservoirs

2.4.1 Infected Shrimp: Shrimp that are infected with EHP act as reservoirs for the disease inside aquaculture systems. In addition to contributing to the pollution of water and sediment, these shrimps are responsible for the release of EHP spores into the environment. Infected shrimp have the ability to act as carriers of the parasite, which allows them to spread EHP into new or existing populations of shrimp.

2.4.2 Environmental Reservoirs: Shrimp cultivation ponds have the potential to act as environmental reservoirs of EHP due to the presence of water and sediment. Infectious spores are retained in contaminated water sources and sediments, which makes it easier for the parasite to spread to shrimp that are vulnerable to it.

2.5 Biosecurity Risks: It is possible for the threat of EHP transmission and persistence within aquaculture facilities to increase if biosecurity controls are not properly implemented. It is possible to develop and sustain EHP reservoirs by the introduction of infected shrimp or water that has been polluted inside of industrial systems. When it comes to shrimp farming, having a solid understanding of the transmission channels and reservoirs of EHP is absolutely necessary in order to successfully apply management and control measures. The impact of EHP on shrimp health and production can be mitigated by the use of strategies that aim to reduce horizontal and vertical transmission, improve biosecurity, and minimize environmental reservoirs. In addition, continuing research is required in order to further clarify the epidemiology of EHP and to create therapies that are specifically targeted for its controlled management.

2.6 Geographical Distribution in India: EHP has been documented in locations of India where shrimp farming is done as of January 2022. These regions include a number of different locales. The presence of the parasite has been reported in a number of states along the coastline of India, including Andhra Pradesh, Tamil Nadu, Gujarat, Maharashtra, Kerala, Odisha, and West Bengal. The geographical distribution of EHP in India is not consistent, and the presence of the parasite has been confirmed in these states (Geetha *et al*., 2022) [2].

3. Pathogenesis and impact on shrimp health

3.1 Histopathological changes: Histopathological alterations that are linked with an infection caused by EHP in shrimp, particularly in the hepatopancreas, might give useful insights into the pathology implications of the parasite (Tourtip *et al*., 2009; Tangprasittipap *et al*., 2013) [16, 14].

3.2 Hepatopancreatic disorganization: EHP infection frequently results in the disarray and degradation of the tubules that are seen in the hepatopancreas. It is possible for the hepatopancreas to display structural abnormalities, such as the breakdown of tubule integrity and the departure from the typical design of the tissue (Thitamadee *et al*., 2016) [15].

3.3 Vacuolation of Hepatopancreatic Cells: There is a possibility that infected hepatopancreatic cells would exhibit vacuolation, which is defined by the presence of vacuoles or clear areas inside the cytoplasm. Vacuolation is a potential consequence of cellular damage and changes in metabolic processes that are brought about by an infection with EHP.

3.4 Cellular Hypertrophy and Hyperplasia: Hypertrophy, also known as enlargement, and hyperplasia, also known as enhanced cell proliferation, are outcomes that may occur in hepatopancreatic cells as a result of an infection with EHP. Cellular stress and compensatory mechanisms in damaged tissues are shown by these cellular alterations, which are symptomatic of the stress.

3.5 Granulomatous Responses: It is common for the hepatopancreas to present with granulomatous inflammation as a reaction from the host to an EHP infection. Granulomas, which are formed of aggregates of immune cells such as hemocytes and melanized cells, have the potential to develop around those cells or spores that are infected with EHP.

3.6 Degenerative Changes: There is a possibility that hepatopancreatic cells would exhibit degenerative alterations such as necrosis, pyknosis (which refers to the shrinking of the nucleus), and karyorrhexis (which refers to the nuclear fragmentation). These alterations are a reflection of the damage and death of cells that occurred as a result of the EHP infection.

3.7 Intracellular Parasite Development: Necrosis, pyknosis (a shrinking of the nucleus), and karyorrhexis (a fragmentation of the nucleus) are all examples of degenerative alterations that may be observed in hepatopancreatic cells of a patient. The infection caused by EHP is responsible for these alterations, which are a reflection of cellular damage and death.

