Prevalence of *Enterocytozoon hepatopenaei* (EHP) in Vinh Long Province, Vietnam and Potential of Bamboo Charcoal in EHP Control in White Leg Shrimp (*Litopenaeus vannamei*) Culture

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ABSTRACT

Background: *Enterocytozoon hepatopenaei* (EHP) has been caused the substantial lost of production in shrimp farms in Vietnam. This study investigated the prevalence of EHP in white leg shrimp *(Litopenaeus vannamei*) culture in Vinh Long province, Vietnam and examined the effect of bamboo charcoal on survival, growth and EHP control in white leg shrimp.

Methods: A total of 58 shrimp samples collected randomly from 58 shrimp ponds at 53 shrimp farms in Vinh Long province was examined for the prevalence of EHP. Beside that, feed supplemented bamboo charcoal (FSBC) at 0%, 1% and 2% was examined for their effects on survival and growth of white leg shrimp and EHP control in white leg shrimp culture.

Result: This study found that EHP can be detected in 28/58 (48.28%) in white leg shrimp samples collected from shrimp ponds. Common clinical symptoms of EHP infected shrimp showed slow growth, size variation, segmented intestinal food and pale hepatopancreas (HP). Several EHP positive samples were found with white feces syndrome. After 3 weeks of culture, FSBC showed no effect on survival and growth of white leg shrimp. After 2 weeks fed EHP infected tissue, shrimp fed bamboo charcoal showed significantly higher survival rates compared to animals fed feed without bamboo charcoal and the cycle threshold value by real time PCR in shrimp fed FSBC at 2% was significantly higher than that in shrimp fed feed without bamboo charcoal. In conclusion, EHP was prevalent relatively high in white leg shrimp ponds in Vinh Long province, Vietnam and bamboo charcoal could be a potential source in EHP control in white leg shrimp culture.

Key words: Bamboo charcoal, *Enterocytozoon hepatopenaei, Litopenaeus vannamei,* Prevalence.

INTRODUCTION

Enterocytozoon hepatopenaei (EHP) is a causative agent of hepatopancreatic microsporidiosis (HPM) in shrimp. It was detected in 2004 as an unidentified microsporidian in slow growth black tiger shrimp *Penaeus monodon* from Thailand and was identified in 2009 (Tourtip *et al*., 2009). After that, it has been reported in many countries, including Korea, China, Taiwan, Indonesia, India, Vietnam, Malaysia, Philippines, Venezuela and Australia (Ibarra-Gámez *et al*. 2023, OIE, 2022). EHP spores are oval-shaped and measure approximately 0.7 to 1.1 µm. It can affect both white leg shrimp (*Litopenaeus vannamei)* and black tiger shrimp (*P. monodon)* and resulted in a substantial lost of production in shrimp farms. It is transmitted directly from shrimp to shrimp by oral rout, fecal means or contaminated water (Singh and Singh, 2018; Ibarra-Gámez *et al.,* 2023). There are no specific clinical signs for EHP infection. EHP infected shrimp is usually related to slow growth and severe size variation (Kim *et al*., 2021; Dieu-An *et al*., 2023). EHP infected shrimp also showed whitish appearance of the hepatopancreas (HP) due to the presence of spores and tissue damage, lethargy, reduced feed intake, an empty midgut and chronic mortality (Tang *et al.*, 2015; Khushbu *et al*., 2022). The histology of EHP infected shrimp shows irregular/regular basophilic inclusion bodies within the cytoplasm with or without spores, sloughing of the tubular epithelial cells, usually with the presence of mature spores (Singh and Singh, 2018; Dieu-An *et al*., 2023). Diseases in shrimp farming are usually controlled by using chemicals or antibiotics. Currently, there are no specific treatments on EHP infection, although several successful studies reported the inhibition of polar filament extrusion potassium

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permanganate ($KMnO_4$) at a concentration of 15 ppm for 15 min, active chlorine at 40 ppm for 15 min, ethanol at 20% (v/v) for 15 min and formalin at 200 ppm for 24 h almost 100% of the spores (Aldama-Cano *et al*., 2018). The use of antibiotics or chemicals in aquaculture products is usually related to public health concern. Therefore, alternative materials of chemicals or antibiotics in shrimp disease control have been extensively investigated. Several research investigated natural materials as the promising candidates for disease control in aquaculture (Pu *et al*., 2017; Hong To *et al*., 2022; Kaur *et al*., 2022; Jha *et al*., 2022).

