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Presumptive Detection of EHP-Like Structures by Hepatopancreatic Wet Mount in Intensive Pacific White Shrimp Culture

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ABSTRACT

The productivity of Pacific white shrimp (*Litopenaeus vannamei*) can be constrained by hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP), a chronic infection associated with growth retardation. This descriptive field case report documented the operational use of unstained hepatopancreatic wet mounts for presumptive microscopic detection of EHP-like structures in two intensive shrimp ponds in Probolinggo Regency, East Java, Indonesia, during October-November 2023. For each pond, 10 shrimp were collected during routine feed-tray inspection for body weight and length assessment, and six individuals were selected for hepatopancreatic wet mount examination at magnifications from 10× to 1,000×. Observations included tubule morphology, lipid droplets, and spore-like structures. The earliest retained observation of EHP-like structures occurred at DOC 19, when the sampled shrimp weighed 0.97 g and measured 5.1 cm. Mean lipid-droplet values were 48.3% in VIP A1 and 29.2% in VVIP A2. EHP-like structures were observed in 3/6 samples (50.0%) from A1 and 5/6 samples (83.3%) from A2. These observations illustrate the potential operational use of wet mounts to select samples requiring confirmatory testing; diagnostic performance remains to be validated against PCR/qPCR or other specific methods.

INTRODUCTION

Aquaculture is a major component of the global aquatic food supply, with production growth accompanied by increasing demands for efficiency, biosecurity, and sustainability (Food and Agriculture Organization of the United Nations [FAO], 2025). Cultured shrimp, particularly Pacific white shrimp (*Litopenaeus vannamei*), is strategically important because of its short production cycle, adaptability to intensive systems, and integration into high-value international trade (Villarreal & Juarez, 2022). In Indonesia, shrimp remains the largest fishery export commodity; in 2025, shrimp exports reached USD 1.87 billion, accounting for 29.8% of total national fishery exports (Kementerian Kelautan dan Perikanan [KKP], 2026). This economic importance makes production consistency, harvest-size uniformity, and disease control critical to maintaining the competitiveness of the national shrimp industry (Virapat, 2023).

Culture intensification increases production per unit area, but at the same time magnifies the biological consequences of health disturbances because high stocking density, intensive host-pathogen interactions, and environmental stress can accelerate the spread of disease agents (Villarreal & Juarez, 2022). Economic losses caused by disease in shrimp farming arise not only from acute mortality but also from reduced growth, increased feed conversion

ratio, prolonged culture periods, size heterogeneity, and lower market value at harvest (Patil *et al.,* 2021). One increasingly important chronic disease is hepatopancreatic microsporidiosis (HPM) caused by EHP, because infection may persist without mass mortality while still causing substantial productivity losses (Chaijarasphong *et al.,* 2021). Recent surveys also indicate that EHP remains relevant in commercial farms and may occur together with other health disorders, making rapid detection an important component of shrimp health management (Kumar *et al.,* 2024).

EHP is an obligate intracellular microsporidian that primarily proliferates in hepatopancreatic epithelial cells, which are central to digestion, absorption, nutrient storage, and metabolism in shrimp (Chaijarasphong *et al.,* 2021). EHP infection can damage hepatopancreatic tubules, disrupt metabolism, increase oxidative stress, and reduce nutrient-use efficiency, collectively contributing to growth retardation (Cao *et al.,* 2023). Controlled infection experiments have shown that infected shrimp may survive but exhibit reduced growth and feed-use efficiency compared with controls, emphasizing EHP as a chronic production disease rather than solely an acutely lethal disease (Kumar *et al.,* 2022). Microbiome studies have also linked EHP infection intensity with changes in hepatopancreatic and intestinal microbial communities that may aggravate host physiological disturbances (López-Carvallo *et al.,* 2022). Taxonomically, *Enterocytozoon hepatopenaei* has been proposed for reclassification as *Ecytonucleospora hepatopenaei* based on its morphology, life cycle, pathology, and molecular characteristics; the term EHP is retained here because it remains widely used in diagnostic and industry literature (Wang *et al.,* 2023).

