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Investigation of parasites in the digestive tract of white leg shrimp (*Litopenaeus vannamei*) cultured at coastal farms in the Mekong Delta

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ABSTRACT

A total of 291 white leg shrimp samples were collected from 70 cultured ponds in Soc Trang, Bac Lieu and Ca Mau provinces in the Mekong Delta and subjected to endoparasitic detection in the digestive tract. Collected shrimps displayed unhealthy behaviors such as stop or less feeding and lethagic swimming. Pathological signs in the gastrointestinal tract include (1) empty midgut and stomach together with pale and atrophy hepatopancreas; (2) empty, little or discontinued food in the midgut; (3) slow growth and variation in sizes; and (4) white feces. The results from fresh and Giemsa stained smears methods revealed that 96.5% of sampled shrimps were infected by gregarine parasite at different developmental stages. A prevalence infection of 24.7% was recorded with Vermiform present in the hepatopancreas by fresh smear and histology. Histopathological analysis noted that 7.9% of collected shrimp samples had Enterocytozoon hepatopenaei (EHP) spores in hepatopancreas and midgut and confirmed by PCR analysis.

1. INTRODUCTION

Aquaculture has contributed to helping people solve many difficulties and increase income for their life. In addition to the achieved results, shrimp farming in the Mekong Delta of Vietnam still faces with many difficulties such as water pollution, high stocking densities, erratic weather conditions and use of antibiotics that create favorable conditions for pathogens to develop. Recently, *Enterocytozoon hepatopenaei*, gregarine and vermiform are reported to be found in shrimp.

Enterocytozoon hepatopenaei (EHP) in cultured *L. vannamei* has been reported to cause severe growth retardation and as one of the pathogenic agents of associated with white faeces disease in cultured shrimp *(*Sriurairatana *et al*., 2014).

Gegarines is a protozoan parasite, usually parasitise in the hepatopancreas and the midgut of shrimp (Chakraborti & Bandyopadhyay, 2010). Infected shrimps do not show no typical pathological sign but often grow slowly as the parasite take nutrients from shrimp to grow.

Vermiform is a Gregarine-like organism that appears in shrimp hepatopancreas tubes and midgut and usually have the length and diameter proportional to the tubule lumens of hepatopancreas (Sriurairatana et al., 2014). Infected shrimps do not have specific pathological signs, however, vermiform infection with high intensity in shrimp may cause shrimp to reduce feeding and growth (Sriurairatana et al., 2014).

Endoparasites in digestive tract of shrimp are also a major concern for local farmers in the Mekong

Delta. However, published information about these agents from shrimp farms in the Delta is lacking. Thus, this study was undertaken to investigate the present of endoparasites in digestive tract of shrimp samples collected from farms in the Mekong Delta, providing additional information for health management in shrimp farming in the studied locations.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 291 shrimp samples from 70 intensive shrimp farms in Soc Trang, Bac Lieu and Ca Mau provinces of the Mekong Delta were randomly collected from March to December, 2019. Samples were transported alive in oxygenated foam container to the laboratory at College of Aquaculture and Fisheries, Can Tho University.

At each sampling location, agreement with farmers was made so that they would call for sampling when signs of diseases (such as slow growth, variation in sizes, lethargic swimming, empty gut and stomach, pale or atrophy of hepatopancreas and white feces) were detected. Alive shrimp samples (5 to 10 shrimps per pond per sampling time), after being noted with pathological signs, were kept on oxygenated nylon bag and transported to the laboratory of aquatic pathology at Can Tho university for further analysis.

2.2. Parasitological examination

The endoparasites present on the hepatopancreas and midgut were detected by fresh tissue scrapings with a drop of sterile physiological saline water (0.85% NaCl), covered with a clean cover slip (wet mount preparation) and examined by light microscope. For stained smears, tissue scrapings from hepatopancreas and midgut were taken on clean slides, smeared, air dried and fixed in acetone free methanol solution and stained with Giemsa (Chakraborti & Bandyopadhyay, 2010) or hematoxylin and eosin (H&E) (Lightner, 1996). The slides were examined under light microscope to observe the gregarine (Chakraborti & Bandyopadhyay, 2010) and Vermiform (Sriurairatana et al., 2014).

