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Field Study on Transmission of Acute Hepatopancreatic Necrosis Syndrome (AHPNS) in Infected Ponds

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Abstract

A field transmission experiment for acute hepatopancreatic necrosis syndrome (AHPNS) in whiteleg shrimp (*Penaeus vannamei*) was set up in two AHPNS-infected ponds. Six hapas were placed in each pond; of these, three were stocked with healthy shrimp (to investigate transmission via water) and the other three were stocked with both healthy and AHPNS-infected shrimp (to investigate transmission via water and cohabitation). At 10 days post stocking, healthy shrimp in both treatments showed typical signs of AHPNS pathology as seen in naturally infected shrimp in ponds. Histopathological analysis revealed rounding and sloughing of hepatopancreatic (HP) epithelial cells, reduction in epithelium height, loss of certain cell types (B-, F- and R-cells), and severe haemocytic infiltration around HP tubules. Mortalities were noted in all experimental hapas; however, mortality rates in hapas stocked with both healthy and AHPNS-infected shrimp were higher than in hapas stocked with healthy shrimp only. Isolates of *Vibrio* bacteria obtained from infected shrimp were identified as *V. parahaemolyticus*. Polymerase chain reaction (PCR) analysis detected the *thl* gene from isolates of *V. parahaemolyticus* but not the *thd* or *trh* genes.

Keywords: AHPNS, *Penaeus vannamei*, histopathology, transmission, mortalities

Introduction

Early mortality syndrome (EMS)/acute hepatopancreatic necrosis syndrome (AHPNS) is an emerging threat in the Asian shrimp industry, causing high mortalities in shrimp aquaculture and economic losses to both small farmers and commercial producers (Eduardo and Mohan 2012). AHPNS first appeared in farmed penaeid shrimp in the coastal provinces of the Mekong Delta of Viet Nam in 2010. In 2011 and 2012, it continued to occur, causing high mortality in farmed shrimp in the Mekong Delta and also affecting shrimp farms in some northern coastal

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provinces. The disease occurred all year round, with greatest severity from April to July.

EMS/AHPNS was considered as an unknown disease before the breakthrough finding of Prof. Donald Lightner's team pointing to a strain of *V. parahaemolyticus* as its causative agent (Tran et al. 2013). It affected farms culturing both giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*P. vannamei*), mainly in areas of intensive and semi-intensive shrimp farming. The incidence of AHPNS seemed to be higher in farms with high salinity and during the dry season with associated high temperature. The disease was considered idiopathic, and it was not known whether the cause was infectious or toxic. Earlier hypotheses suggested a range of possible causes, including cypermethrin (an insecticide), other pesticides, pollution, contaminated feed, parasites, harmful algae, probiotics and inbreeding. This field study on disease transmission in AHPNS-affected ponds was carried out during the FAO TCP/VIE/3304 (E) Emergency Assistance to Control the Spread of an Unknown Disease Affecting Shrimps, which was being implemented by Viet Nam's Ministry of Agriculture and Rural Development (MARD). The objective of the study was to determine if AHPNS is transmissible by water and by co-habitation of infected and healthy shrimp in AHPNS-affected ponds.

Materials and Methods

Experimental set up

The study was carried out from April to May, 2013, and the experiment was set up in two AHPNS-infected ponds at Cong ap 10, Tan Duyet Commune, Dam Doi District, Ca Mau Province (Fig. 1a). AHPNS-infected ponds were determined by histopathological examination of infected shrimp showing typical AHPNS pathology (Figs. 1b and 3b). Healthy broodstock of *P. vannamei* held at the College of Aquaculture and Fisheries, Can Tho University were induced to spawn, and the resulting larvae were reared to a size of ~ 1–1.5 gram. Ten individuals with apparently healthy hepatopancreas (HP) and full gut content (Fig. 2) were checked by histology to make sure that they showed no AHPNS pathology (Fig. 3a) prior to being transported to the experimental site.