In Indonesia, EHP has been reported in major shrimp-farming areas and associated with growth problems and pond environmental conditions (Nkuba *et al.,* 2021). Surveillance data from Bali showed high EHP prevalence in shrimp samples from several regencies examined by PCR, indicating that the agent is a genuine health problem in national farming systems (Aras *et al.,* 2022). In super-intensive ponds in East Java, ammonia and nitrite concentrations were correlated with EHP prevalence, reinforcing the need to integrate shrimp health assessment with environmental monitoring (Nkuba *et al.,* 2021). EHP can also be detected in environmental components or organisms that may act as mechanical carriers, including polychaetes and pond macrofauna; therefore, control should not focus only on shrimp showing clinical signs (Desrina *et al.,* 2020). Findings from Malaysia further support the presence of EHP in several pond macrofaunal organisms, although their epidemiological roles should be distinguished between true infection and mechanical carriage (Wan Sajiri *et al.,* 2023). Salinity can also influence EHP infectivity, indicating that environmental factors may modulate transmission risk (Aranguren Caro *et al.,* 2021).

A major challenge in EHP management is the absence of a single specific macroscopic clinical sign, because slow growth, size variation, a pale hepatopancreas, or white feces may also occur under other conditions and therefore must be interpreted cautiously (Govindasamy *et al.,* 2024). An association between EHP and white feces syndrome (WFS) has been reported in Indonesia and Venezuela, but WFS is regarded as a multifactorial syndrome; consequently, the presence of white feces cannot be used as sole evidence of EHP infection (Aranguren Caro *et al.,* 2020). Reviews of WFS have also emphasized the need to distinguish clinical indicators, microbiota changes, and specific diagnostic evidence to avoid overdiagnosis (Piamsomboon & Han, 2022). Therefore, an ideal EHP detection system should combine routine farm observations with examinations that improve diagnostic specificity (Chaijarasphong *et al.,* 2021).

Molecular methods such as PCR and qPCR are important diagnostic approaches because they detect EHP DNA targets and can provide higher sensitivity and specificity than unstained morphological examination (Chen *et al.,* 2022). qPCR combined with microscopic staining has been used to detect and quantify EHP, indicating that molecular and microscopic examinations should be regarded as complementary approaches (Wang *et al.,* 2020). Isothermal recombinase polymerase amplification (RPA) has also been developed to accelerate molecular detection of EHP and reduce dependence on conventional thermal cycling (Ma *et al.,* 2021). Previous farm-

level microscopy work demonstrated that fresh wet mounts can reveal EHP spores, although identification was improved by concentration and staining procedures and was evaluated together with other diagnostic approaches (Praveena *et al.,* 2018). Calcofluor white staining can further improve visualization of the EHP spore wall compared with unstained preparations (Zhao *et al.,* 2020). Thus, routine unstained wet mounts remain attractive for farm laboratories because they are rapid and simple, but their very small target structures and resemblance to debris limit diagnostic certainty when staining or molecular confirmation is unavailable (Raj *et al.,* 2023).

Published EHP diagnostic studies have predominantly emphasized molecular assays, histopathology, or staining-enhanced microscopy, whereas evidence is more limited on how routine unstained hepatopancreatic wet mounts should be interpreted operationally when they are used only for presumptive triage at farm level (Praveena *et al.,* 2018; Wang *et al.,* 2020; Zhao *et al.,* 2020). The present study does not claim to establish a standardized diagnostic protocol or to measure sensitivity and specificity. Instead, it is explicitly framed as a descriptive field case report documenting how morphometric observations, hepatopancreatic tubule condition, lipid droplets, and EHP-like structures were considered together in two intensive ponds to identify samples that warranted confirmatory testing. Accordingly, the objective was to describe the operational use and limitations of unstained wet mount examination for presumptive detection of EHP-like structures under routine farm-laboratory conditions, while clearly separating microscopy-based observations from confirmatory diagnosis.

METHOD

Study Design, Time, and Location

This work was a descriptive field case study based on routine operational health-monitoring observations from two intensive Pacific white shrimp production ponds, coded VIP A1 and VVIP A2, during October-November 2023 in Probolinggo Regency, East Java, Indonesia. It was not designed as a diagnostic-accuracy trial or as a prospectively standardized longitudinal experiment. The same two pond codes were the source of the observations reported during the study period, but individual shrimp were not repeatedly tracked. Sampling was conducted during routine farm monitoring rather than according to a pre-specified fixed research schedule. The retained dataset identifies the earliest EHP-like microscopic finding at DOC 19; however, exact calendar dates and DOC values for every sequential morphometric observation, as well as complete pond descriptors such as pond area, stocking density, initial biomass, and a full water-quality series, were not preserved in the available records and therefore were not reconstructed.