2.3. Histopathological technique

Shrimp samples were injected with Davidson's AFA (alcohol-formalin-acetic acid) fixative for 48 hours then transferred to 70% ethyl alcohol, then processed and stained with hematoxylin and eosin (H&E) using routine histological methods described by Lightner (1996). Histological sections of hepatopancreas (HP) and mid gut were examined by light microscopy for gregarine (Chakraborti & Bandyopadhyay, 2010) and Vermiform (Sriurairatana *et al*., 2014) and EHP (Tourtip et al., 2009).

2.4. Detection of EHP by PCR

Total DNA from collected samples was extracted by using Dneasy Blood and Tissue Kit (Qiagen) following the instruction from manufacturer. PCR procedure to detect EHP was performed according to the procedure of Tang *et al.* (2015). Ten µL PCR product was run on 1% agarose gel (Promega, USA) in 1x TAE (10 mM Tris, 5 mM acetate, 0.1 mM EDTA). Electrophoresis products were visualized under UV illumination. The 100 bp DNA DNA ladder (Promega) was used to determine the size of DNA fragments. Amplification product of EHP is 510 bp.

2.5. Data analysis

The prevalence of infection (number of sample infected with parasite species/ number of samples examined) by each group of parasites was determined by using Excel software.

3. RESULTS AND DISCUSSIONS

3.1. External pathological signs of sampled shrimp

Collected shrimps showed common behavior of an unhealthy shrimp such as stop feeding (or less feeding) and lethagic swimming. Pathological signs in the gastrointestinal tract include (1) empty midgut and stomach along with pale and atrophied hepatopancreas (Figure 1); (2) empty midgut or there was a little or discontinued food in the midgut (Figure 2); (3) slow growth and variation in sizes; and (4) white feces (Figure 3). The number of samples per clinical signs in studied locations is presented in Table 1.

Collected shrimps, which were noted as slow growth, did not show specific pathological signs but growth retardation and variation in sizes about 2-3 months after stocking.

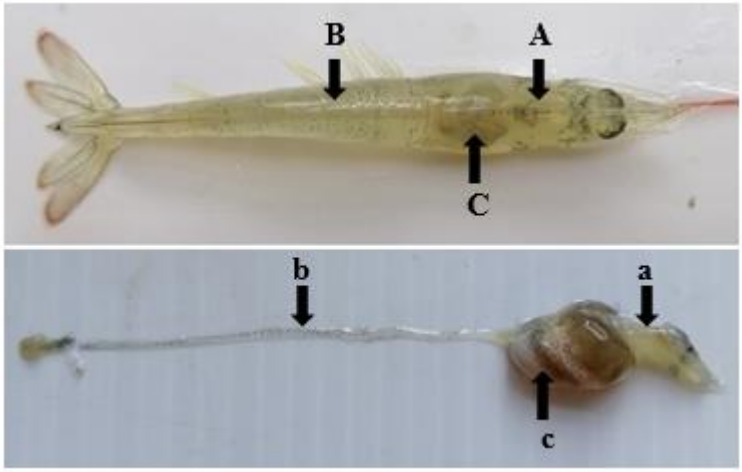

Figure 1. Pathological signs of collected shrimp showing empty stomach (A/a), empty midgut (B/b) and significant atrophied and pale hepatopancreas (C/c)