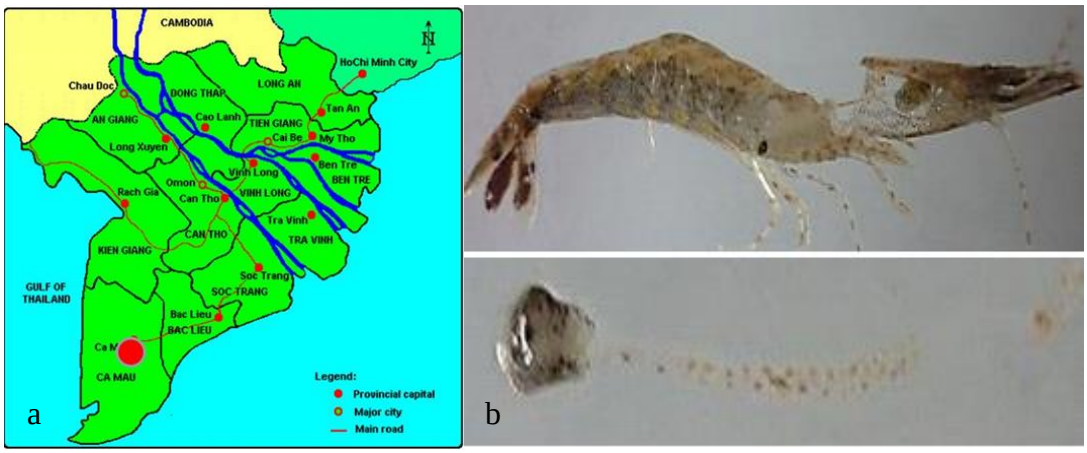

Fig. 1. (a) Location of the experimental study on AHPNS transmission, and (b) Gross signs of AHPNS in shrimp from experimental ponds.