Biochar/activated charcoals are reported as potential materials for heavy metal, organic pollutants, microbe and parasite removal because is's absorptance characteristics (Gęca *et al*., 2022). A number of research have been reported positive effects of biochar application in animal husbandry including chicken, cow, fish, pig and sheep such as detoxification, reduction of pathogens, enhancement of growth when using biochar as a feed additive (Quaiyum *et al*., 2014; Schmidt *et al*., 2019). Of biochar/activated charcoal types, bamboo biochar/activated charcoal has micro porous structure and high recovery efficiency. It is considered to have a higher adsorption capacity than wood charcoal because it has about 4 times more cavities, 3 times more mineral content and 4 times better absorption rate (Chaturvedi *et al*., 2024).

Up to now, the information on the prevalence of EHP in shrimp culture in Vinh Long province, the Mekong Delta of Vietnam is limited. Additionally, no report on the effect of bamboo charcoal in EHP control has been published. This study investigated the prevalence of EHP in Vinh Long province, Vietnam and examined the effect of bamboo charcoal on survival, growth and EHP prevention in white leg shrimp.

MATERIALS AND METHODS

This study was conducted from September 2024 to January 2026 at Tra Vinh University, Vinh Long province, Vietnam.

Prevalence of EHP in Vinh Long province

Sample collection

A total of 58 white leg shrimp samples (6-10 shrimps/sample) was randomly collected from 58 white leg shrimp ponds at 53 farms at Duyen Hai ward, Truong Long Hoa ward, Long Huu commune, Dong Hai commune, My Long commune, Nhi Truong commune, Tra Cu commune and Hoa Minh commune of Vinh Long province, Vietnam during 2025. Shrimp samples were contained in plastic bag, kept on dry ice and transferred to laboratory for immediately analysis. Shrimp was recorded for clinical signs and day of culture.

EHP detection

One gram of the mixture of HP and gut of shrimp was used for DNA extraction. DNA was extracted using commercial test kit (ABT Solutions, Vietnam) following the instruction of manufacturer. EHP was detected by nested PCR according to method described by Jaroenlak *et al*. (2016).

Histopathology

HP of some EHP infected shrimp by nested PCR was subjected to histopathology using protocol described by Lightner (1996).

Effect of bamboo charcoal on survival, growth and EHP control of white leg shrimp

Charcoal preparation

Bamboo tree was shredded into small pieces. Then, the small pieces were carbonized at 900°C in vacuum condition to form activated charcoal. Charcoal was kept in plastic bag at room temperature for further use.

Feed preparation

Commercial feed was milled to have powder. Bamboo charcoal was added to the powder at two different levels including 1% and 2%. The powder without charcoal supplementation was used as control feed. Water was added into the powder to form a paste, then the paste was extruded through mini extrusion machine to have pellet. The 2 mm pellet was dried at 55°C in 24 h and stored at 4°C until use.

Experiment designs for investigating the effect of bamboo charcoal on survival and growth of white leg shrimp: Shrimp at PL15 was reared to reach experimental size (1±0.13 g) and screened for EHP, AHPND (Acute hepatopancreatic necrosis disease), IHHNV (Infectious hypodermal and haematopoietic necrosis virus), WSSV (White spot syndrome virus) and YHD (Yellow head disease) by realtime PCR (ABT solution, Vietnam). The free pathogen shrimp confirmed EHP negative by nested PCR (Jaroenlak *et al*., 2016) was used for experiment. Three experiments were examined including shrimp fed feed supplemented bamboo charcoal (FSBC) at 0%, 1% and 2%. Each experiment was in triplicates. Fifty shrimp were placed in each 150 L containers with aeration. Water was exchanged at 20% every 2 days. The experiment was carried out in 3 weeks. Experimental shrimp was observed for growth and survival. During the experiment, water parameters were maintained at optimal level for white leg shrimp (Salinity: 13‰; pH: 7.5-8.5; Dissolved oxygen: above 5 ppm; Ammonia and hydrogen sulfide: undetectable). Survival and growth [weigh gain (WG) and specific growth rate (SGR)] of shrimp after 3 weeks fed FSBC were determined as formula below:

$$\text{SR (\%)} = \frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}} * 100$$

$$\text{WG (\%)} = \frac{\text{(Final weight - Initial weight)}}{\text{Initial weight}} * 100$$