Observation Units and Sampling

Shrimp samples originated from the two ponds coded VIP A1 and VVIP A2. During a sampling event, shrimp were collected during routine feed-tray (anco) inspection, which is normally used to evaluate uneaten feed. For each pond, 10 shrimp were collected for body weight and length assessment, and six individuals from those samples were selected for hepatopancreatic wet mount examination. Because sampling followed routine operational monitoring rather than probability-based population sampling, the observed positive proportions describe only the examined shrimp and were not used to estimate pond-population prevalence.

Morphometric Monitoring

Body weight was recorded in grams and body length in centimeters during routine monitoring. The retained monitoring figure contained 12 sequential paired summary observations of weight and length, which are presented descriptively in Figure 2 to provide growth context. Individual-level replicate measurements, exact observation dates, and the sampling structure required for formal inferential analysis were not retained in the available dataset. Therefore, the previously reported linear regression equation, coefficient of determination, p-value interpretation, and causal wording were removed from the revised

manuscript. No alternative allometric length-weight model was fitted because the available summary records were insufficient for a defensible individual-level analysis.

Hepatopancreas Wet Mount Preparation and Examination

Shrimp selected for wet mount examination were documented, after which the hepatopancreas was separated from the body and placed on the examination medium. The fresh preparation was then examined under a microscope at magnifications of 10×, 40×, and up to 1,000× to evaluate hepatopancreatic tubule structure, lipid droplets, and small structures that morphologically resembled EHP spores/microsporidia. Microscopic identification was treated as presumptive because definitive etiological confirmation of EHP requires confirmatory methods such as specific staining, histology, *in situ hybridization*, PCR, or qPCR (Zhao *et al.,* 2020).

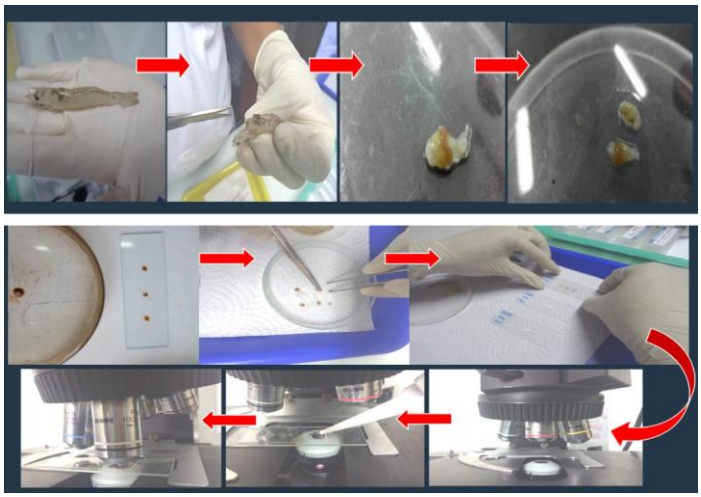


Figure 1. Sample preparation and hepatopancreas examination steps using the wet mount method.

Observation Parameters and Data Analysis

The observed parameters included body weight, body length, hepatopancreatic tubule morphology, including swelling (swollen) and shrinkage (shrunken), the percentage of lipid droplets, and the presence of structures interpreted as EHP-like spores/microsporidia. A wet mount sample was categorized as presumptively positive when structures morphologically consistent with EHP spores were observed in the hepatopancreas. Lipid-droplet percentages were summarized as the mean of six examined samples per pond, whereas wet mount findings were presented as the number and proportion of presumptively positive samples among the six examined shrimp in each pond. The 12 sequential length-weight records shown in Figure 2 were treated descriptively only; no inferential regression or diagnostic-accuracy statistics were calculated.

RESULTS AND DISCUSSION

Shrimp Length-Weight Pattern

The sequential monitoring records showed a general increase in body length and body weight over the observation sequence (Figure 2). The earliest displayed summary value was 2.5 cm and 0.070 g, whereas the final displayed value was 12.7 cm and 11.865 g. These records provide descriptive context for growth progression but should not be interpreted as individual longitudinal trajectories or as a basis for inferential regression because individual-level replicates and exact sampling intervals were not retaine.