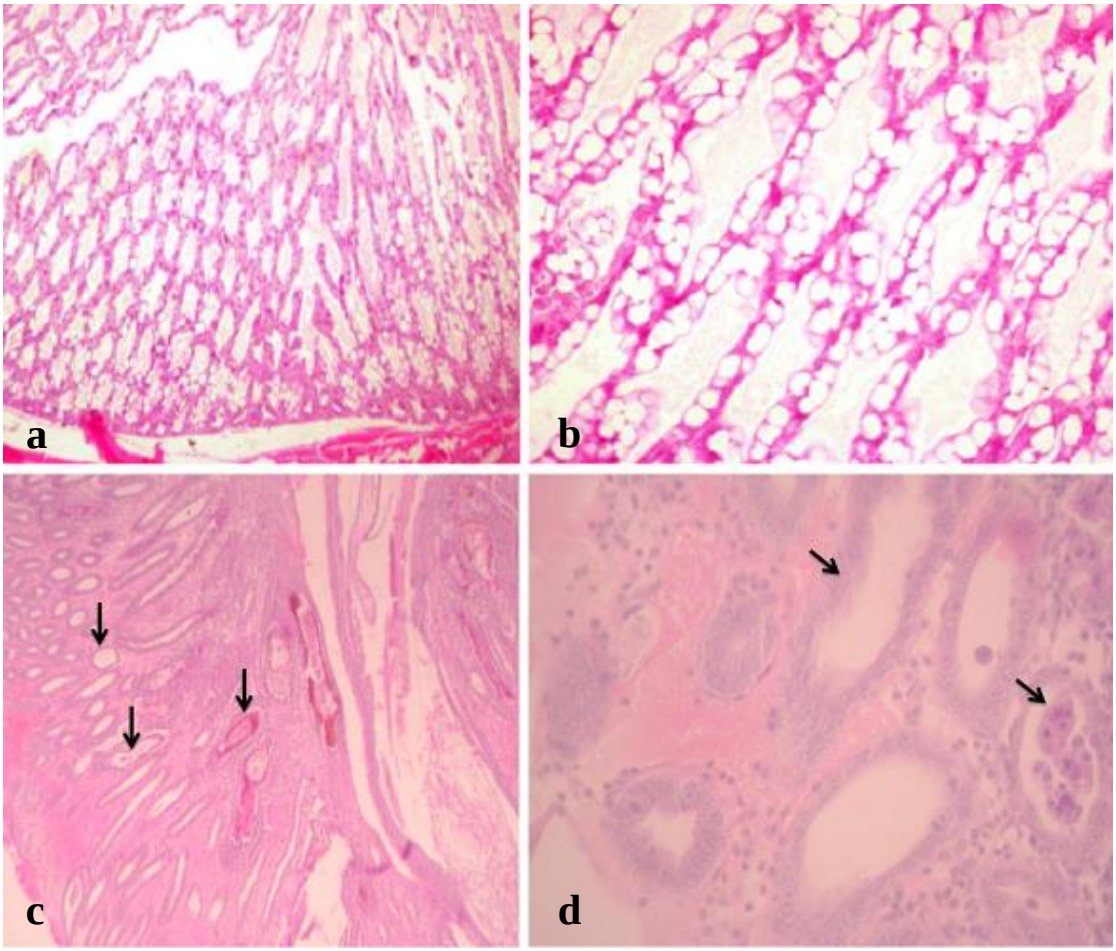

Fig. 2. Histological sections showing the hepatopancreas (HP) of healthy experimental shrimp (a: 10X; b: 40X magnification) and infected shrimp; (c) arrows (L to R): distruction of HP tubules, melanization (10X magnification) and (d) arrows (L to R): distruction of HP tubule, sloughing cells (40X magnification). H&E stain.

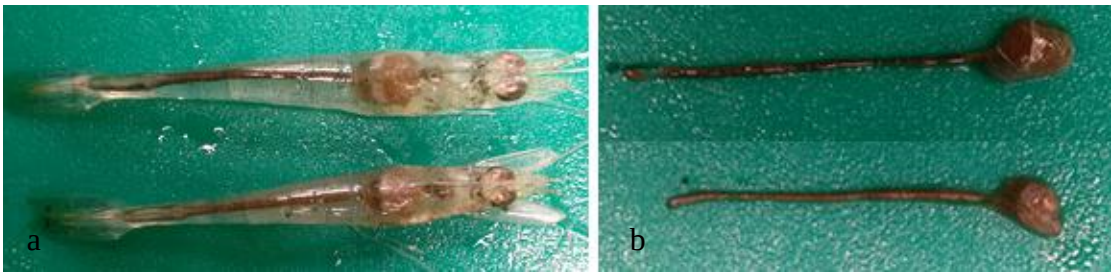

Fig. 3. (a) Healthy shrimp with full gut content used in the study and (b) hepatopancreas and gut from healthy shrimp.

Both the water transmission and the cohabitation experiments were conducted in a series of 6 hapas (1m x 1m x 1m) aligned as shown in Figure 4 (Pond 1), with a second, duplicate pond (Pond 2) also with a similar 6 hapas. Each of the ponds contained 2 experimental groups as follows:

- Group 1 (hapas 1, 2 and 3): stocked only with healthy shrimp (100 shrimp per hapa) that had been reared under laboratory conditions at Can Tho University to study of the transmission of AHPNS via water.
- Group 2 (hapas 4, 5 and 6: stocked with 100 healthy shrimp per hapa and 15 AHPNS-infected shrimp collected from an AHPNS-infected pond to study transmission of AHPNS by cohabitation.

The experiment was followed for up to 10 days post-stocking of healthy shrimp in the experimental hapas.

Fig. 4. Experimental hapas were set up from 1–6. Hapas 1, 2 and 3 contained healthy shrimp only, while hapas 4, 5 and 6 were stocked with both healthy and AHPNS-affected shrimp.

Histopathology

During the experiment, moribund shrimp (shrimp that are dying, but are still alive) were collected from experimental hapas and injected with Davidson's AFA (alcohol-formalin-acetic acid) fixative and processed and stained with hematoxylin and eosin (H&E) using the routine histological methods described by Lightner (1996). The histological sections were examined by light microscopy for AHPNS lesions in the hepatopancreas (HP). At the end of the experiment, 10 shrimp from each hapa were randomly collected and examined by histology for pathology due to AHPNS.