$$\text{SGR (\% /day)} = \frac{[\ln w_f - \ln w_i]}{t} * 100$$

EHP challenge experiment

After 3 weeks fed FSBC at three different levels (0%, 1% and 2%), shrimp from the three experiments were exposed to EHP *via* oral challenge (feeding EHP infected tissue). Shrimp fed tissue (HP and intestine) from healthy shrimp was used as negative control group. Each experiment was in triplicates. Twenty-five shrimp were placed in each 80l container. Shrimp samples collected from farms were screened for EHP, AHPND, IHHNV, WSSV and YHD by realtime PCR (ABT solution, Vietnam). Only shrimp from culture pond confirmed EHP positive by realtime PCR and nested PCR (Jaroenlak *et al*., 2016) was applied in challenge experiment. Fresh HP and intestine of EHP positive shrimp were mixed to form a paste. One gram of mixture [cycle threshold (CT) = 25.3] was added into each experimental container. No water exchange was carried out in the first two days. After that, the sediment was removed by siphoning every day. The survival and CT values in experimental shrimp were observed after 2 weeks.

RESULTS AND DISCUSSION

The prevalence of EHP in Vinh Long province

Of 58 shrimp samples collected and examined for EHP prevalence, 28/58 (48.28%) was EHP positive by nested PCR (Table 1). The common clinical signs of EHP infected shrimp showed slow growth, size variation, segmented intestinal food and pale HP (Table 2 and Fig 1). White feces syndrome (WFS) was recorded in several EHP infected samples (Table 2). Histopathology of HP of EHP infected shrimp showed the present of EHP spore in the cytoplasm of HP tubular epithelial cells and sloughing of tubular epithelial cells (Fig 2).

Effect of bamboo charcoal on survival, growth and EHP control of white leg shrimp

Effect of bamboo charcoal on survival and growth of white leg shrimp.

Table 3 indicated growth and survival of shrimp after 3 weeks fed FSBC. There were no significant differences in the mean values of WG, SGR and survival of shrimp among treatments (P>0.05).

EHP challenges

Fig 3 indicated that after 2 weeks fed fresh EHP infected tissue, shrimp fed FSBC showed significantly lower survival rates (69.33%-70.67%) compared to shrimp fed tissue from healthy shrimp (85.33%); however, they showed significantly higher survival rates in comparison to shrimp in positive control group (60%).

Fig 4 indicated the CT values of shrimp after 2 weeks fed EHP infected tissue. The CT value of shrimp fed FSBC at 2% was significantly higher than that of shrimp fed feed without bamboo charcoal, although it was not significant difference to that of shrimp fed FSBC at 1%. There was no significant difference in the mean CT values between shrimp fed FSBC at 0% and 1%.

This study found the prevalence of EHP in shrimp farms in Vinh Long province, Vietnam was relatively high. The high prevalence of EHP in shrimp farms had been reported elsewhere. Dieu-An *et al*. (2023) examined the prevalence of EHP in white leg shrimp farm in Taiwan and indicated the proportion of EHP-positive cases was 48% (31/65). In Korea, the prevalent of EHP infected shrimp was calculated to be 25.5%. Sajiri *et al*. (2021) examined the infection of EHP in white leg shrimp farm in Malaysia and showed that the EHP infection was initially detected in the hatchery and increased to 96.6% after the shrimp were transferred to the pond. Biju *et al*. (2016) examined shrimp

Table 1: The prevalence of EHP in collected shrimp samples.

Day of culture	Number of samples	Number of positive samples (%)
≤ 50	48	28/48 (58.33%)
≥ 51	10	0/10 (0%)
Total	58	28/58 (48.28%)

A: Shrimp with different growth pattern and segmented intestinal food (black arrow);
B: Shrimp with pale HP (black arrow).

Fig 1: Cross observation of EHP infected shrimp.

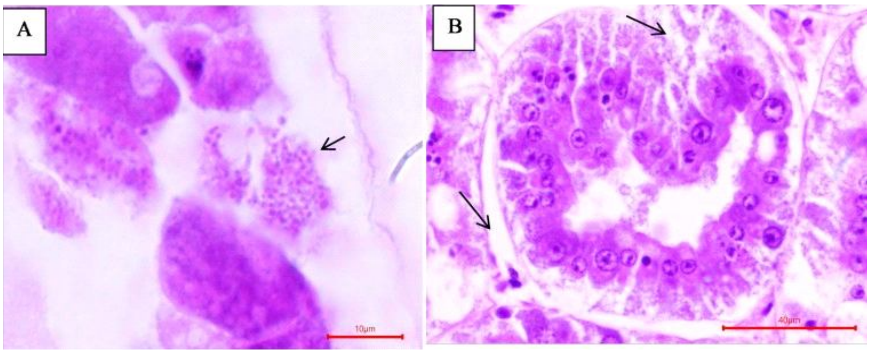
samples from ponds affected growth retardation in India and reported 69% white leg shrimp samples and 49% black tiger shrimp samples were EHP positive *via* nested PCR. Clinical signs of EHP infected shrimp recorded in this study were similar to that described in previous studies such as slow growth, size variation, segmented intestinal food and whitish HP (Tang *et al*., 2015; Dieu-An *et al*., 2023).

