Acute Hepatopancreatic Necrosis Disease (AHPND) and Hepatopancreatic Microsporidiosis (HPM): two threats to sustainable shrimp aquaculture

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Abstract

Infectious diseases caused by viruses and bacteria are a major threat to sustainable shrimp farming globally. Since early 80's viral diseases such as White Spot Disease, Taura Syndrome disease have caused enormous losses to shrimp aquaculture both in eastern and western hemisphere. As the shrimp industry tried to recover from the onslaught of these diseases, a bacterial, Acute Hepatopancreatic Necrosis Disease (AHPND), also known as Early Mortality Syndrome, and a fungal disease Hepatopancreatic Microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) are now posing new threat to shrimp aquaculture.

Acute Hepatopancreatic Necrosis Disease is caused by *Vibrio spp.* expressing plasmid-borne binary toxins, PirA and PirB that is similar to entomopathogenic bacterium, *Photorhabdus* encoded toxin. In 2009, AHPND emerged in China and since then spread to many countries in East Asia and in the Americas. Another disease that has caused alarm in recent year is Hepatopancreatic Microsporidiosis (HPM) caused by Enterocytozoon penaei (EHP), a microsporidium. While AHPND causes acute infection and large-scale mortalities, EHP causes chronic infection and results growth retardation and size variation in population reducing marketability of the infected shrimp. Both diseases affect hepatopancreas, an organ involved in metabolism and humoral immunity in shrimp. The binary toxin, PirA/ PirB are the primary virulence factor for AHPND, but specific virulence factor(s) for EHP is not known. It is, however, known that EHP does not have mitochondria and appears to transport ATP from the cytoplasm of infected cells as it contains ATP transporter genes in its genome. EHP has been shown to be a risk factor for AHPND. Due to lack of therapeutics, preventative measures remain as a corner stone for managing these diseases and efforts are underway to develop genetically improved lines of shrimp having resistance to AHPND and EHP.

Key words: AHPND, EHP, Penaeus vannamei

Introduction

The global shrimp industry has grown rapidly since it started in 1970s. Currently, shrimp aquaculture contributes to more than 50 percent of the world's production (nearly 4.5 million metric tons, MMT) (Anderson *et al*., 2018) and shrimp ranks third in the value chain of total aquaculture production after salmon and tilapia (FAO, 2020). Pacific white shrimp, *P. vannamei* and black tiger shrimp, *P. monodon* are the two most cultivated penaeid shrimp. Currently, *P. vannamei* accounts for approximately 73 % of farmed shrimp globally. The worldwide expansion of Pacific white shrimp farming has been possible due to the ease of farming this species in a wide range of salinity levels, development of captive breeding programs, and availability of genetically superior broodstock and postlarvae (PL). Unlike *P. vannamei*, captive breeding program is not well established in *P. monodon*. As a result, despite high commercial value of this species, farming of *P. monodon* remains restricted to some countries in Asia (e.g. India, Bangladesh, Thailand, Taiwan) and East Africa (e.g. Madagascar) (Thornber *et al*., 2019). As shrimp farming expanded from a backyard small farming to a major industrial operation, it faces myriads of challenge, and infectious diseases caused by viruses, bacteria and fungi remain as the primary bottleneck for further expansion of the industry. In fact, emerging diseases are now threatening the long-term sustainability of the shrimp industry worldwide.

One of the most critical issues in aquaculture is the incidence of various diseases that threaten the health of aquatic animals. Virtually, aquatic animals swim in a microbial soup that make them vulnerable to viruses, bacteria, fungi, and protozoa (Santos *et al*., 2019). In shrimp aquaculture, it is estimated that about 60 % of the losses are due to viruses and 20 % due to bacteria with the remaining losses attributed to fungi and parasites (Flegel, 2019). Viruses that have caused serious economic losses since their emergence include white-spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), shrimp hemocyte iridovirus (SHIV), yellow-head virus (YHV), and Taura syndrome virus (TSV) (Karunasagar and Ababouch, 2012; Lightner *et al*., 2012; Santos *et al*., 2019), and almost 80 % of these economic losses have occurred in Asia (Santos *et al*., 2019). Among bacterial diseases acute hepatopancreatic necrosis disease (AHPND), also known as Early Mortality Syndrome (EMS), is the most important diseases that have caused major economic losses (Tran *et al*., 2013). In fact, since its emergence in 2009 in China, AHPND has caused over USD $10 billion to shrimp aquaculture worldwide and today White spot syndrome disease AHPND are the two most economically important diseases threatening shrimp aquaculture worldwide. Among fungal disease, *Enterocytozoon hepatopenaei* (EHP), a microsporidium is the most economically important disease that has caused serious losses in recent years (Rajendran *et al*., 2016). This review will provide an overview of AHPND and EHP diagnosis and strategies to managing these diseases to prevent continued losses in shrimp aquaculture due to these diseases.

