Exploring EHP infections in shrimp aquaculture and the potential of neem as a natural solution

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Abstract

Enterocytozoon hepatopenaei (EHP) infection in shrimp aquaculture, highlighting its impact on the industry and potential mitigation strategies. It covers EHP's life stages, transmission pathways, and clinical manifestations, emphasizing growth retardation, lethargy, reduced feed intake, and soft shells. The study also explores diagnostic methods such as PCR and qPCR, and examines the use of natural products like neem for treatment. EHP's implications for shrimp health and the challenges of control are discussed, underscoring the need for effective diagnostic tools and management approaches to prevent economic losses.

Introduction

Aquaculture plays a vital role in global food security by providing a significant portion of the world's seafood consumption. It encompasses a wide range of activities, from raising fish in freshwater ponds to cultivating oysters in coastal waters, and it can take place in natural or artificial environments. Proper management and sustainable practices are crucial to ensure the health of the aquatic ecosystem and the production of safe and high-quality seafood products. India holds the second position globally in aquaculture production and is a significant shrimp supplier to the United States and the European Union. With a 17.5% market share in farm-raised shrimp aquaculture, India shifted from Black tiger shrimp (Penaeus monodon) to Pacific white leg shrimp (Litopenaeus vannamei) due to white spot disease. L. vannamei exhibits superior