Bacterial isolation and characterization

Bacterial isolates (primarily *Vibrio* spp.) were obtained from the HP of shrimp specimens. Strains were stored at -70 °C in tryptic soy broth (Merck) containing 25 % glycerol supplemented with 1.5 % (w/v) sodium chloride. The strains were identified using API 20E (BioMerieux, France) and detection of the *tlh*, *tdh* and *trh* genes was carried out by PCR (Nishibuchi et al. 1986; Bej et al. 1999).

Data analysis

Differences in final mortality between experimental groups were analysed using Student's t-test ($P < 0.05$).

Results

Gross clinical signs in experimental shrimp

Experimental shrimp had changes in the HP colour from the first day after being released into the experimental hapas. From the second day, shrimp displayed the first signs of AHPNS as described by Lightner et al. (2012), including anorexia, lethargy and pale colouration of the body and HP (Fig. 5). Almost from day 4 onwards, the HP of experimental shrimp displayed gross signs similar to those seen in diseased shrimp collected from the same ponds (Fig. 6). AHPNS pathology was more evident in samples which were collected at end of the experiment (10 days after the shrimp were released into experimental hapas).

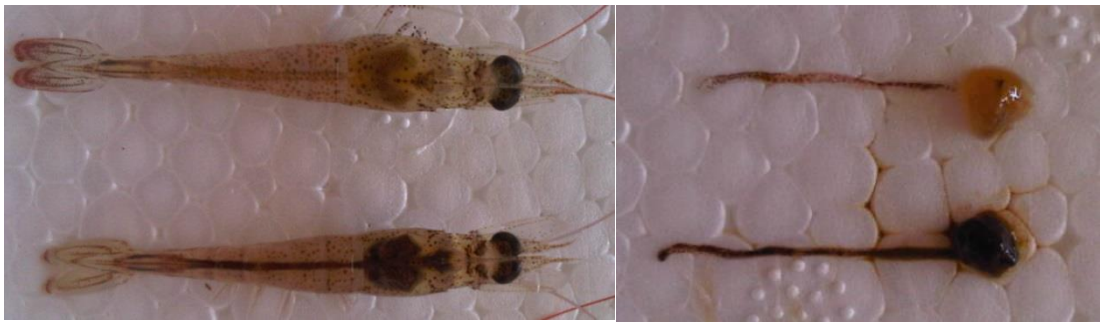

Fig. 5. Experimental shrimp collected on day 2 after release into hapas.

Fig. 6. Experimental shrimp showing change of colour of the hepatopancreas and empty gut four days after release into hapas.

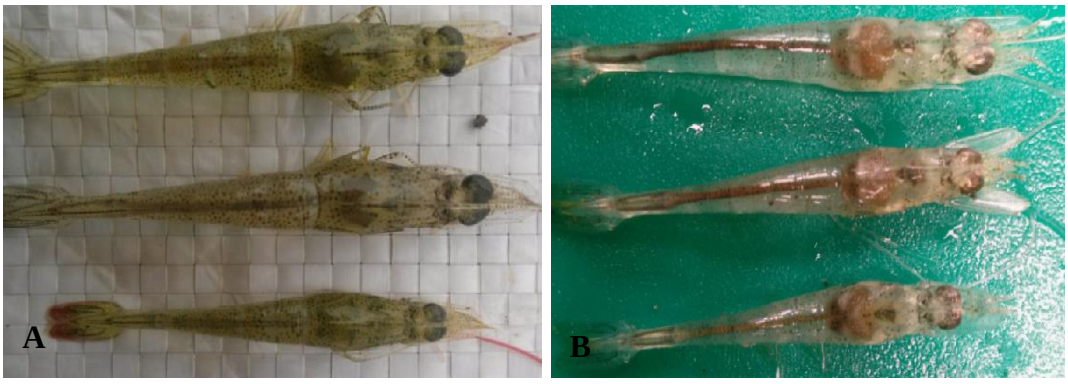

Fig. 7. A. Shrimp collected from experimental hapas. B. Shrimp collected from the same stock kept in the laboratory.

