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Review

A Review on Pears of Nutrition Vs Shadows of Enterocytozoon Hepatopenaei

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Abstract

Shrimp aquaculture is a rapidly expanding sector that plays a vital role in global seafood production and trade. However, the industry faces significant challenges from emerging pathogens, among which Enterocytozoon hepatopenaei has gained increasing attention. EHP is a microsporidian parasite that infects the hepatopancreas of farmed shrimp, particularly Penaeus vannamei and Penaeus monodon. Unlike other pathogens that cause acute mortality, EHP does not directly kill shrimp but severely impairs growth, leading to stunted populations, increased production costs, and reduced farm profitability. The parasite spreads rapidly through contaminated broodstock, feed, and pond environments, making its control extremely difficult. Conventional disease management strategies, including antibiotics and chemotherapeutics, are ineffective against EHP. Early detection methods such as PCR and histopathology are critical for farm-level surveillance and biosecurity. Preventive strategies focus on strict hatchery screening, biosecure farming practices, and pond management to minimize horizontal transmission. As EHP infections often occur in combination with other opportunistic pathogens, they further exacerbate production losses and threaten the sustainability of shrimp aquaculture. Given its "silent" yet severe economic impacts, urgent attention is required to enhance farmer awareness, strengthen diagnostic capacity, and develop sustainable management strategies. A comprehensive approach involving improved biosecurity, breeding programs for resistant strains, and international cooperation will be essential to mitigate the long-term threat posed by EHP in global shrimp farming.

Keywords: EHP, hepatopancreas, growth retardation, biosecurity.

INTRODUCTION

Shrimp aquaculture, also known as shrimp farming, is a business that cultivates various types of shrimp. Shrimp cultivation is one of the fastest-growing financial exercises in coastal zones of the Asia-Pacific regions, contributing more than 85% of the world's cultivated shrimp, where Bangladesh is the fifth biggest producer on the planet [1]. Marine shrimps and prawns for human consumption, with farmed shrimp now representing more than half of the world's total shrimp supply [2]. *Enterocytozoon hepatopenaei* (EHP) belongs to microsporidia and parasitizes the hepatopancreatic epithelial cells of *L.* vannamei [3,4]. According to the World Wildlife Fund (WWF), 80% of shrimp farming is dominated by two penaeid shrimp species, *Penaeus monodon* (giant tiger prawn) and *Penaeus vannamei* (white leg shrimp) [5]. A newly emerging disease, hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei*, has raised concerns in the shrimp aquaculture industry. This disease is named HPM mainly because the target organ of EHP is the hepatopancreas, in which the microsporidians infect the hepatopancreatic tubule epithelial cells of crustaceans [6]. These days, shrimp is the second most significant food item in Bangladesh. Accordingly, shrimp cultivation became one of the key economic activities in various seaside areas. It is much of the time named as 'blue revolution' [7].

Fig 1: Shrimp farming pond

Oxidative phosphorylation and glycolysis are the main pathways for energy production in eukaryotic organisms. That made them lose the mitochondrial genome and the ability to generate ATP via oxidative phosphorylation [8]. The negative effects of shrimp cultivation have emerged from a lack of common sense and management practices. Objections are normally related to environmental consequences (loss of mangroves, loss of agro-biodiversity, disease outbreak, and pollution), and negative impact on the economic system due to decline in land for crop production [9,10]. The other microsporidian reported from Thailand in penaeid shrimp was a newly described species [11], which was found restricted to tubule epithelial cells of the hepatopancreas. The parasite was isolated from P. monodon in Thailand and was described and named as Enterocytozoon hepatopenaei [12]. It hampers the digestive and absorptive ability of shrimp, leading to growth retardation in P. vannamei and P. monodon [13] and does not cause mortality in the population. Studies conducted at CIBA during the last year also indicated the widespread occurrence of EHP in Indian shrimp farming systems. In India, EHP was first reported [14].