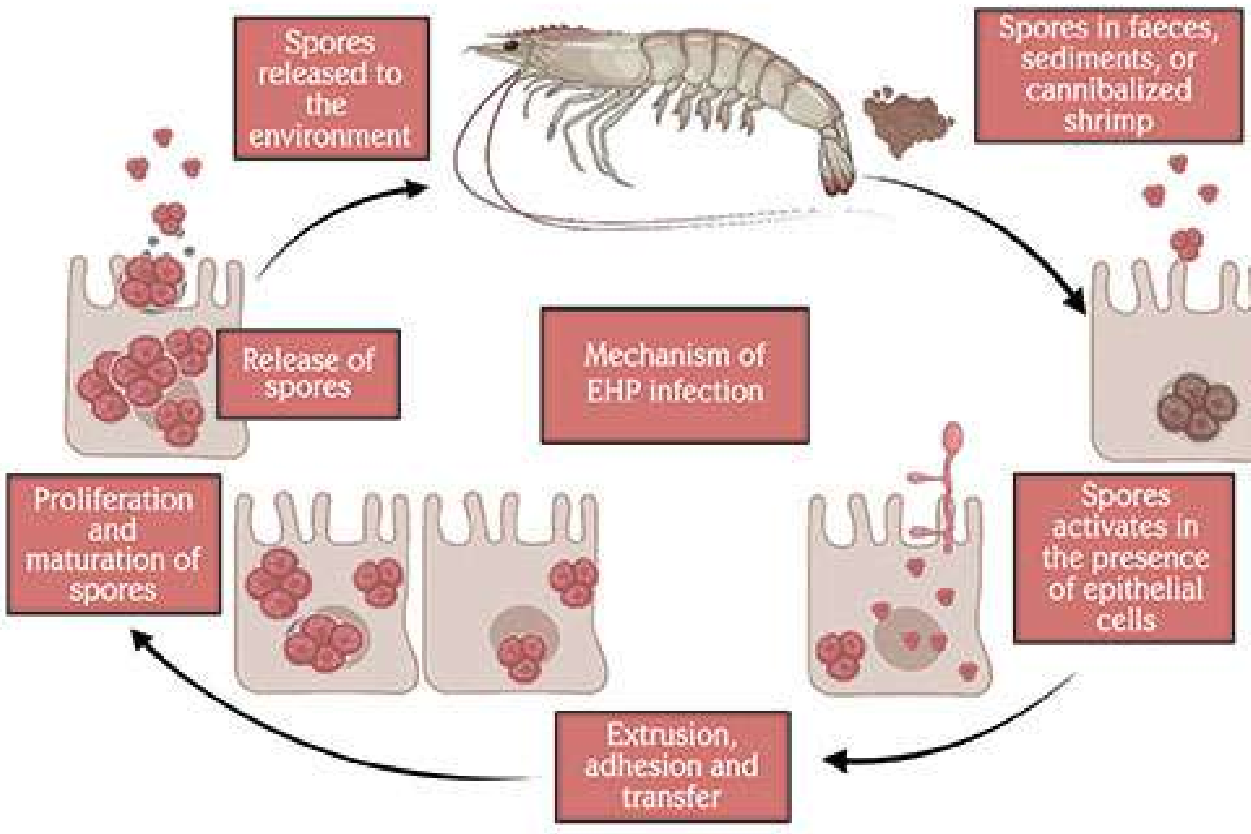

Fig. 1. General Life cycle and transmission of EHP

Table 1. Clinical manifestations of EHP in shrimp

Clinical Manifestations	Description
Growth Retardation	EHP-infected shrimp often exhibit reduced growth rates, leading to size variability.
Lethargy	Infected shrimp may show decreased activity levels and overall sluggishness.
Reduced Feed Intake	Shrimp with EHP infections tend to consume less feed than their healthy counterparts.
Empty Midgut	Infected shrimp may have an empty midgut, indicating reduced feeding and digestion.
Chronic Mortalities	Long-term infections can lead to recurring mortalities within the shrimp population.
Soft Shells	Infected shrimp often exhibit soft shell conditions, which can impact overall health.
White Feces Syndrome (WFS)	EHP has been linked to WFS, characterized by white GI tract discolouration and white faecal threads.

aquaculture traits to P. monodon and Fenneropenaeus indicus, including better survival, rapid development, adaptability, and disease resistance. The shift resulted in 5.4 million tonnes of seafood in 2019.

Shrimp aquaculture

Shrimp aquaculture plays a pivotal role in driving economic growth and generating significant employment opportunities, particularly in the developing countries of Asia. With a substantial market share of 24.9% in the global trade of farmed shrimp, India holds the position of the second-largest aquaculture product producer. In 2018, India stood as the primary prawn supplier to the United States and the second-largest to the European Union. In response to the threat of white spot disease, Litopenaeus vannamei emerged as a replacement for the Black tiger prawn (Penaeus monodon) in India. The Pacific whiteleg shrimp (L. vannamei) has emerged as the preferred crustacean species for human consumption through aquaculture. This notable crustacean, known for its prominence in aquaculture, yielded a farm production of 5.4 million tonnes and contributed significantly to the economic value of 2.6 million rupees in 2019.

Current affairs

Challenges of unknown origin have impacted shrimp farmers' expectations in L. vannamei production, hindering the industry's ability to meet global demand. Infectious ailments induced by microorganisms, such as bacteria, viruses, and microsporidia, threaten the shrimp farming sector across Asia. A microsporidian named EHP has afflicted Penaeus monodon in Thailand since 2003. Initially identified as EHP in 2009, it infects shrimp's hepatopancreas cells.

In nations where EHP occurrences have been recorded, including China, Indonesia, Malaysia, Vietnam, and Venezuela, primary shrimp cultivation occurs across diverse environmental settings, encompassing coastal regions, brackish zones, and rural locales. Initially, there were reports of early-stage shrimp mortalities attributed to early mortality syndrome (EMS) in several parts of India. Nevertheless, a comprehensive investigation conducted by the Central Institute of Brackishwater Aquaculture (CIBA) in Chennai, India, effectively debunked the presence of EMS in the country. Consistent shrimp deaths within culture ponds have also been linked to suspected cases of running mortality syndrome, yet the precise causative agent for this phenomenon remains unidentified.

During 2016, instances emerged where L. vannamei, cultivated in ponds, exhibited the presence of EHP across multiple regions in India. Consequently, the shrimp farming sector encountered considerable financial losses. Notably, specific eastern Indian states, such as Andhra Pradesh, feature ponds comprising a borehole and estuary water blend, resulting in varying salinities ranging from 0 to 30 ppt, with an average of approximately 10 ppt. Conversely, western regions like Gujarat experience pond water salinities ranging from 30 to 44 ppt. It is worth highlighting that EHP occurrences have been documented across both high and low-salinity conditions. Reports indicate a staggering production loss of 0.77 million tonnes in Indian prawn farms due to EHP infection, translating to a revenue decline of $567.62 million.