Although the experiment lasted for only ten days, it is long enough to recognize the abnormal growth (which was slower than normal), lethargic swimming, relatively weak, soft shells, and the darkened, atrophied and pale HP of AHPNS-affected shrimp as compared to those control shrimp that were maintained in the laboratory (Fig. 7).

As can be clearly seen in Figure 8, the HP of experimental shrimp displayed clinical signs that were quite similar to the gross signs of AHPNS as described by Lightner et al. (2012).

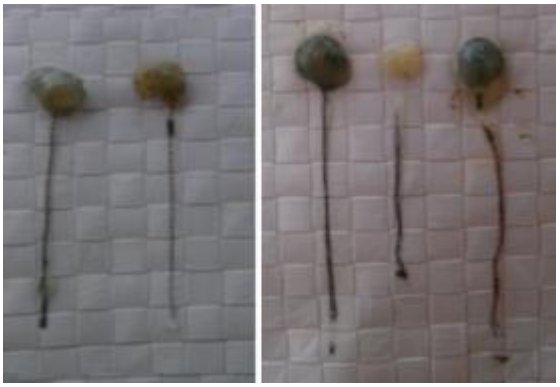

Fig. 8. Hepatopancreas of infected shrimp at the end of the experiment.

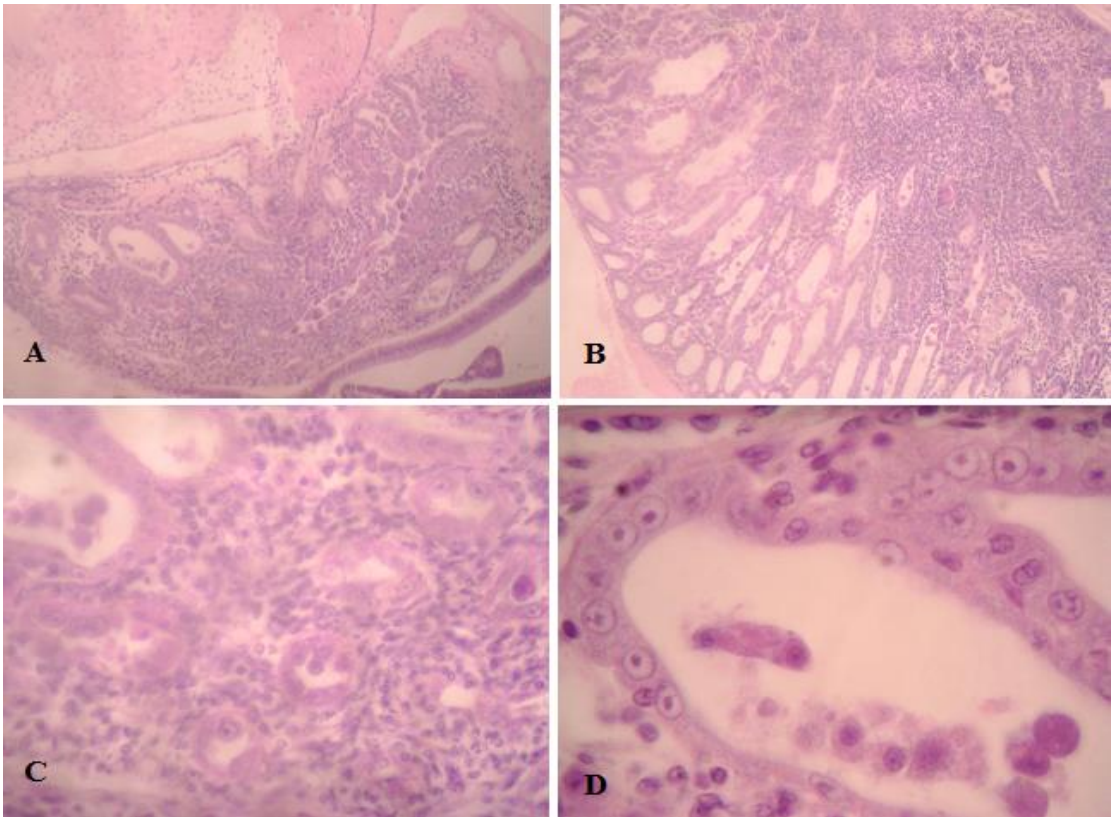

Fig. 9. Histopathological preparations of the hepatopancreas (HP) of infected shrimp collected from experimental hapas. A & B: distruction of HP cells, lack of E, B and R cells (10X & 20X magnification. C&D: HP sloughing of tubule epithelium, significant proximal haemocytic inflammation, some tubules with putative vibriosis (40X & 100X magnification). H&E stain.