Fig 2: White leg shrimp affected by white feces syndrome

Shrimp Culture in Asian Country History

For a long time, the people of Bangladesh have been connected to the vast water catchment of inland and marine finfish and the cultivation in perennial and seasonal tanks and lakes [15]. By 1940, the government of Bengal had made proposals to improve the shuway of life fisheries, comprising shrimp, and there would not be a local trade in open catch shrimp from coastal estuaries with Myanmar and East Bengal. By 1945, broil and produce were raised by government export availability authorities for sale [16]. The Pakistan Government paid considerable attention to shrimp manufacturing in the course of the 1950s [17]. By the mid-1960s, wild shrimp (22 species) were being sold for the domestic market, both fresh and preserved by boiling and sun-drying [18]. During the 1970s, shrimp aquaculture in Bangladesh began in ghers (ponds) [19].

3. PATHOPHYSIOLOGY

3.1. Causative agent

Enterocytozoon hepatopenaei is a microsporidian parasite belonging to the phylum Microsporidia, family Enterocytozoonidae [12]. Microsporidia are obligate intracellular spore-forming parasites that infect a wide range of invertebrates and vertebrates. EHP is highly specific to hepatopancreatic epithelial cells of penaeid shrimp, including Penaeus vannamei and P. monodon [20]. The parasite exists in the environment as resistant spores, which serve as the infectious stage and facilitate horizontal transmission among shrimp via contaminated water, feed, or broodstock. The spores are small, oval, and contain a polar tubule, which is essential for injecting the infective sporoplasm into host cells [21]. EHP infections result in hepatopancreatic microsporidiosis (HPM), leading to growth retardation without causing acute mortality [21].

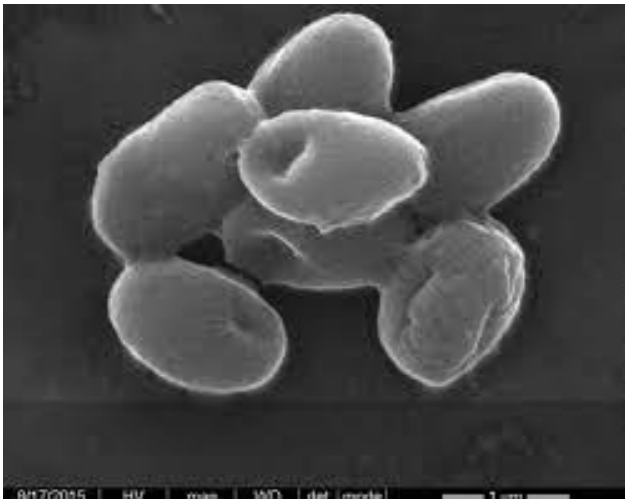

Fig 3: Hepatopancreatic Microsporidiosis Of Shrimp

3.2. Infection and Entry

The infection of penaeid shrimp by Enterocytozoon hepatopenaei (EHP) occurs mainly through the oral ingestion of spores present in contaminated pond water, feces, or via cannibalism of infected shrimp [22]. Once ingested, the spores reach the hepatopancreas, the primary target organ.EHP spores possess a unique infection apparatus called the polar tubule. Upon activation, the polar tubule rapidly extrudes and penetrates the hepatopancreatic epithelial cell membrane, injecting the sporoplasm directly into the host cell cytoplasm [12]. Inside the cell, the sporoplasm develops within parasitophorous vacuoles, undergoes merogony, and produces new spores. These spores eventually cause host cell rupture and are released into the hepatopancreatic and shrimp populations [21].