Length-weight observations are useful for describing growth status, but they cannot by themselves determine the etiology of growth impairment. EHP is biologically relevant because chronic infection can reduce digestive performance, absorption, and nutrient utilization without necessarily causing high mortality (Kumar *et al.,* 2022). Metabolic studies have shown that EHP infection alters multiple metabolic processes and increases oxidative stress in *Penaeus*

vannamei, providing a plausible mechanism for impaired growth performance (Cao *et al.,* 2023). Changes in hepatopancreatic microbial communities in infected shrimp also indicate interactions among the parasite, target organ, and microbiome (López-Carvallo *et al.,* 2022). A size-based study further reported an association between higher EHP burden and smaller shrimp, although causal interpretation requires longitudinal or experimental designs that were not available in the present field case (Duan *et al.,* 2021).

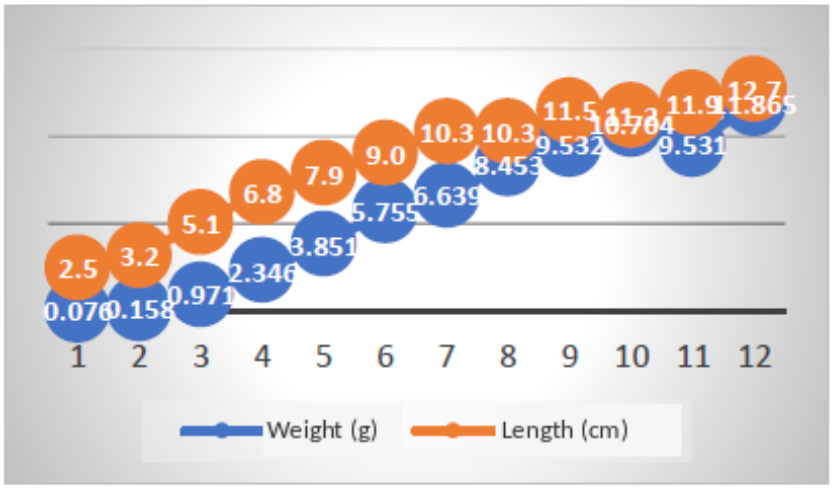


Figure 2. Sequential summary records of shrimp body weight and length available from routine farm monitoring.

In the available field record, EHP-like microscopic structures were first documented in the third week, at DOC 19, when the sampled shrimp weighed 0.97 g and measured 5.1 cm in length. This represents the earliest retained observation in this case report, not the biological onset of infection. A subclinical phase may have preceded microscopic recognition, and molecular methods can detect target DNA at burdens that are not readily visible in unstained wet preparations, depending on sample quality, extraction efficiency, primer/probe targets, inhibitors, and assay detection limits (Chen *et al.,* 2022).

Shrimp Morphology and Hepatopancreatic Tubule Condition

At the time EHP-consistent indications were observed, shrimp showed size variation, pale and relatively soft carapaces, and slow movement responses (Figure 3). These signs may accompany health or nutritional disturbances, but they are nonspecific and should not be used as a stand-alone diagnosis of EHP. Growth retardation and size heterogeneity are consistent consequences of EHP infection, whereas other clinical signs are influenced by host condition, environmental factors, and possible co-infections (Chaijarasphong *et al.,* 2021). Surveillance reports from Bali also documented pale and soft hepatopancreases in some samples that were subsequently tested by PCR, indicating that macroscopic observations can help select individuals for further examination but still require specific diagnostic evidence (Aras *et al.,* 2022).



Figure 3. Variation in shrimp size and morphology when EHP-consistent indications were observed.

The hepatopancreatic preparations showed swollen and shrunken tubules (Figure 4). These changes are biologically relevant because EHP proliferates in the hepatopancreatic epithelium and can cause tissue damage, impaired digestive function, and altered metabolism in this organ (Chaijarasphong *et al.,* 2021). Controlled infection experiments have reported hepatopancreatic tubule lesions and reduced digestive-enzyme activity in EHP-infected shrimp compared with controls (Kumar *et al.,* 2022). Recent research also indicates that the severity of EHP infection is associated with altered microbiota function and host physiological processes, suggesting that hepatopancreatic impairment should be viewed as part of broader systemic dysfunction rather than merely a change in tubule morphology (Lu *et al.,* 2025).