Shrimp with gross signs of AHPNS were subjected to histological analysis. The HP of these shrimp displayed histopathological changes typical of AHPNS, including distruction of HP cells, lack of E, B and R cells, sloughing of HP tubule epithelium, significant proximal haemocytic inflammation, and some tubules with putative vibriosis (Fig. 9).

A total of 120 shrimp (10 from each hapa) was randomly collected at the end of the experiment. Among the 60 shrimp sampled from hapas stocked with healthy shrimp, only 18 displayed typical AHPNS pathology, whereas 27 of the shrimp collected from hapas stocked with both healthy and AHPNS-affected shrimp displayed AHPNS pathology.

Mortality

During the experimental period, shrimp were observed daily to check for abnormal signs and to record mortality. The mortality data (Fig. 10) clearly showed the difference in the number of shrimp which survived in the different experimental groups, as well as between the two experimental ponds.

In Pond 1, the lowest mortality of experimental shrimp was noted in hapa 1.4 (19 %), and the highest was in hapa 1.6 (57 %). In Pond 2, the lowest mortality of experimental shrimp was noted in hapa 2.2 (2 %) and the highest was in hapa 2.2 (44 %). Although the mortality rates between hapas with the same treatment, as well as between treatments were different, these differences were not statistically significant ($P > 0.05$).

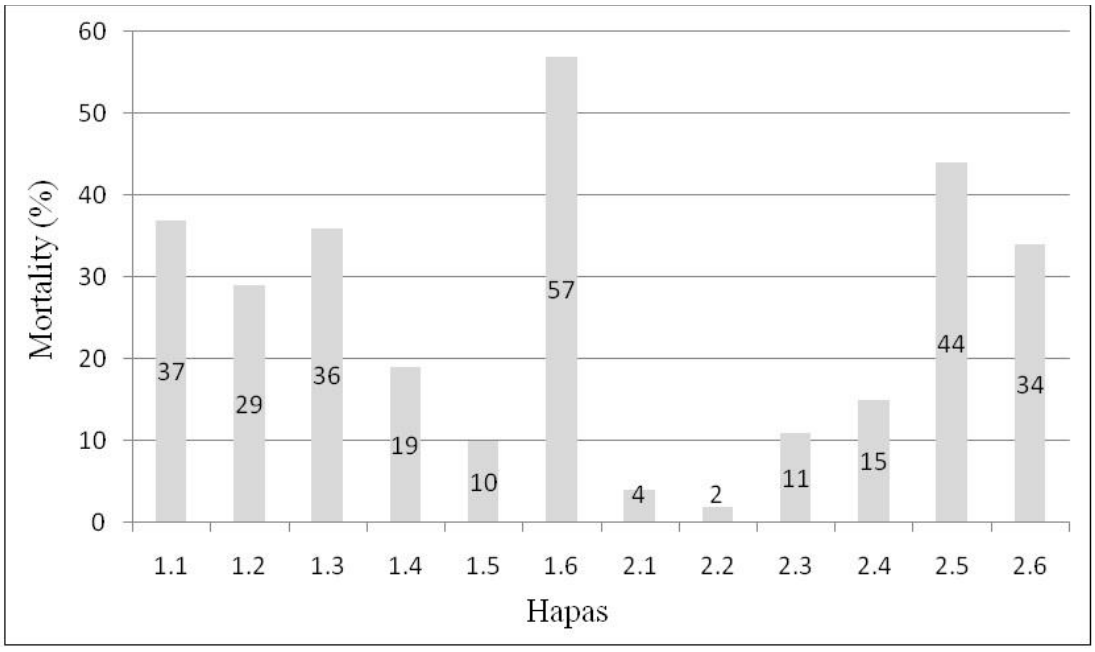

Fig. 10. Mortality of experimental shrimp in hapas in the two ponds. Numbers 1.1–1.6 indicate hapas 1–6 in Pond 1 and numbers 2.1–2.6 indicate hapas 1–6 in Pond 2.