This study found WFS in several cases of EHP infected shrimp. The prevalence of EHP in WFS infected shrimp has been reported (Tang *et al*., 2016; Jithendran *et al*., 2018;

Table 2: Clinical signs of EHP infected shrimp.

Number of infected samples	Geographic location	EHP by nested PCR	Clinical signs
1	Duyen Hai ward	+	Slow growth, size variation, pale HP, segmented intestinal food, white feces
2	Duyen Hai ward	+	Slow growth, size variation, segmented intestinal food
3	Duyen Hai ward	+	Slow growth, pale HP
4	Duyen Hai ward	+	Slow growth, pale HP
5	Duyen Hai ward	+	NA*
6	Duyen Hai ward	+	Slow growth, size variation, segmented intestinal food, pale HP
7	Duyen Hai ward	+	Slow growth, size variation, segmented intestinal food, pale HP
8	Truong Long Hoa ward	+	Slow growth, pale HP
19	Long Huu commune	+	Slow growth
10	Long Huu commune	+	Slow growth
11	Long Huu commune	+	NA
12	Long Huu commune	+	Slow growth, size variation, pale HP
13	Long Huu commune	+	NA
14	Long Huu commune	+	Slow growth, size variation
15	Dong Hai commune	+	Slow growth, size variation, pale HP
16	Dong Hai commune	+	Slow growth, size variation, pale HP, segmented intestinal food, white feces
17	Dong Hai commune	+	Slow growth, size variation, segmented intestinal food, pale HP
18	My Long commune	+	Slow growth, size variation, segmented intestinal food, pale HP
19	My Long commune	+	Slow growth, size variation, segmented intestinal food, pale HP, white feces
20	Nhi Truong commune	+	NA
21	Nhi Truong commune	+	NA
22	Nhi Truong commune	+	Slow growth, size variation, segmented intestinal food, pale HP
23	Tra Cu commune	+	Slow growth, size variation, segmented intestinal food, pale HP
24	Tra Cu commune	+	Slow growth, size variation, segmented intestinal food, pale HP
25	Tra Cu commune	+	Slow growth, size variation, segmented intestinal food, pale HP, white feces
26	Tra Cu commune	+	Slow growth, size variation, segmented intestinal food, pale HP
27	Hoa Minh commune	+	NA
28	Hoa Minh commune	+	Slow growth, size variation, segmented intestinal food, pale HP

*: Clinical signs of disease were not found.

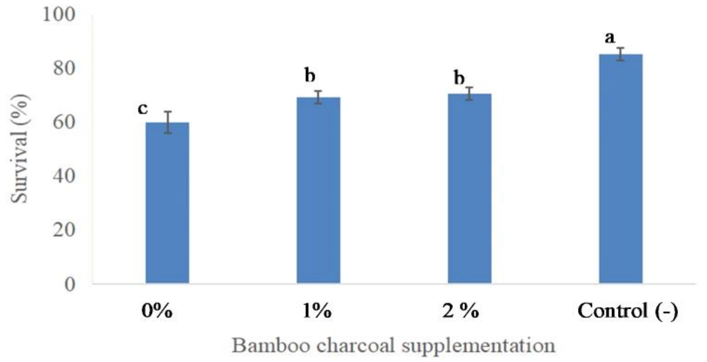


A: EHP spore in the cytoplasm of HP tubular epithelial cells (black arrow); B: Sloughing of tubular epithelial cells (black arrow). Scale bar 10 µm.

Fig 2: Histopathology of EHP infected shrimp collected from pond.