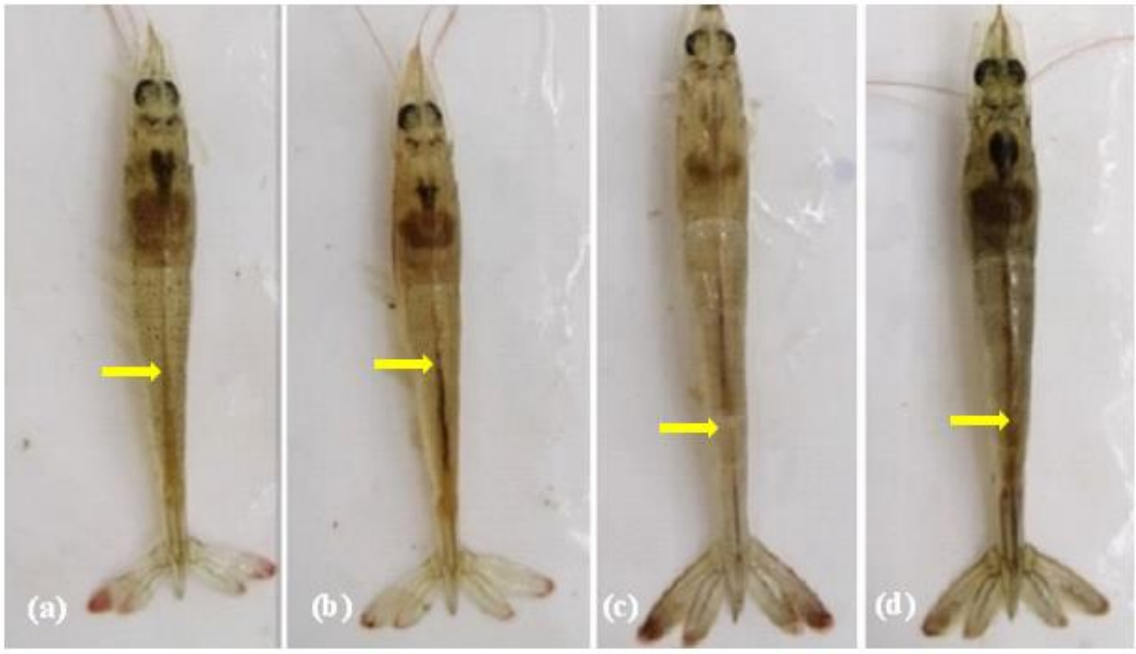

Figure 2. Pathological signs of collected shrimp showing empty midgut (a), discontinued food in the midgut (b and c) and little food in the midgut (d) (arrow)

Shrimp with white feces symptoms (Figure 3) showed opaque white in the midgut (Figure 3a). Shrimps fed less or stop eating and the midgut has an incomplete, intermittent (broken) or empty with pale yellow or milky spots, especially at the gut connecting the shrimp's stomach and hepatopancreas (Figure 3b). The hepatopancreas of diseased shrimp is soft, pale yellow or milky white. Shrimp release opaque white (or yellowish white) feces floating on the water surface especially at the corner of the pond where ending of the wind and were often seen in the feeding trays (Figure 3c).

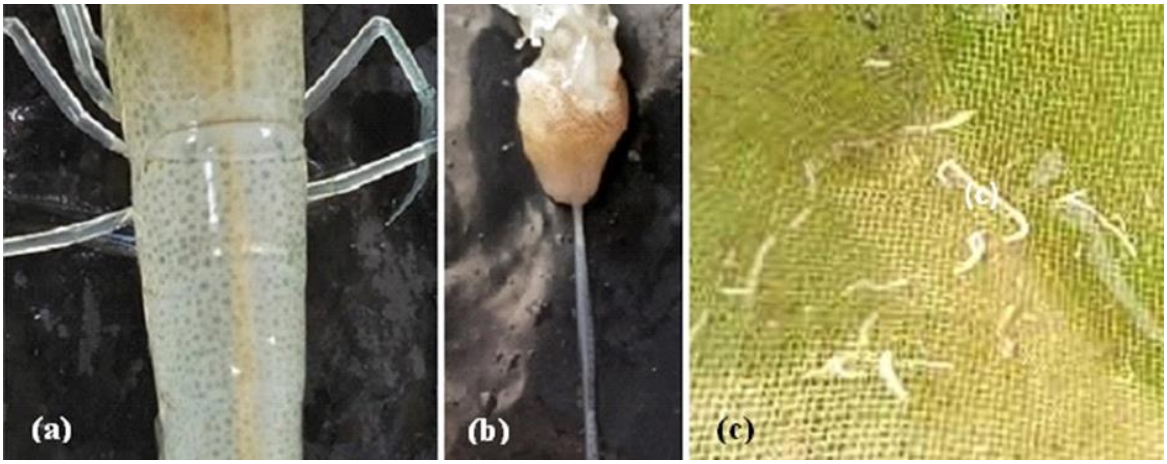

Figure 3. Pathological signs of collected shrimp with white feces disease

(a) The midgut has light yellowish colour; (b) hepatopancreas and midgut of white feces diseased shrimp; (c) white feces segments in the feeding tray.