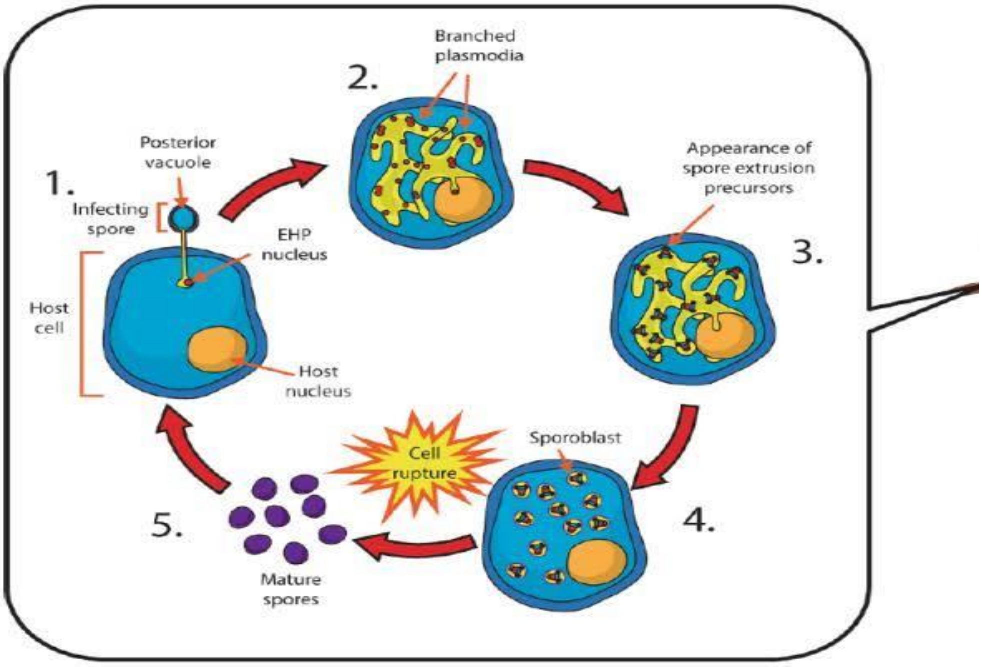

Fig 4: Entry of Spore

3.3. Site of infection

Enterocytozoon hepatopenaei is an intracellular microsporidian parasite that infects the hepatopancreas of penaeid shrimp. Within the hepatopancreas, EHP specifically targets the tubule epithelial cells, especially the embryonic (E-cells), which are essential for tissue renewal and digestive enzyme production [12]. The parasite develops within parasitophorous vacuoles in the cytoplasm of these cells, leading to cellular hypertrophy, dysfunction, and eventual sloughing of epithelial cells into the hepatopancreatic lumen [20]. Importantly, EHP is restricted to the hepatopancreas and has not been reported in other tissues such as muscle and gills [23]. Damage to the hepatopancreatic epithelium results in impaired digestion and nutrient absorption, ultimately leading to growth retardation and size variation in farmed shrimp [23].