EHP

Microsporidia are parasitic microorganisms that can infect various hosts, including mammals, invertebrates, certain protists, fishes, and crustaceans, by forming spores. The distribution of microsporidians has been extensively documented in freshwater, brackish water, and marine environments. Among these microorganisms, EHP has gained prominence as a pathogen. Taxonomically, EHP belongs to the Enterocytozoonidae family, suborder Apansporoblastina, phylum Microsporidia, and kingdom Fungi. Various shrimp species like Penaeus japonicas, Penaeus monodon, and Penaeus vannamei, commonly raised in shrimp farming, can harbour EHP in their hepatopancreas.

Life cycle

EHP follows a life cycle with two distinct stages. The intracellular spore-forming stages colonize the shrimp hepatopancreas's digestive epithelial cells, causing harm to the hepatopancreatic (HP) tubule epithelial cells. The extracellular stage involves an active spore phase within the digestive tubule, with multiplication occurring in the hepatopancreatic cell cytoplasm. When infected with EHP, host cells display irregular or regular basophilic inclusion bodies in the cytoplasm, resembling the Plasmodium sporogony stage, with or without spores. Host cells are frequently infected as the spore is released from its polar tube, breaching the plasma membrane and releasing its contents (sporoplasm) into the cytoplasm. The sporoplasm subsequently develops adjacent to the host cell's cytoplasm, resulting in a spreading plasmodium. Upon separation from sporoblasts, this plasmodium generates complete spores while remaining proximate to the host cell cytoplasm. Sporogony involves synthesizing spore extrusion precursors, including the polar filament and anchoring disc.

EHP spores are found within the gastrointestinal tract and the lumen of HP tubules. Horizontal transmission in shrimp farming occurs as spores are ingested through contaminated shrimp excrement or via cannibalism of infected shrimp, where ruptured HP epithelial cells produce mature spores. EHP has been documented across a spectrum of salinity conditions **(Fig. 1)**.

The hepatopancreas is pivotal in digestion, absorption, lipid, glycogen, and mineral storage. An EHP-infected hepatopancreas can significantly impact shrimp's health and physiology, potentially disrupting nutritional processes, metabolic pathways, and enzyme production. While EHP infections do not cause substantial mortality, they are linked to size discrepancies and growth retardation. Correlations have been observed between the detection of elevated EHP gene levels and increasing growth retardation in shrimp.

Clinical manifestations

Clinical manifestations of EHP include growth retardation, which contributes to size variability, lethargy, reduced feeding, and empty midguts. Infected shrimp often exhibit soft shells. EHP is also associated with White Feces Syndrome (WFS), characterized by white gastrointestinal tract discolouration and whitish faecal threads floating on the water's surface.

Despite the availability of EHP-free post-larvae for stocking shrimp farms, feeding them locally captured marine organisms positive for EHP can result in their infection. Additionally, the faeces of infected shrimp carry numerous spores that facilitate horizontal transmission to healthy shrimp. (EHP) exhibits complex life cycles involving various spore types generated according to the host species, often spanning multiple phyla. While some spores may appear physically similar under a light microscope, their functionality can differ, with one type infecting one host while another targets an alternative host. These intricate cycles may involve transitions between monokaryotic (haploid) and diplokaryotic nuclei, where the latter consists of two tightly enclosed haploid nuclei. In certain species, the diplokaryotic nucleus may merge to form a conventional diploid nucleus, which then undergoes eukaryotic meiosis, yielding four haploid nuclei.

EHP produces oval-shaped, monokaryotic spores measuring around 1.1 ± 0.2 µm x 0.6 ± 0.2 µm. These spores exhibit 5-6 coils of the polar filament on one side and an anchoring disc on the other. Recent PCR results have revealed EHP spores within various shrimp cellular components, including R (reserve), B (blister), and F cells of the hepatopancreas, as well as in midgut epithelial cells and shrimp faecal specimens. Embryonic cells (E-cells) near the hepatopancreas typically remain unaffected **(Table 1)**.