Acute Hepatopancreatic Necrosis Disease (AHPND)

Clinical signs and histopathology

The AHPND infected shrimp show pale or discoloration hepatopancreas, empty or interrupted digestive tracts. Generally, the mortality occurs within the initial 20–30 days after stocking a pond with postlarvae (PL), although there is a report that the disease can occur in late stage juveniles even at 94-day post-culture (De La Peña

et al., 2015) (**Fig. 1A**). Histopathology of AHPND susceptible shrimp primarily shows two phases of disease development defined as acute and terminal phases (Tran *et al*., 2013), and more recently, in AHPND tolerant lines a third phase, a chronic phase has been described (Aranguren *et al*., 2020c) The acute phase is characterized by the medial to distal dysfunction of HP tubule cells such as B (blister like), F (fibrillar), and R (resorptive) cells, prominent karyomegaly, and lack of mitotic activity in E cells (embryonic). In the terminal phase, the hemocytic infiltration and a secondary bacterial infection were observed in infected hepatopancreases (Lai *et al*., 2015; Lightner, 2012). In the chronic phase, melanized granuloma and hemocytic nodules in the HP that resembles septic hepatopancreatic necrosis (SHPN) are observed (Aranguren *et al*., 2020c; Mai *et al*., 2021) (**Figure1B**).

Geographical distribution

Acute Hepatopancreatic Necrosis Disease emerged in China in 2009 (Lightner and Flegel, 2012). Since then, AHPND was reported in several shrimp producing countries from Asia to Latin America such as Viet Nam (Tran *et al*., 2013), Malaysia in 2011 (Lightner and Flegel, 2012), Thailand in 2012 (Joshi *et al*., 2014), Mexico in 2013 (Nunan *et al*., 2014), the Philippines in 2014 (De La Peña *et al*., 2015), and more recently in Bangladesh (Eshik *et al*., 2017), Myanmar (Lai *et al*., 2015), USA (in Texas, 2017)(Dhar *et al*., 2019), South Korea in 2019 (Han *et al*., 2020) and Japan in 2020 (OIE, 2021)(**Figure 2**).

Etiology

The etiologic agent of AHPND was originally shown to be a specific strain of *Vibrio parahaemolyticus* called the AHPND-causing *V. parahaemolyticus* (VP_{AHPND}) (Han *et al*., 2017; Lee *et al*., 2015). *Vibrio parahaemolyticus*, is a Gram-negative, halophilic, rod-shaped bacterium which is widely present in marine environments. *Vibrio parahaemolyticus* becomes virulent after acquiring a plasmid (pVA1) that expresses a deadly binary toxin Pir^{vp} (Dong *et al*., 2017). The toxin consists of two subunits, $PirA^{vp}$ and $PirB^{vp}$, and is homologous to the Pir (*Photorhabdus* insect-related) binary toxin (Dong *et al*., 2017). The plasmid pVA1 that carries the toxin genes is 69-73 kb in size and was found to contain a cluster of genes related to conjugative transfer indicating that the