Table 1. Number of samples per clinical signs in studied locations

Province	District	Empty midgut or a little or discontinued food in the midgut	Empty midgut and stomach along with pale and atrophied hepatopancreas	Slow growth and variation in sizes	White feces
Soc Trang	Long Phu	15	18	12	7
	Tran De	17	19	16	6
Bac Lieu	Bac Lieu	18	13	14	7
	Hoa Binh	17	12	12	8
Ca Mau	Dam Doi	15	15	15	9
	Cai Nuoc	19	15	18	6
	Phu Tan	18	19	16	5
Total		119	111	103	48

3.2. Parasitological and histopathological examinations

Gregarines

The parasitological examination of midgut and hepatopancreas by fresh smears (Figure 4A) and Giemsa stained smear (Figure 4B) methods revealed that 96.5% of sampled shrimp were infected by gregarine parasite at different developmental stages (Figure 4B) and intensities.

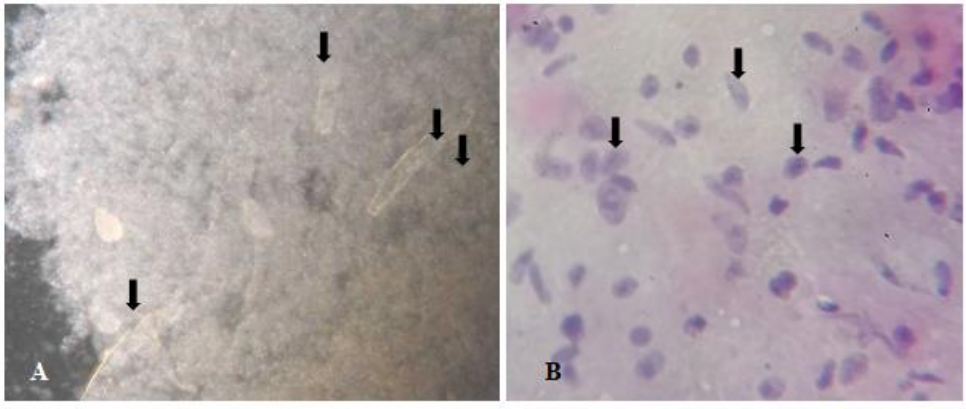

Figure 4. (A) Fresh smear showing Gregarine in the midgut (arrow); (B) giemsa stained smear showing Gregarine in the hepatopanceas (arrow) (20X, H&E)

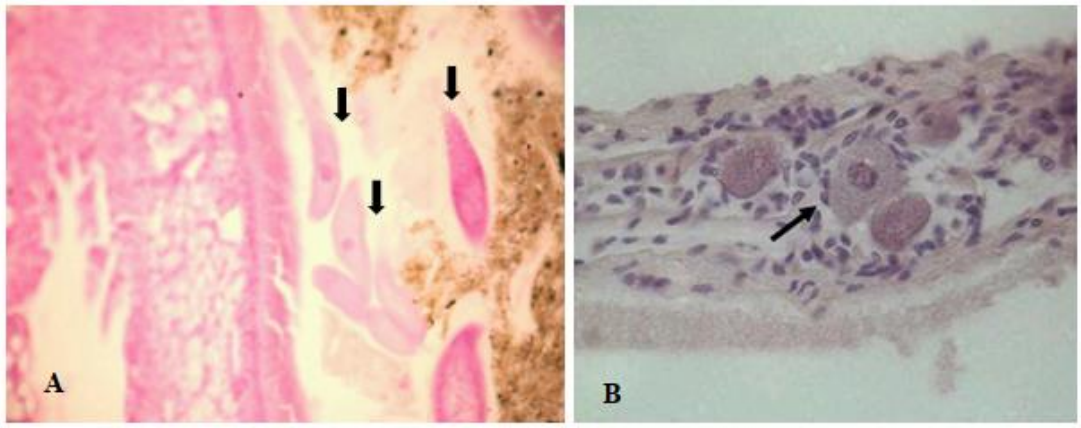

Figure 5. Histopathological photographs showing Gregarine in the midgut of sampled shrimp (arrow) (A) and Gregarine in the epithelial cells of the midgut (arrow), (40X, H&E) (B)