Similar to other microsporidians, EHP spores possess a double-layered spore wall. The outer layer (exospore), approximately 10 nm thick, is electron-dense, while the inner layer (endospore), approximately 2 nm thick, is electron-lucent. This spore wall offers protection against harsh conditions, rendering the spore components environmentally resilient during dissemination. EHP's spore wall proteins are categorized within the SWP12 clade, responsible for host cell attachments. The first EHP spore wall protein (EhSWP1) was identified through whole-genome sequencing and is associated with host cell attachment function. Additionally, these proteins contribute to spore protection. This evolutionary trait likely provides advantages for EHP to infect other shrimp species or expand its host range.

Like other invertebrates, shrimp employ their innate immune system to counter invading pathogens. This defence mechanism encompasses both humoral and cell-mediated immunity. Non-specific enzymes like lysozyme (LZM) and superoxide dismutase (SOD) in bodily fluids collaborate to ward off bacterial infections. Shrimp physiological and immunological status is influenced by metabolic factors such as triglycerides (TG), total protein (TP), cholesterol (CL), glucose (GLU), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as parameters like total hemocyte count (THC), prophenoloxidase (PO), superoxide anion (SOA), total antioxidants capacity (T-AOC), and catalase (CAT).

Table 2 . Different diagnostic methods to detect EHP in shrimp

Diagnostic Method	Description
PCR (Polymerase Chain Reaction)	Amplifies specific DNA segments for EHP detection; highly sensitive and specific.
qPCR (Quantitative PCR)	Measures EHP DNA concentration quantitatively; aids in assessing infection severity.
LAMP (Loop-Mediated Isothermal Amplification)	Rapid amplification method, cost-effective and suited for field use.
In Situ Hybridization Assay	Uses labelled probes to detect EHP-specific RNA in tissue sections; offers spatial information.
Histopathology	Microscopic examination of tissue sections for EHP-induced cellular changes provides visual evidence.
Nested PCR	Two-step PCR that enhances specificity targets specific EHP genes to avoid cross-reactions.

Diagnosis

Numerous diagnostic methodologies have been documented for the detection of EHP to date. These include loop-mediated isothermal amplification (LAMP), in situ hybridization assay, histopathology, polymerase chain reaction (PCR), and quantitative PCR (qPCR), which relies on the small subunit rRNA (SSU rRNA) gene. Nested PCR targeting the spore wall protein gene (*swp*) or b-tubulin gene may also mitigate potential misinterpretations stemming from sequence similarities among SSU rRNA genes among closely related microsporidia.

EHP has been recognized as a significant threat to shrimp farms in various countries. Preventative strategies involve treating prawn ponds with quick lime (CaO) at six tonnes per hectare to eliminate residual spores and potential carriers before introducing EHP-free post larvae (PL). The main focus of EHP infection control efforts is these measures. However, their effectiveness in preventing the spread of the EHP virus to healthy shrimp is limited. Unfortunately, no curative treatments are known for EHP infection, and clear clinical symptoms are absent in infected prawns, posing challenges for surveillance and containment. As a result, developing a rapid and straightforward diagnostic approach for EHP infection is imperative to avert disease outbreaks and economic losses **(Table 2)**.

Treatment

To address EHP infections or enhance the immune response of affected shrimp against EHP, natural products such as herbal compounds like neem have been investigated. Neem, scientifically known as Azadirachta indica, is deeply rooted in Ayurveda, the ancient medicinal system of India. Ayurveda extends beyond mere treatments and procedures; it represents a holistic way of life that aligns with fundamental truths. Traditional Ayurvedic remedies often feature ingredients like aloe vera (Aloe barbadensis), neem, and sandalwood (Santalum album), which possess antibacterial properties and cool the blood, benefiting conditions related to Pitta.

Neem, a versatile plant, has been utilized for over two millennia in India and neighbouring regions. Its diverse biological functions are well-documented through scientific research. Various parts of the neem tree, including the seeds, flowers, kernel, twigs, bark, roots, and leaves, have been employed in traditional and folk medicinal systems for their therapeutic attributes. Neem leaves, for instance, exhibit anti-inflammatory, antibacterial, antiviral, antioxidant, hepatoprotective, antimutagenic, anticarcinogenic, and other properties, making them integral to traditional healing practices. Neem seeds possess antimalarial, antipyretic, and antifungal qualities and properties similar to the leaves. The neem flower contributes medicinal benefits such as phlegm clearance, bile regulation, and intestinal worm treatment, containing sesquiterpenes, hydrocarbons, fatty acids, steroids, and aromatic compounds.