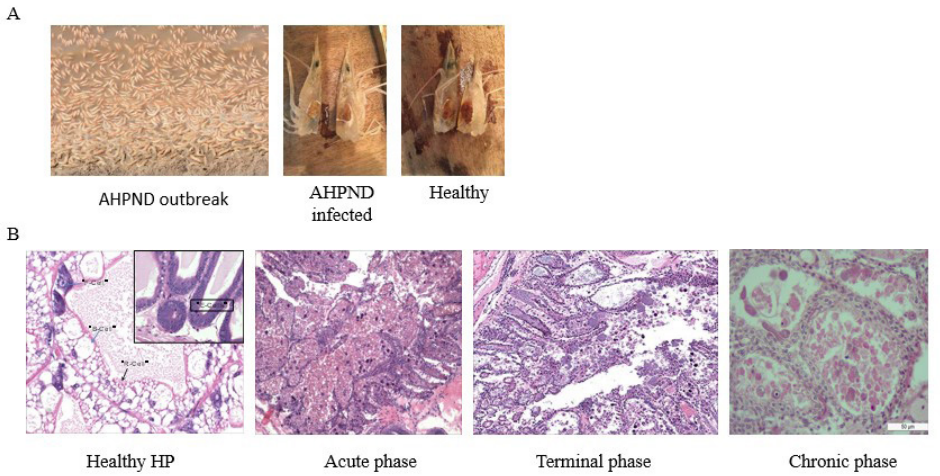

Figure 1. Clinical signs and histopathology of AHPND. (A) Clinical signs of AHPND and (B) Histopathology of AHPND (Mai *et al*., 2021)

Figure 2. Graphical distribution of AHPND. Arrows and colors indicate the countries (Tang *et al.*, 2020)

plasmid may potentially be able to transfer not only among *V. parahaemolyticus* strains, but also to different bacterial species (Lee *et al*., 2015). In fact, since the isolation of AHPND-causing *V. parahaemolyticus*, number of other *Vibrio* species causing AHPND were isolated including *V. harveyi*, *V. campbellii*, *V. owensii*, and *V. punensis* (Dong *et al*., 2017; Han *et al*., 2017; Kondo *et al*., 2015; Restrepo *et al*., 2018). The tertiary structure of $PirA^{vp}$ and $PirB^{vp}$ toxins have similarity to Cry insecticidal toxin-like protein that has a pore-forming activity leading to cell deaths in insects (Prachumwat *et al*., 2019). It is believed that AHPND-causing *Vibrio parahaemolyticus* colonizes shrimp stomach and releases binary toxin which enters hepatopancreas via the gastric sieve. The molecular mechanisms by which toxins induce cell necrosis and severe sloughing in hepatopancreas tissue in shrimp are yet to be determined.

Recently, Aranguren and colleagues identified two novel isolates of *V. parahaemolyticus* from Latin America while screening these isolates to detect binary toxin genes, *pirAB(+)* by PCR (Aranguren *et al*., (2020b). These authors reported a unique stain of *V. parahaemolyticus* (i.e., R13) that carries only *pirA* and not *pirB* while there was a second strain (i.e., R14) that carries both *pirAB* (+) genes. Interestingly, neither strain caused clinical signs of AHPND in experimental bioassay including the strain R14 that contains both binary toxin genes (Aranguren *et al*., (2020b). Genomic analysis revealed a complete deletion of *pirA* gene in R13 resulting in loss of virulence. It is possible that the deletion of *pirA* is mediated by transposase elements (**Figure 3**) which may play a role in transferring toxin genes between cells through conjugation or plasmid uptake (Lee *et al*., 2015; Tang *et al*., 2020). In the R14 strain, there is an insertion of transposase element upstream of the promoter region of *pirA* and *pirB* genes. Although insertion of transposase element did not disrupt transcription of these genes, translation of $PirA^{vp}$ and $PirB^{vp}$ toxins was inhibited as determined by western blot analysis using polyclonal antibodies against $PirA^{vp}$ and $PirB^{vp}$ toxins (Aranguren *et al*., 2020b) (**Figure 3**). This finding has a major implication in PCR-based screening of *Vibrio* isolates. Because R14 is tested positive for pirA and pirB genes by duplex PCR following OIE-recommended protocol for screening AHPND-causing *Vibrio spp.* The finding suggests DNA based detection such as PCR is not enough to conclude the pathogenicity of AHPND-causing *Vibrio* spp., and experimental bioassay needs to be conducted to delineate the pathogenic potential of the bacterial isolates(s).

