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RESEARCH ARTICLE

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Application of the wet mount method as an early prevention of Enterocytozoan hepatopenaei (EHP) disease that infects vanname shrimp (*Litopanaeus vannmaei*)

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Abstract

The cultivation of vannamei shrimp faces persistent challenges, particularly due to various disease outbreaks. One emerging and common disease is Enterocytozoon hepatopenaei (EHP). This disease is caused by the microsporidian parasite, which hinders shrimp growth by infecting the hepatopancreas. This study aims to evaluate the application of the wet mount method as an early prevention strategy for EHP infection in whiteleg shrimp. The research employs a laboratory-based experimental approach using the wet mount method to detect spores/microsporidia in the shrimp hepatopancreas, observed under a microscope at 1.000x magnification. Results from this method revealed a 50% prevalence of spores/microsporidia in shrimp hepatopancreas samples from VIP pond section A1 and 90% prevalence in very very important person (VVIP) pond section A2. Thus, the wet mount method is a viable technique for identifying spores/microsporidia in the hepatopancreas of *Litopenaeus vannamei*, offering an effective early detection approach for EHP infection.

Keywords: Enterocytozoon hepatpenaei, hepatopancreas, *Litopanaeus vannamei*, microsporidia, wet mount

Introduction

Vannamei shrimp (*L. vannamei*) cultivation represents a highly promising sector within the fisheries industry, driven by strong market demand[1–3]. In Indonesia, vannamei shrimp (*L. vannamei*) ranks as the leading aquaculture export, contributing 14.13% to the nation's export volume and 42% to the fisheries trade balance[4,5]. A significant challenge in vannamei shrimp (*L. vannamei*) farming is the frequent occurrence of disease outbreaks, which can lead to major cultivation failures. Shrimp pathogens include viruses, bacteria, and parasites. White spot syndrome virus (WSSV) is caused by the white spot baculovirus complex and EHP. EHP is a microsporidian fungus that causes hepatopancreatic microsporidiosis (HPM) and stunts shrimp growth[6,7]. First discovered in 2004 in the hepatopancreas tissue of cultured black tiger shrimp in Thailand, EHP was later detected in vannamei shrimp (*L.*

vannamei) in Indonesia in 2015. In both India and Indonesia, EHP infection has been associated with white feces syndrome (WFS) in cultured shrimp[8].

Currently, there is limited information on environmental and shrimp health conditions before EHP infections occur in intensive shrimp farms. However, this early information is critical for traditional farmers in planning preventive actions to avoid severe losses[9]. So far, preventive measures for EHP in shrimp are limited to selecting high-quality fry and ensuring good management practices. Although polymerase chain reaction (PCR) testing is frequently used to detect EHP, it remains inadequate for early detection. Therefore, preventive efforts involving early warning and monitoring of EHP presence in the farming environment are essential throughout the cultivation period[10]. One alternative is early-stage wet mount screening of shrimp during early stocking. This allows farmers to make prompt and informed decisions to effectively manage EHP risks.

Material and methods

Research period and location

This study was conducted at a vannamei shrimp (*L. vannamei*) farm laboratory in Probolinggo Regency, East Java, Indonesia, from October to November 2023.

Figure 1. Stages of EHP identification using the wet mount method

Procedure

The experimental approach used was laboratory-based, applying the wet mount method. This technique involves microscopic analysis of spores or microsporidia present in the hepatopancreas of vannamei shrimp at a magnification of 40x. **Figure 1** illustrates the stages of EHP examination using the wet mount method. Sampling was carried out by collecting shrimp from ponds during feed checks (anchoring), as this method is useful for assessing feed consumption during vannamei shrimp (*L. vannamei*) cultivation[11]. A total of 10 shrimp were sampled for weight and length measurements, and six shrimp were then selected for further analysis using the wet mount method[12]. Hepatopancreas samples from these shrimp were examined under microscopes with magnifications of 10x, 40x, and 1.000x to detect the presence or absence of spores or microsporidia causing EHP[13].

Research and discussion

This study assessed shrimp health and environmental conditions by sampling multiple shrimp ponds, producing the following result (**Figure 2**). **Figure 2** demonstrates a significant correlation ($p = 0.00$) between shrimp length and weight, with length explaining 89.2% of the weight variance, and other factors accounting for 10.8%. The regression equation is as follows:

Y = 1,1X – 2,9
Which stands for:
X = length (cm) and Y = weight (g).