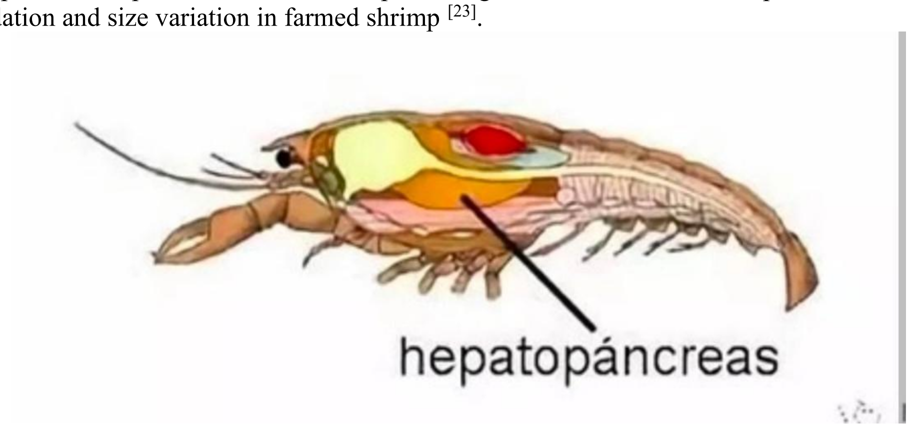

Fig 5: Shrimp with hepatopancreas

3.4. Cellular pathology

Enterocytozoon hepatopenaei specifically infects the tubule epithelial cells of the shrimp hepatopancreas. Once the sporoplasm enters the host cell through the polar tubule, it develops inside a parasitophorous vacuole in the cytoplasm [12]. Within these vacuoles, the parasite undergoes merogony and sporogony, producing large numbers of spores. The infected epithelial cells become hypertrophic and packed with spores, which disrupts their normal functions of digestion and nutrient absorption [20]. Eventually, these cells undergo degeneration and slough off into the hepatopancreatic lumen, releasing spores that continue the infection cycle. This localized cellular pathology leads to structural damage of hepatopancreatic tubules, reduced enzyme secretion, and chronic growth retardation in infected shrimp [23]. Importantly, EHP infection is chronic and non-lethal, but its cumulative damage makes it a major economic threat [23].

3.5. Functional consequences

Infection with Enterocytozoon hepatopenaei disrupts the normal physiology of the shrimp hepatopancreas. The parasite infects and destroys tubule epithelial cells, leading to a loss of their secretory and absorptive functions [12]. As a result, digestive enzyme production is reduced, and nutrient absorption is impaired, causing poor feed utilization and energy diversion toward parasite replication [20]. This leads to growth retardation, size variation among shrimp, and poor feed conversion ratios (FCR), which are hallmark signs of EHP outbreaks [23]. Although EHP does not usually cause acute mortality, the chronic impairment of hepatopancreatic function results in silent but severe production losses. Additionally, infected shrimp may be more vulnerable to secondary infections, compounding the negative effects on aquaculture yields [22].'

Fig 6: Growth comparison of healthy vs infected shrimp

3.6. Clinical Manifestations

Enterocytozoon hepatopenaei (EHP) infection in shrimp is generally chronic and non-lethal, making it a "silent" disease. The most notable clinical signs include growth retardation, size variation among shrimp, and poor feed conversion ratios (FCR) [12,20]. Affected shrimp may appear outwardly healthy, without obvious gross lesions, but hepatopancreatic epithelial cells are heavily infected, causing impaired digestion and nutrient absorption [23]. Secondary infections may occur more frequently due to chronic stress and weakened physiological condition [22].