Neem is rich in over a hundred primary and secondary metabolites, making it a valuable source of bioactive compounds. Primary constituents encompass protein molecules and derivatives of fats or carbohydrates, while secondary compounds include flavonoids, steroids, saponins, and alkaloids. Neem's therapeutic components are broadly categorized into isoprenoids and nonisoprenoids. The primary active component is tetranortriterpenoids, limonoids or C-seco meliacins. Azadirachtinoids, isomeric meliacins, are a significant subgroup, with azadirachtin E standing out for its efficacy. Other constituents include phenolic compounds, carotenoids, steroids, ketones, and terpenoids.

Over time, diverse techniques have been developed to extract neem with varying potency for personal and commercial applications. Indigenous methods involve water extraction, cold maceration, steam distillation, and solvent extracts. Aqueous extracts, achieved with minimal equipment, are comparably effective. Crushing neem seeds, kernels, or leaves, soaking them overnight, and filtering yields extract. Alternatively, granular material can be soaked in a cotton bag for hours, producing a refined extract containing water-soluble amino acids, bitters, and carbs.

In conclusion, neem's multifaceted therapeutic attributes, documented through ancient practices and scientific research, make it a promising natural compound for combating EHP infections and enhancing the immune response in affected shrimp.

Conclusion

The introduction of Litopenaeus vannamei revolutionized shrimp aquaculture and led to Enterocytozoon hepatopenaei (EHP) infections. These infections, caused by microsporidian parasites, challenge the industry globally. Diagnostic methods like PCR and LAMP aid in identifying EHP, but preventive measures and effective treatments are lacking. EHP's intricate life cycle affects shrimp health, causing growth retardation and size variability. With its diverse biological properties, neem shows promise as a potential natural remedy to combat EHP infections and bolster shrimp immunity. While advancements in diagnostics provide early detection, the industry faces ongoing challenges in managing EHP and minimizing economic losses. Understanding EHP's impact and exploring remedies like neem offer avenues for further research and innovation. The quest for sustainable shrimp aquaculture necessitates continuous scientific exploration and collaboration.

References

1. Aldama-Cano, D.J., Sanguanrut, P., Munkongwongsiri, N., Ibarra Gámez, J.C., Itsathitphaisarn, O., Vanichviriyakit, R., Flegel, T.W., Sritunyalucksana, K., Thitamadee, S., 2018. Bioassay for spore polar tube extrusion of shrimp Enterocytozoon hepatopenaei (EHP). Aquaculture 490, 156–161. https://doi.org/10.1016/j.aquaculture.2018.02.039

2. Aranguren Caro, L.F., Alghamdi, F., De Belder, K., Lin, J., Mai, H.N., Millabas, J., Alrehaili, Y., Alazwari, A., Algetham, S., Dhar, A.K., 2021. The effect of salinity on Enterocytozoon hepatopenaei infection in Penaeus vannamei under experimental conditions. BMC Vet. Res. 17, 65. https://doi.org/10.1186/s12917-021-02778-0

3. Chayaburakul, K., Nash, G., Pratanpipat, P., Sriurairatana, S., Withyachumnarnkul, B., 2004. Multiple pathogens found in growth retarded black tiger shrimp Penaeus monodon cultivated in Thailand. Dis. Aquat. Organ. 60, 89–96. https://doi.org/10.3354/dao060089

4. Hashim, N., Abdullah, S., Hassan, L.S., Ghazali, S.R., Jalil, R., 2021. A study of neem leaves: Identification of method and solvent in extraction. Mater. Today Proc. 42, 217–221. https://doi.org/10.1016/j.matpr.2020.11.726

5. Jithendran, K.P., Alavandi, S.V., Vijayan, K.K., 2021a. Economic loss due to diseases in Indian shrimp farming with special reference to Enterocytozoon hepatopenaei (EHP) and white spot syndrome virus (WSSV). Aquaculture 533, 736231. https://doi.org/10.1016/j.aquaculture.2020.736231