In addition, genomic comparison reveals that the genome of AHPND and non-AHPND causing *V. parahaemolyticus* strains were 50-100 % identical to clinical strain of *V. parahaemolyticus* (**Figure 4**). It is worth noting that like other Gram negative bacteria, *V. parahaemolyticus* also deploys two conserved secretory systems (T3SS1 and T3SS2) to deliver virulence factors into the cytoplasm of the infected host cells (Galán *et al*., 2014). The function of T3SS1 and T3SS2 as a secretary system and its role in pathogenicity was demonstrated by Park and colleagues using a series of deletion mutants *V. parahaemolyticus* (Park *et al*., 2004). The pVA1 plasmid contains two type-II secretion systems (T2SSs), two type-III secretion systems (T3SS1 and T3SS2), two type-IV secretion systems (T6SS1, T6SS2), and two T2/4SSs (Gomez-Gil *et al*., 2014; Henke and Bassler, 2004). Interestingly, the genes responsible for T3SS1 in A3 strain (AHPND causing strain) are different from the genes in T3SS1 in non-AHPND causing strains. It is widely

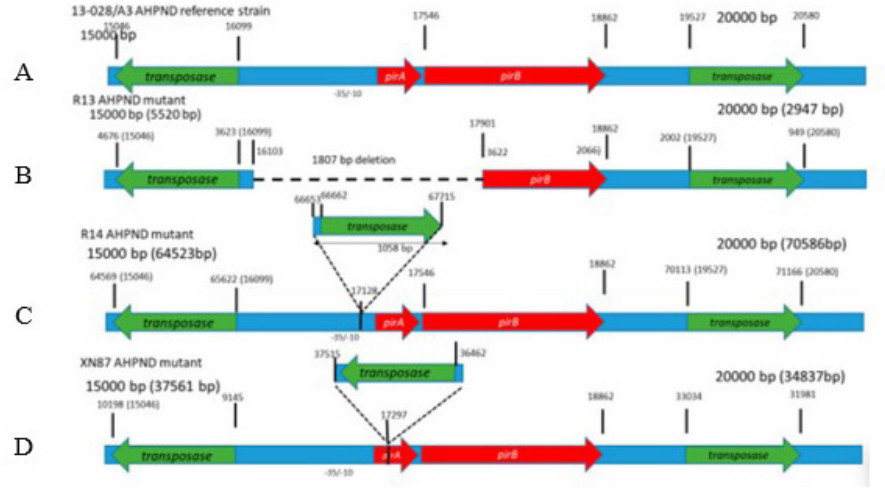

Figure 3. Comparison of the normal pVA3 plasmid (strain A3) and their mutated forms in isolate R13 and R14. (A) Scaled diagram of the pirA and pirB toxin gene regions in the normal pVA3 plasmid. (B) Mutant strain R13. Dash lines denote the absence of pirA. (C) Mutant strain R14. Notice insertion upstream of pirA. (D) XN87 AHPND mutant. The numberx over the gene organization schematics indicate the nucleotide position (Aranguren et al., 2020)

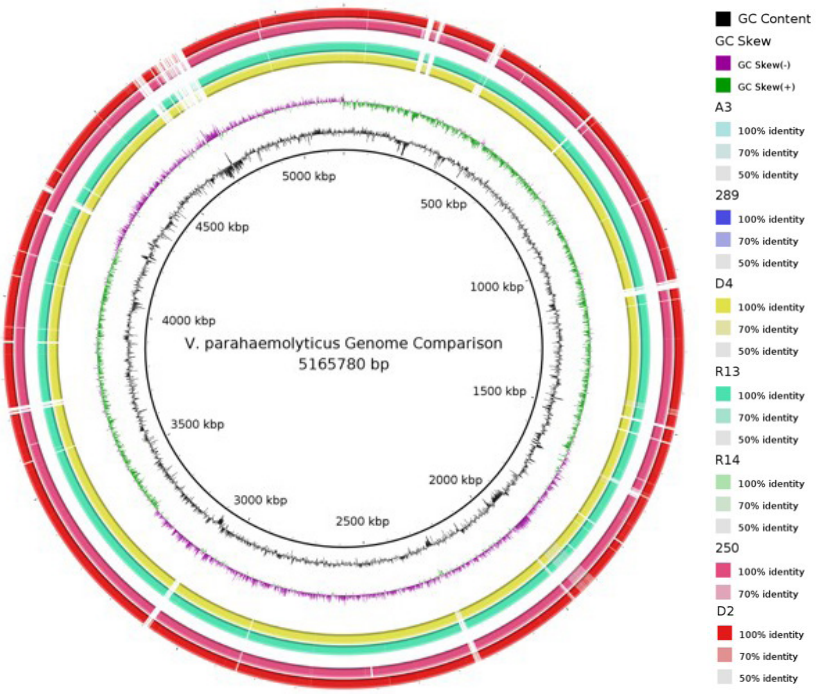

Figure 4. Genomic comparison between AHPND and non-AHPND *V. parahaemolyticus* and *V. parahaemolyticus* clinical strain RMID2210663. The graph was generated by Blast Ring Image Generator (BRID)