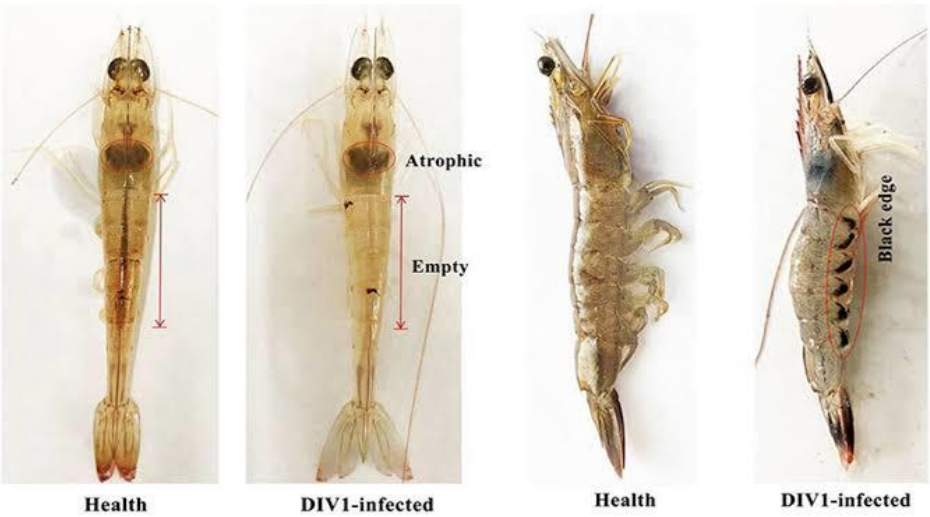

Fig 7: Infected shrimp vs Healthy shrimp

3.7. Silent Economic Threat

Enterocytozoon hepatopenaei (EHP) poses a significant economic threat to shrimp farming due to its chronic, subclinical nature. Infected shrimp usually survive without showing acute mortality, but their growth is severely retarded, and size variation within the stock increases [12]. This impaired growth leads to poor feed conversion ratios (FCR) and reduced harvest yield, resulting in economic losses for farmers [20,23]. The hidden or "silent" progression of the disease makes early detection difficult, allowing EHP to spread between ponds and farms via contaminated water, feed, or broodstock [22].

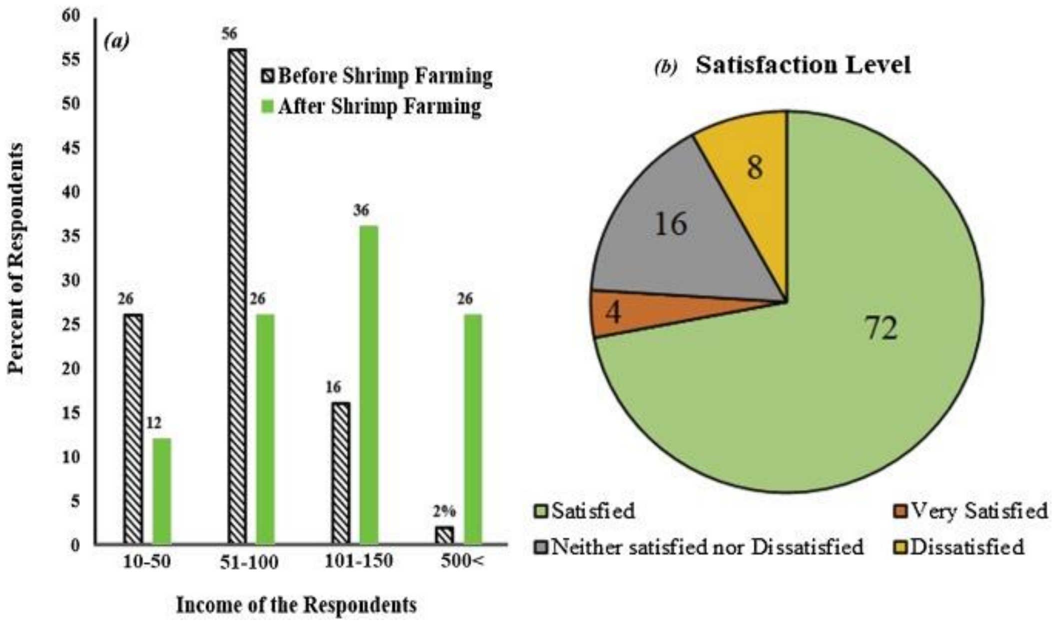

Fig 8: Economic impact on shrimp farms

Table 1: Nutritional Comparison Summary [24,25,26]

Nutrient	Healthy Shrimp	EHP-Affected shrimp
Protein	20.4g	Decrease
Carbohydrates	0.2g	Decrease
Total Fat	0.2g	Decrease
Glycogen	Normal	Decrease
ATP	Normal	Decrease
Glucose	Normal	Decrease
Digestive Enzymes	Normal	Decrease
Immune Response	Normal	Increase
Growth Rate	Normal	Decrease
Shell Hardness	Normal	Decrease