3.8 Hemocytic Infiltration: Hepatopancreatic hemocytic infiltration may be linked to EHP infection. Shrimp immune cells called hemocytes have the ability to go to the infection site and take part in the host's defensive mechanism against the parasite.

These histopathological changes collectively contribute to the pathological manifestations of EHP infection in shrimp. Histological examination of infected tissues plays a crucial role in the diagnosis and characterization of EHP-associated lesions, aiding in disease detection and management efforts in shrimp aquaculture.

4. Diagnostic Methods for EHP Detection

4.1 Microscopic Examination: Microscopic inspection is a useful method for diagnosing EHP and can be enhanced by molecular methods like polymerase chain reaction (PCR) for confirming the diagnosis. Moreover, interpreting histological data necessitates competence and experience in shrimp pathology.

4.2 PCR-Based Techniques: EHP detection may be done using nested PCR techniques that target the SWP1 gene. The tests have been altered to enhance their sensitivity and specificity in identifying EHP strains across various shrimp species. The SWP-PCR technique was specifically created to address the problem of incorrect positive outcomes caused by cross-reactivity with closely similar microsporidia. This approach has demonstrated more sensitivity than the current SSU-PCR method, making it appropriate for detecting EHP in feces, feed, and environmental materials. A diagnostic kit or device has been created for real-time gene amplification of the hepatitis E virus (HEV) in isolated biological samples. These diagnostic approaches are important for early diagnosis and monitoring of EHP and HEV infections in shrimp farming and human populations, respectively. One-step PCR (Tourtip, S. *et al*., 2009) [6], Nested PCR (Tangprasittipap *et al*., 2013) [14] (Tang *et al*., 2017) [11] (Jaroenlak, P *et al*., 2016) [4], LAMP (Suebsing *et al*., 2013) [10] qPCR (Liu *et al*., 2018) [6].

5. Management practices and biosecurity measures

Potential Treatment and Control Strategies: Shrimp farmers should implement biosecurity measures before and after stocking post larvae (PL) to avoid EHP infection from environmental sources and guarantee that the PL supply tests negative by PCR. Currently, there is a lack of effective or authorized treatments for controlling HPM. Once EHP has entered the system, it can be very challenging to mitigate the problem, regardless of the etiology. We suggest that realistic management measures on farms should be based on knowledge from molecular and observational studies. This section offers a summary of current and future research directions that might help in developing control measures for EHP. EHP infections include the release of spores, suggesting that direct interaction with EHP spores might be a viable control strategy. Spore preparations that were purified and germinated in a controlled environment before being orally administered shown dramatically reduced infectivity when compared to spores that were not treated in the same way. Stimulating polar tube extrusion, together with disinfection, can be a successful method for deactivating EHP spores, particularly in ponds or hatcheries that have experienced previous infections. To control ecofriendly extracts can be used (Madesh *et al*., 2023) [7] To prevent EHP infections in shrimp ponds with a previous history, it is recommended to apply quick lime (CaO) to the pond bottom to encourage spore removal and deactivation before adding new shrimp (Pattarayingsakul *et al*., 2022) [8]. Extreme temperatures have been shown to be able to destroy EHP.

5.1 Integrated approaches and best management practices

Biosecurity and Management Practices: Implementing adequate biosecurity controls and management methods is crucial to avoid the transmission and expansion of EHP in shrimp farms. Enforcing adherence to biosecurity regulations can be difficult, particularly in areas with a wide range of farming methods and varying amounts of infrastructure.

6. Conclusion

This review aims to provide a comprehensive understanding of the current status of *Enterocytozoon hepatopenaei* (EHP) in the Indian shrimp farming scenario, addressing its implications, challenges, and potential mitigation strategies. By synthesizing available knowledge and identifying research gaps, this review seeks to support the development of sustainable management practices and facilitate collaborative efforts among stakeholders to safeguard the future of the Indian shrimp farming industry.

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