Bacterial isolation and characterization

Seventy-four bacterial isolates (primarily *Vibrio* spp.) were obtained from the HP of shrimp specimens. Of these, 31 isolates were identified as *V. parahaemolyticus* by API 20E. PCR analysis detected the *thl* gene from isolates of *V. parahaemolyticus* (Fig. 11) but not the *thd* or the *trh* genes.

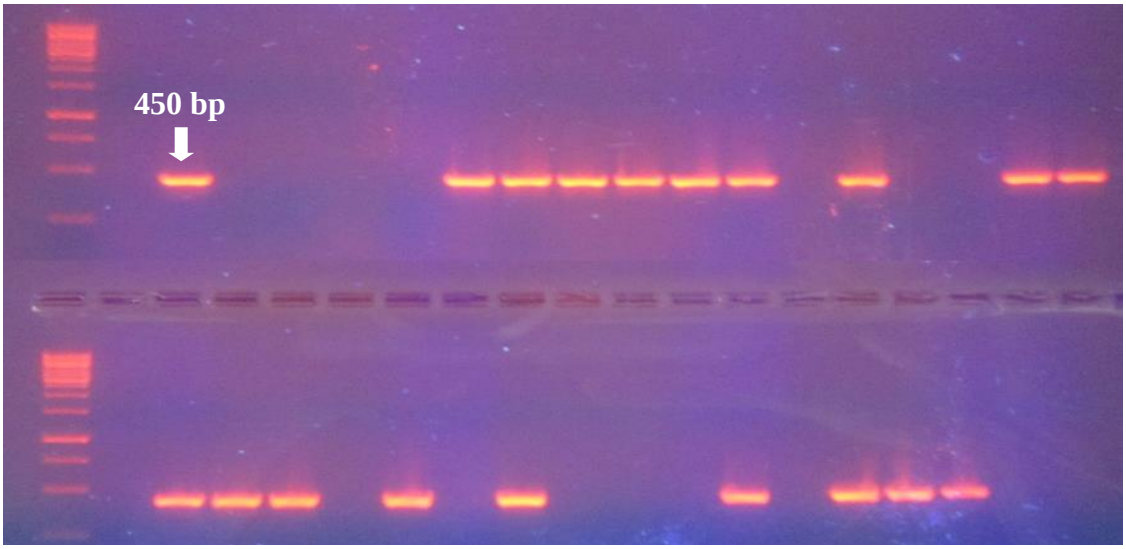

Fig. 11. Polymerase chain reaction (PCR) detection of *tlh* gene from isolates of *Vibrio parahaemolyticus*.

Discussion

This study indicates that AHPNS can be transmitted through infected water and via co-habitation. It also confirms that AHPNS is an infectious disease as reported by Tran et al. (2013). Although not statistically significant, the data obtained from the experiment suggest that the mortality rate in cohabitation was higher than that for water transmission only (average mortality of 26.7 % vs 13.7 %). Higher mortality in cohabitation may be due to AHPNS infections resulting from direct contact between healthy and infected shrimp, via water and/or by cannibalism.

Conclusion

AHPNS is transmissible both via water and by cohabitation of uninfected and infected shrimp in AHPNS-affected ponds. Healthy shrimp experimentally infected with AHPNS displayed pathological and histopathological characteristics typical of AHPNS as seen in natural infections in shrimp ponds. The mortality rate in cohabitation was higher than in water transmission alone. Isolates of *V. parahaemolyticus* from AHPNS-infected shrimp did not carry the *thd* of *trh* toxin genes.

Acknowledgements

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