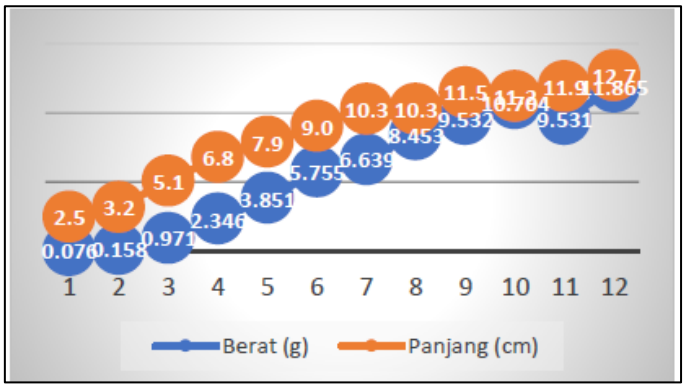

Figure 2. Growth patterns in weight and length of shrimp

According to the regression equation, a shrimp measuring 3 cm in length has an estimated weight of 0.4 g. It reports that shrimp body weight and length are linked to EHP infections, as shrimp infected with EHP exhibit stunted growth despite maintaining normal appetite and feed intake. This results in what is commonly termed *stunted shrimp*[14]. In addition, **Figure 3** reveals that EHP-infected shrimp exhibit variable lengths, indicative of slowed growth. EHP infections were first detected in shrimp at three weeks of age, with an average weight of 0.97 g and length of 5.1 cm. Characteristics of EHP infection include pale, soft carapaces with signs of incomplete molting and reduced movement response[15].

Figure 3. Condition of shrimp detected with WSSV and EHP infection

Figure 4 reveals evidence of *swollen* and shrunken tubules in the hepatopancreas of vannamei shrimp. *Swollen* indicates damage or swelling in the tubules, while *shrunken* refers to tubule damage marked by plasmolysis (cell shrinkage)[16]. The presence of swollen and shrunken tubules correlates with EHP infection, suggesting significant internal organ damage, including in the hepatopancreas. Additionally, lipid droplet analysis in EHP-infected shrimp samples shows an average lipid droplet content of 48% in VIP Pond A1 and 29% in VVIP Pond A2. Lipid droplets, which serve as energy reserves in the hepatopancreas, are crucial for shrimp health, with optimal levels above 30% under microscopic examination (**Figure 5**). This energy reserve is essential for immunity, environmental

adaptation, and growth. Thus, lipid droplet content serves as an indicator of shrimp health during cultivation[8].

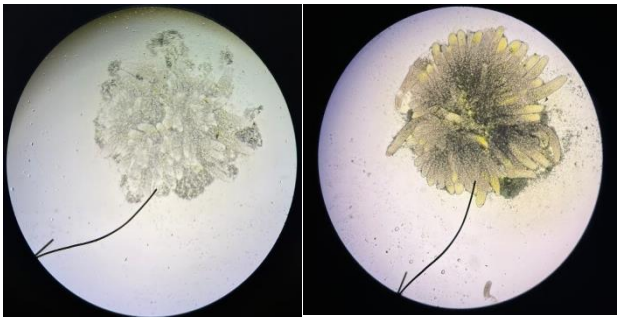

Figure 4. Condition of tubules in the hepatopancreas of vannamei shrimp (*L. vannamei*) identified with EHP

Microscopic observations at 1.000x magnification revealed EHP infection prevalence through the presence of spores/microsporidia in hepatopancreatic tubules. As shown in **Figure 6**, infection rates were 50% in VIP Pond A1 (3 out of 6 shrimp tested positive) and 90% in VVIP Pond A2 (5 out of 6 shrimp tested positive). Microscopic observations at 1.000x magnification revealed EHP infection prevalence through the presence of spores/microsporidia in hepatopancreatic tubules. As shown in Figure 6, infection rates were 50% in VIP Pond A1 (3 out of 6 shrimp tested positive) and 90% in VVIP Pond A2 (5 out of 6 shrimp tested positive). These results indicate that the wet mount method effectively detects EHP infections, offering an alternative to PCR, which may not accurately identify EHP if spores are not fully mature. This limitation of PCR can lead to neglect of EHP signs, as farmers may rely solely on PCR results. Both mature and immature EHP spores pose pathogenic risks, as EHP transmission occurs through spores released in shrimp feces, the infectious stage capable of surviving outside the host[9,10,17–20].

Conclusions

In conclusion, EHP infection cases in intensive vannamei shrimp (*L. vannamei*) ponds can be identified using the wet mount method, enabling early preventive actions against EHP infections.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest

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