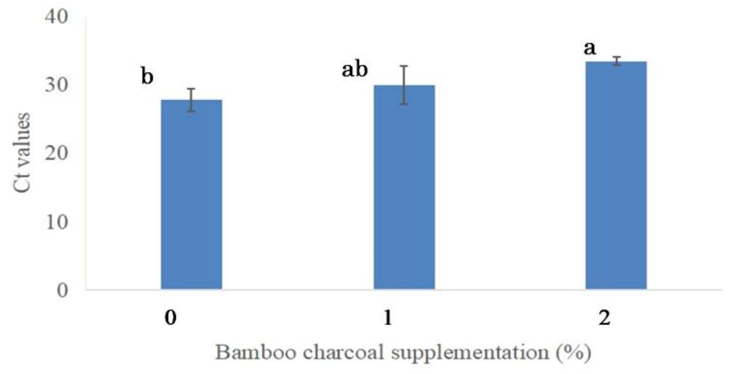
Piamsomboon and Han, 2022). The details of the correlation between EHP and WFS are still discussed although WFS occasionally detected in EHP infected ponds. According to Aranguren *et al*. (2019), in EHP-endemic areas, shrimp displaying clinical signs of WFS indicated a very active EHP infection process in grow-out ponds. Aranguren Caro *et al*. (2021) also indicated that there is a synergistic relation between EHP and *V. parahaemolyticus* isolate that led to the manifestation of WFS. As suggested by Piamsomboon and Han (2022), the disrupted hepatopancreatic tubules and sloughing cells caused by EHP infection move through the gastrointestinal tract, supporting opportunistic bacteria growth. The changes in microbial community may lead to gut dysbiosis, metabolic disorder and resulting in white fecal formation. Consequently, infected shrimp shows slow growth along with hepatopancreatic discoloration.

Bamboo charcoal is preferred over regular charcoal due to it's various advantages (Chaturvedi *et al*., 2024). This study found that diets supplemented bamboo charcoal do not affect on survival and growth performance of shrimp after 3 weeks culture. Additionally, FSBC enhances survival rate of EHP infected shrimp and might reduce EHP load in infected shrimp due to higher CT value compared to animals fed feed without bamboo charcoal. Similar to this study, Kumar *et al*. (2021) examined the effect of bamboo charcoal in AHPND control and indicated that the addition of bamboo charcoal (50-100 mg/l) improved survival of brine shrimp larvae upon challenge with AHPND, profound effects on the phenotypic responses of AHPND-causing *V. parahaemolyticus* strains. The AHPND strains grown with bamboo powder developed cellular aggregates or floccules in the culture medium and switch in the pattern of protein production and secrete alkaline phosphatase PhoX instead of PirAVP and PirBVP toxins. The benefits of charcoal in fish culture has also been reported elsewhere. According to Mabe *et al*. (2017), diet supplemented charcoal at different level including 0.5%, 1%, 2% and 4% did not affect on growth and overall muscle fatty acid composition of juvenile common carp and improved several serum indicators such as alanine aminotransferase (ALT), aspartate aminotransferase



0%: Shrimp fed feed without bamboo charcoal and supplied fresh EHP infected tissue; 1% and 2%: Shrimp fed FSBC at 1% and 2%, respectively and supplied fresh EHP infected tissue. Control (-): Shrimp fed tissue of healthy shrimp. Different lowercase letters (a, b, c) indicate statistically significant differences ($P<0.05$).

Fig 3: Survivals of shrimp after 2 weeks fed EHP infected tissue.



Different lowercase letters (a, b, c) indicate statistically significant differences ($P<0.05$)

Fig 4: The CT values of shrimp after 2 weeks challenge.

Table 3: Survival and growth of white leg shrimp after 3 weeks fed bamboo charcoal.

Parameters	Bamboo charcoal supplementation in feed (%)		
	0	1.0	2.0
Initial weight (g)	1.0±0.13	1.0±0.13	1.0±0.13
Final weight (g)	4.23±0.02	4.25±0.11	4.17±0.09
WG (%)	322.89±1.61	321.42±5.78	320.18±3.20
SGR (%/day)	6.87±0.02	6.89±0.13	6.79±0.11
SR (%)	87.33±1.15	88.67±2.31	88.00±3.46

(AST), total protein (TP), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and glucose (GLU) with the 4% inclusion level producing the most beneficial effects. Similarly, Quaiyum (2014) showed that diets supplemented 2% of charcoal enhanced growth performances of catfish and reduced ammonia levels.

CONCLUSION

EHP was prevalent relatively high in Vinh Long province, Vietnam. Bamboo charcoal did not affect on growth and survival rate of white leg shrimp. Additionally, the supplementation of bamboo charcoal up to 2% in feed could improve the survival rate of shrimp infected EHP and reduce EHP load in infected shrimp. Therefore, bamboo charcoal could be a potential source in EHP control in white leg shrimp culture.

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Conflict of interest

No conflicts were declared.

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