4. DIAGNOSTIC METHODS FOR EHP DETECTION

4.1. Molecular Detection Methods

4.1.1 Polymerase Chain Reaction (PCR)

PCR remains a common technique used in the diagnosis of shrimp diseases, as it is simpler and more cost-effective. The types of PCR methods used in EHP detection are one-step PCR [27], qPCR [28], and nested PCR [29]. In this case, one-step PCR is easier to execute and needs only a set of primers; however, the detection threshold frequently varies between 1000 and 10,000 copies per reaction [30]. In a study conducted on developing a PCR assay for the effective detection of EHP and investigation of EHP prevalence in Shandong Province, China, a pair of primers amplifying 358 base pairs of an EHP DNA fragment was designed, and it was shown to have the ability to detect EHP at a copy number as low as 2×10^1 [27]. This method was reported to be more sensitive and specific compared to previous EHP PCR assays. Meanwhile, nested PCR employs two sets of primers to successively amplify the target, which yields at least 10 times the sensitivity of its one-step equivalent [30]. As a result, this leads to the ability to detect low-level infections. Because existing PCR methods targeting EHP SSU rRNA were found to give false positive test results due to the cross-reactivity [29].

This method of nested PCR was found to successfully distinguish EHP and did not yield false positive results from related microsporidia. A modified method of nested PCR to detect EHP in *Macrobrachium rosenbergi*, a giant freshwater prawn, was carried out in which the primers were redesigned to detect the distinct strain of EHP [31]. Overall, this study's method was proposed to be beneficial for investigating EHP mutants [30,32]. In a study conducted to detect and quantify EHP in infected shrimp *Litopenaeus vannamei*, a SYBR Green I fluorescent qPCR assay was designed based on the polar tube protein 2 (PTP2) gene [28]. The study found that the efficiency of amplification was 102%, and the qPCR technique was determined to be highly sensitive and repeatable. However, the fundamental disadvantage of qPCR [30].

4.1.2. Loop-Mediated Isothermal Amplification (LAMP)

The LAMP assay is quick to detect EHP with a constant reaction temperature and by using a simple dry bath without requiring any technical expertise or costly equipment such as a thermocycler [30,33]. Recently, an improved colorimetric EHP LAMP diagnostic assay was developed with primers specific to the EHP spore wall protein (SWP) gene for the visual detection of EHP [33]. In this study, hydroxy naphthol blue (HNB) or phenol red dye was used to achieve the visual detection of LAMP amplicons without opening the tubes to prevent contamination. Moreover, the EHP LAMP assay showed 95.31% sensitivity, 98.98% specificity, and a kappa value of 0.948 compared to the gold standard, SWP-PCR [34].

4.2. Histological methods

4.2.1 In Situ Hybridisation (ISH)

The DIG-labelled 18SrRNA gene probe is employed in ISH assays to identify EHP, as it allows for the assessment of infection severity because all EHP life stages can be seen, even at low magnification levels [35,36]. In addition, ISH was reported to be more sensitive and precise in detecting causative agents, as cells with no visible evidence of microsporidian spores from histological analysis can be determined to be positive [36], as shown in Figure 9.