Gegarines are a diverse group of protozoan parasites belonging to the phylum Apicomplexa (order Eugregarinorida). They usually parasite in the hepatopancreas and the midgut of shrimp with mollusks (mainly bivalve molluscs) and arthropods as intermediate hosts (Tuntiwaranuruk *et al*., 2015). The development process of Gregarine goes through various stages that can be found in hepatopancreas and midgut of shrimp (Chakraborti & Bandyopadhyay, 2010).

Gregarine-infected shrimps do not have obvious pathological sign but often grow slowly. When shrimp is infected with high density of Gregarine, the parsite will damage the epithelial cells of the midgut of shrimp (Figure 5) and create opportunity for harmful bacteria to cause many other diseases in the intestinal tract and causes high mortality.

Vermiform

A prevalence infection of 24.7% was noted with Vermiform in hepatopancreas of sampled shrimp either by fresh smears (Figure 6A) or histological slides staining with H&E (Figure 6B).

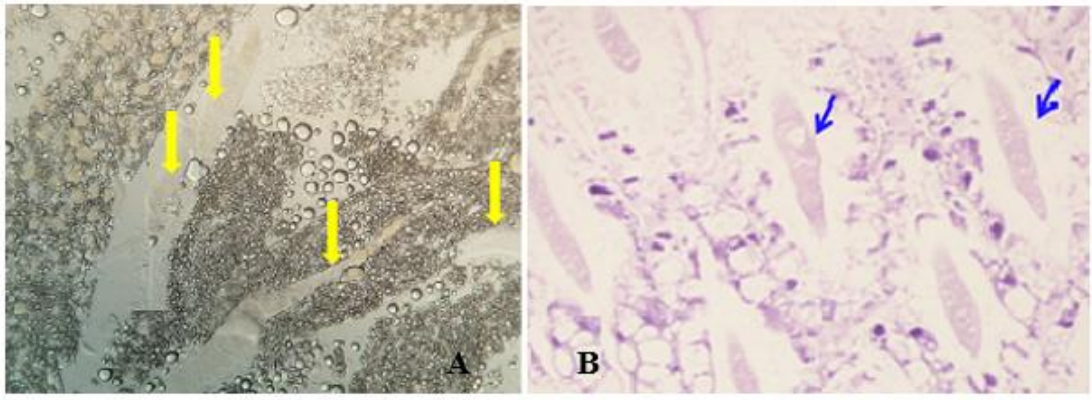

Figure 6. Fresh smear showing vermiform (arrow) in hepatopancreas (40X) (A); histopathological image of Vermiform in hepatopancreas (arrow) (H&E) (40X) (B)

Vermiform infected shrimp sample showed no specific pathological signs even in high intensity. The presence of vermiform in the hepatopancreas and midgut of shrimp has been thought to make shrimps reduce feeding, slowly grow and be associated with white feces disease in shrimp (Sriurairatana *et al*., 2014). The authors suggest that this pathological sign is formed by transformation, peeling and aggregation of microvilli from hepatopancreas epithelial cells and when vermiform occurs in high intensity will lead to the formation of white strings in the midgut and discharged into the environment. However, the mechanism of vermiform pathogenesis has not been determined yet.

Microporidia Enterocytozoon hepatopenaei (EHP)

Histological examinations of collected shrimp samples showed the presence of EHP spores in the cytoplasm of hepatopancreas tubule epithelial cells (Figure 7A) and midgut lumen (Figure 7B) of collected shrimp. The EHP spores were stained purple by hematocyline showing single or clustered oval shapes. PCR analysis result revealed EHP positive in collected samples (Figure 8). A prevalence infection of 7.9% was noted EHP spores in hepatopancreas and midgut of sampled shrimp and confirmed by PCR with specific primers for EHP.