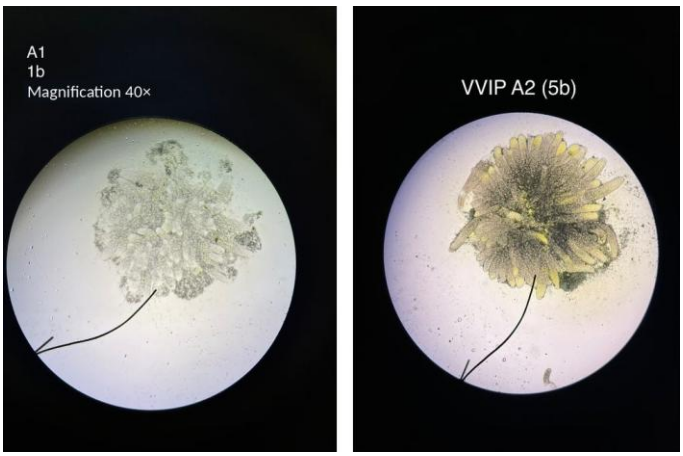


Figure 4. Hepatopancreatic tubules in a wet mount preparation showing swollen and shrunken morphology.

Nevertheless, swelling, shrinkage, vacuolization, or changes in tubule transparency are not pathognomonic lesions of EHP. Nutritional factors, molting, water quality, toxicity, other infections, and post-sampling conditions may produce overlapping morphological changes. Therefore, the diagnostic value of tubule abnormalities lies primarily in their ability to identify an abnormal hepatopancreas as a signal for further examination, rather than to independently establish EHP etiology (Govindasamy *et al.,* 2024).

Lipid Droplets as an Indicator of Hepatopancreatic Energy Status

The observations showed lipid-droplet values of 65%, 80%, 30%, 15%, 70%, and 30% in VIP A1 and 40%, 30%, 30%, 20%, 35%, and 20% in VVIP A2 (Figure 5). The arithmetic mean for the two ponds were approximately 48.3% and 29.2%, respectively. The between-pond difference indicated variation in hepatopancreatic lipid reserves, but it could not be directly attributed to EHP without a comparison group, longitudinal measurements, and control of feeding-related factors and molting stage.

The interpretation of lipid droplets is biologically supported by proteomic studies showing reduced lipid metabolism and increased use of energy reserves in the hepatopancreas of shrimp with both light and heavy EHP infections (Wu *et al.,* 2022). Subsequent research has shown that EHP can cause lipid-droplet depletion through an imbalance between lipid breakdown and synthesis, suggesting that reduced lipid accumulation may form part of the pathogenetic mechanism of EHP (Yuanlae *et al.,* 2025). Metabolic and oxidative-stress findings in infected shrimp further support the presence of altered energy homeostasis in the hepatopancreas during infection (Cao *et al.,* 2023). However, the percentage of lipid droplets should be treated as a supportive indicator rather than a universal diagnostic threshold because its interpretation may be influenced by nutritional status, molting stage, environmental conditions, and scoring procedures.