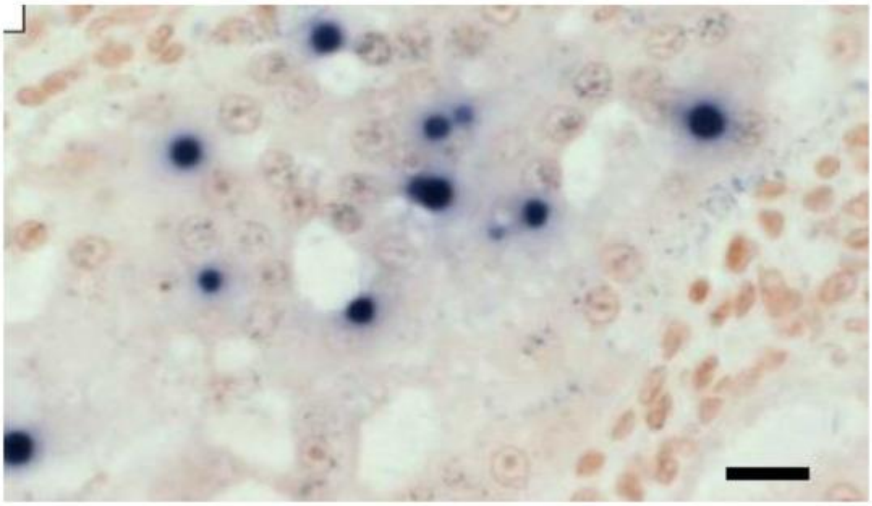

Fig 9: In Situ Hybridisation

In situ hybridisation of HP tissue of *Penaeus vannamei* with a digoxigenin-labelled EHP probe [37]. Scale bar = 25 µm.

4.3. Microscopy

EHP can be diagnosed by using a microscopic method using haematoxylin–eosin (H&E)-stained tissue sections. This method can be carried out by carefully observing spores in hepatopancreatic tissue and faecal samples using microscopic analysis [6]. For light microscopy analysis, the shrimp hepatopancreases need to be fixed in a fixative such as Davidson fixative, processed for histology, and stained with H&E [38]. Histopathological analysis of EHP infections reveals EHP life stages in hepatopancreatic tubule epithelial cells and free spores that have been released into the HP tubule lumens from lysed epithelial cells [30]. This was proven by a study [38] in which the histological analysis showed EHP life stages, mature spores, as shown in Figure 10.

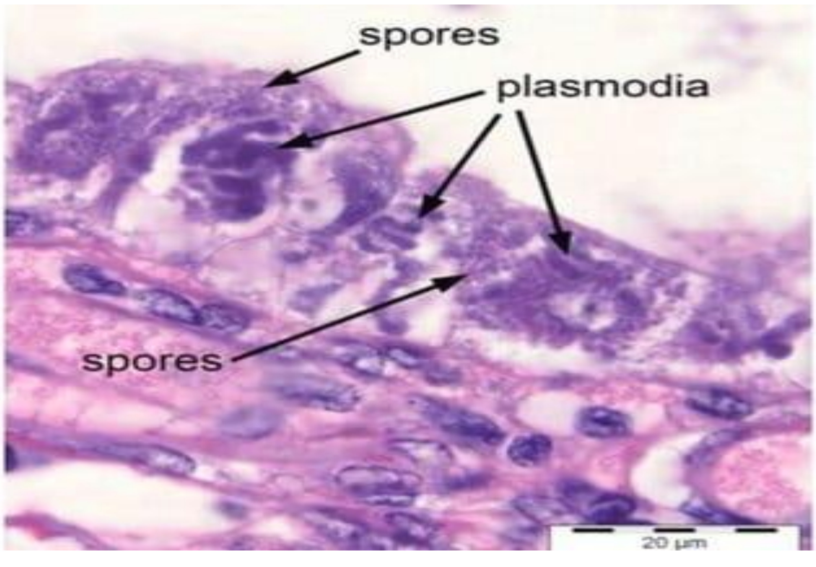

Fig 10: H&E-stained HP tissue showing EHP spores and plasmodia

However, the sensitivity of the H&E approach falls short of the criteria in the EHP preventive plan because spores cannot be distinguished easily from normal host cells in low-level infections [30,35]. Thus, the development of in situ hybridisation (ISH) is needed to confirm the result [30,35].

5. CONCLUSION

In conclusion, EHP has evolved as one of Asia's most significant infections, causing HPM in cultivated white leg shrimp *Penaeus vannamei* [30]. Shrimp diseases have a significant effect on shrimp cultivation, and production sustainability is reliant on the balance between the environment, disease prevention through pathogen diagnostics, and shrimp health [6]. Because there is no treatment available for EHP to date, prevention is the greatest line of defence against EHP. Overall, this study will aid in the understanding of EHP and its diagnosis. As a result, proper guidelines must be followed in shrimp farming systems, and extensive research on the diagnosis of EHP infection is required to ensure and prevent the transmission of EHP to humans [6].

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