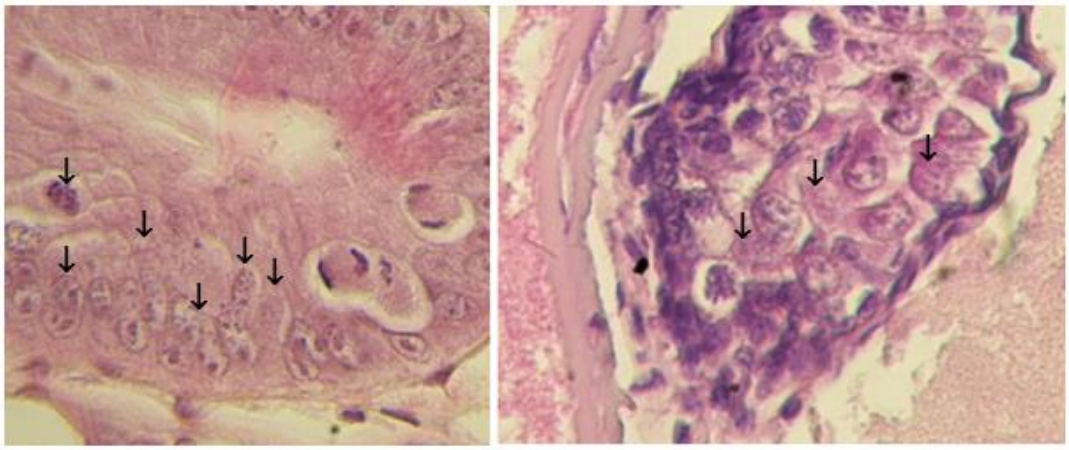

Figure 7. (A) Photograph of hepatopancreas and midgut epithelial cells infected with EHP (arrow), (B) EHP spores in the midgut lumen (arrow), (H&E stain) (100X)

Enterocytozoon hepatopenaei sp. is the name of a species of microsporidia found to be infected in the epithelial cells of shrimp hepatopancreas and midgut (Tourtip et al., 2009; Tangprasittipap et al., 2013; Tang et al., 2016), they have an ultrastructural features unique to the family Enterocytozoonidae (Tourtip et al., 2009). EHP is an intracellular parasite, they use nutrients stored in hepatopancreas and cause slow growth for shrimp due to insufficient nutrients for growth and molting.

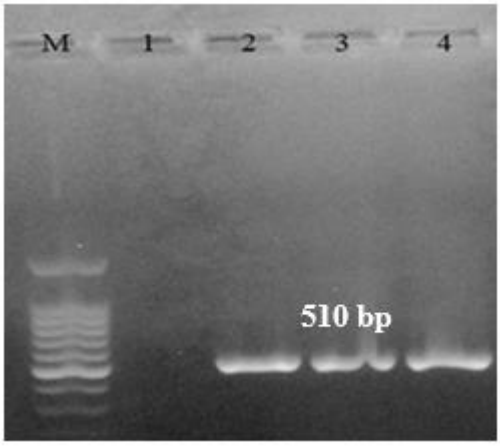

Figure 8. PCR product using specific primers for EHP

(M): 1 kb DNA ladder (Promega); (1) negative control (water), (2) positive control 510 bp, (3 and 4) DNA from collected samples.

Shrimp infected with EHP showed no specific pathological signs, but it was thought to be related to slow growth and thus different in sizes about 2-3 months after stocking, EHP-infected shrimp can only be half of the size of EHP-uninfected shrimp with the same culture period (Tang et al., 2016).

4. CONCLUSIONS

A significant observation made in the present study is that a high prevalence of Gregarine (96.5%) has been detected in the collected shrimp samples which displayed pathological signs in the digestive tract. Vermiform and EHP were also detected in hepatopancreas and midgut of shrimp samples but with low prevalence (24.7% and 7.9%, respectively).

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