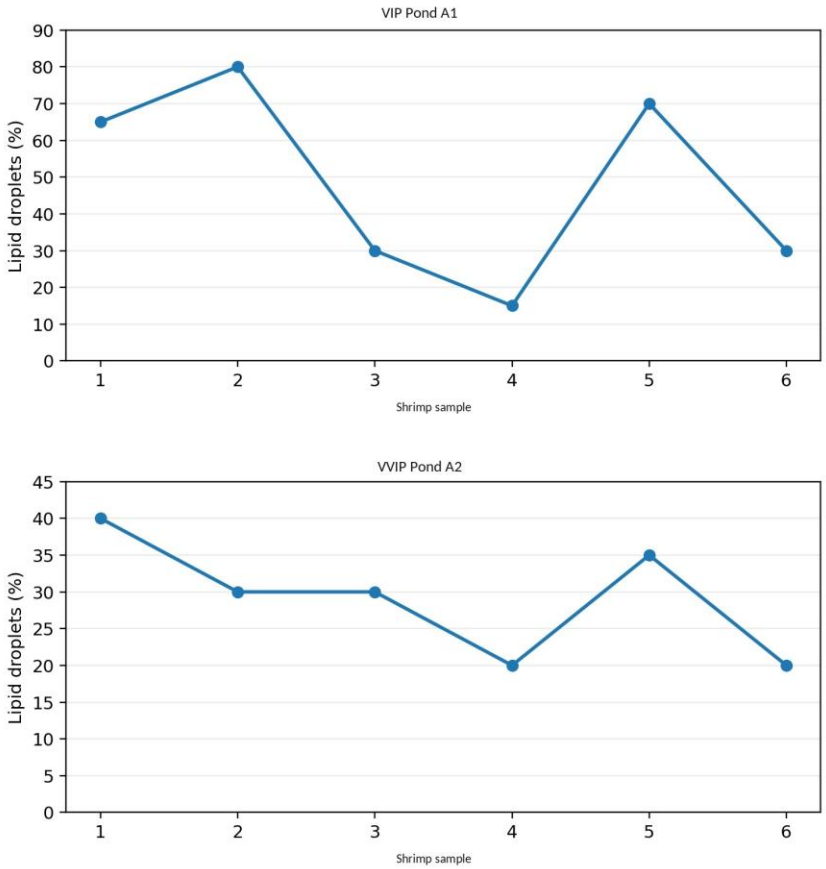


Figure 5. Percentage of lipid droplets in six shrimp samples from VIP A1 and VVIP A2.

Presumptive Identification of EHP Spores by Wet Mount

At magnifications of up to 1,000×, microscopic structures morphologically interpreted as EHP spores/microsporidia were observed (Figure 6). In VIP A1, 3 of 6 samples contained these structures, resulting in a presumptive-positive proportion of 50.0%. In VVIP A2, 5 of 6 samples showed similar structures, resulting in a presumptive-positive proportion of 83.3%. These proportions describe only the examined samples and were not intended as estimates of population prevalence for the entire ponds.

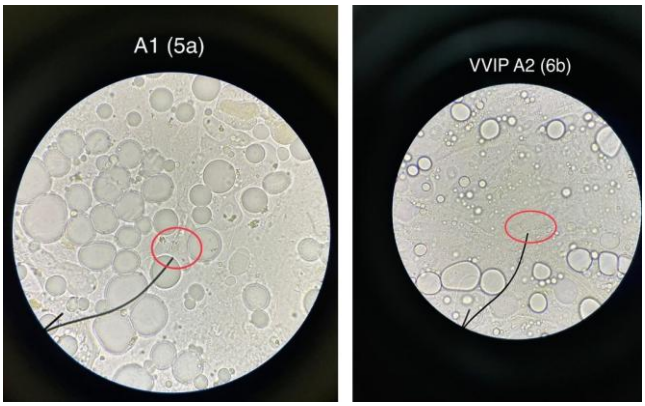


Figure 6. Microscopic structures in the hepatopancreas presumptively interpreted as EHP spores/microsporidia; red circles indicate the observed areas.

Table 1. Summary of the main examination results from the two shrimp ponds.

Parameter	VIP A1	VVIP A2	Interpretation
Mean lipid droplets	48.3%	29.2%	Mean of six samples per pond

Parameter	VIP A1	VVIP A2	Interpretation
Presumptive-positive wet mount	3/6	5/6	Structures morphologically resembling EHP spores; confirmatory diagnosis not performed
Presumptive-positive proportion	50.0%	83.3%	Observed sample proportion only; not a pond-population prevalence estimate
Mean lipid droplets	48.3%	29.2%	Mean of six samples per pond

From a practical perspective, wet mount examination is valuable because it provides immediate information after sampling without DNA extraction or amplification equipment. However, the small size of EHP spores means that morphological detection depends on spore burden, preparation quality, observer skill, illumination, magnification, and the ability to distinguish spores from debris or other cellular structures. *Calcofluor white* staining increases spore-wall contrast and can improve visualization compared with unstained observation (Zhao *et al.,* 2020). Studies integrating staining and qPCR show that the approaches can be complementary, although molecular quantification provides information unavailable from conventional wet mount examination (Wang *et al.,* 2020).

PCR and qPCR detect target DNA sequences; therefore, successful detection does not depend on an observer's ability to recognize mature spore morphology, but rather on the presence of target DNA in the sample, extraction quality, primers/probes, inhibitors, and the assay detection limit (Chen *et al.,* 2022). Various molecular assays have been developed for EHP, including TaqMan-based qPCR and real-time RPA, to improve diagnostic sensitivity, quantification, or speed (Chen *et al.,* 2022; Ma *et al.,* 2021). EHP characterization studies have also shown that wet mount examination can reveal spores, but pathogen identification and localization are strengthened when combined with histopathology, molecular analysis, and ultrastructural methods (Raj *et al.,* 2023). Therefore, wet mount examination is more appropriately positioned as a presumptive screening method than as a substitute for molecular testing.

Operational Implications of Wet Mount Examination at Farm Level

The observations illustrate the potential operational use of wet mounts for selecting samples that require confirmatory testing; diagnostic performance remains to be validated. In practice, farm personnel may use growth anomalies, size heterogeneity, hepatopancreatic condition, lipid-droplet patterns, and EHP-like microscopic structures as reasons to prioritize samples for PCR/qPCR or another specific confirmatory method. This use should be regarded as triage rather than a validated first-tier diagnostic algorithm because the present study did not include paired PCR/qPCR results and therefore cannot estimate sensitivity, specificity, false-positive rates, or false-negative rates (Govindasamy *et al.,* 2024).

Early screening provides meaningful benefits only when linked to biosecurity measures. EHP can be detected in fecal material, the pond environment, and carrier organisms; therefore, post-larval source control, equipment sanitation, pond-bottom management, carrier control, and interruption of contamination pathways are more direct preventive measures than wet mount examination itself (Desrina *et al.,* 2020). Studies of pond macrofauna emphasize the need to evaluate potential reservoirs or carriers, particularly in ponds with recurrent infection (Wan Sajiri *et al.,* 2023). Environmental factors such as salinity can alter infectivity, while poor water quality may increase host susceptibility; diagnostic findings should therefore be interpreted together with environmental and culture-management data (Aranguren Caro *et al.,* 2021). Evidence from East Java linking ammonia and nitrite with EHP prevalence further supports inclusion of water-quality parameters in farm early-warning systems (Nkuba *et al.,* 2021).

Study Limitations

This study had several limitations. It was a descriptive field case report rather than a prospectively standardized diagnostic-accuracy study. Only six shrimp per pond were

examined by wet mount, samples were obtained during feed-tray monitoring, and no probability-based sampling frame was used; therefore, the observed positive proportions cannot be generalized as population prevalence estimates. Exact calendar dates and DOC values for every sequential morphometric observation, complete pond characteristics, and individual-level raw morphometric replicates were not retained in the available dataset. A clearly defined negative-control group was absent, and PCR/qPCR or histopathological confirmation was not performed on the same individuals, so the observed structures remain presumptive. Complete data on water quality, EHP burden, post-larval history, feed, stocking density, molting stage, and possible co-infections were also unavailable, preventing causal attribution of growth or hepatopancreatic changes to EHP.

These limitations do not remove the descriptive value of the field observations, but they restrict the certainty of the conclusions. Future validation should use a prospective comparative diagnostic design with qPCR as a reference method, pre-specified sampling dates and DOC intervals, larger sample sizes, complete pond metadata, repeated early-stage sampling, blinded readings by multiple observers, standardized numbers of microscopic fields, scale-calibrated photographic documentation, and paired results from the same individuals. Such a design would allow calculation of sensitivity, specificity, predictive values, and inter-observer agreement and would determine whether an unstained wet mount protocol can be standardized for farm-level triage.

CONCLUSION

In this descriptive field case, hepatopancreatic wet mount examination revealed tubule-morphology changes, variation in lipid droplets, and structures presumptively interpreted as EHP-like spores/microsporidia in shrimp from two intensive ponds. EHP-like structures were observed in 3 of 6 examined samples (50.0%) from VIP A1 and 5 of 6 samples (83.3%) from VVIP A2; these proportions apply only to the examined shrimp and are not estimates of pond prevalence. The observations illustrate the potential operational use of wet mounts for selecting samples that require confirmatory testing, while diagnostic performance remains to be validated. Consequently, wet mount findings should not be interpreted as definitive EHP diagnosis or as evidence that the method can replace PCR/qPCR. Future studies should compare wet mount and qPCR results in the same individuals using pre-specified sampling schedules, larger samples, complete pond metadata, and standardized microscopic protocols

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