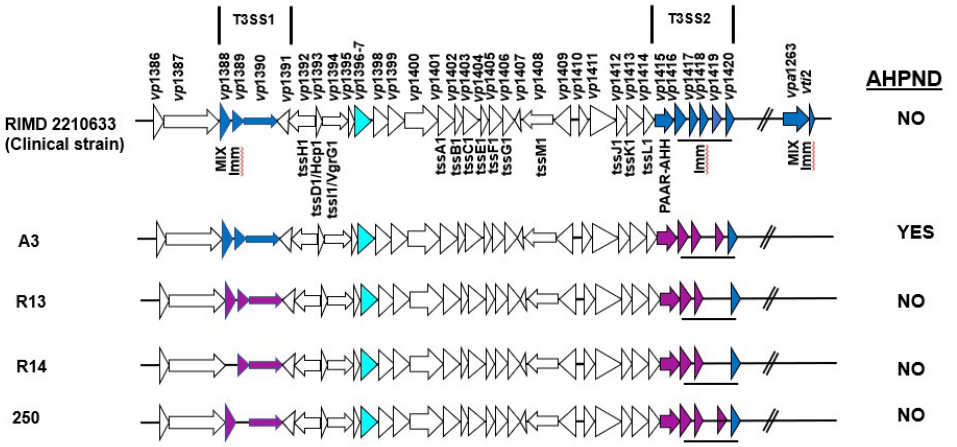

Figure 5. Comparison of genomic architecture of pathogenicity island between *V. parahaemolyticus* clinical strains versus non-AHPND and AHPND causing *V. parahaemolyticus*. Same genes were indicated by same colors.

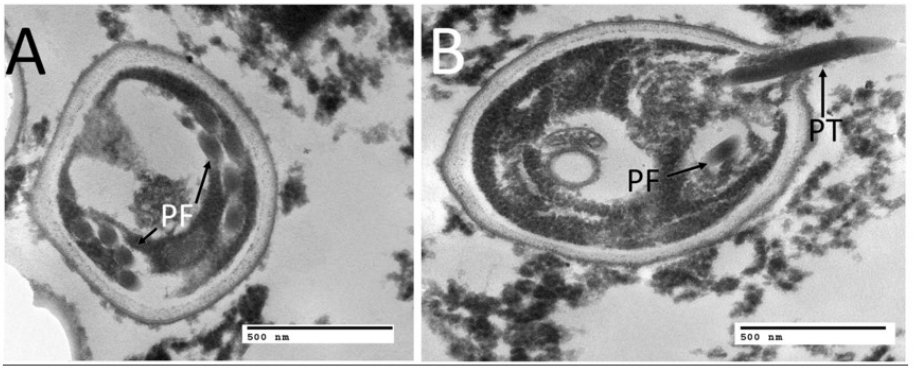

Figure 6. Electron microscopy of *Enterocytozoon hepatopenaei* from the pelleted biopsy samples. (A) Mature spore showing a section of the coiled part of the polar filament (PF), five coils are observed on one side and four coils are observed on the other side. (B) Cross section of a mature spore were a coiled portion of the PF and the polar tube (PT) are observed. Magnification of both images is 43,000× (Cruz-flores et al., 2019)

accepted that the binary toxin PirAB*vp* is a secreted protein, thus, the secretion systems, especially T3SS, found in pVA1 may contribute to the virulence of AHPND causing strains.

Diagnosis

AHPND is a threat to sustainability of global shrimp aquaculture. Therefore, early detection via screening of broodstock and post larvae, shrimp feed and feed ingredients are necessary to prevent further spread and disease outbreaks. Since 2013, several methods have been developed to detect AHPND. Initially, AHPND has been detected by histopathology (Tran *et al*., 2013) but histopathology alone is not enough to unequivocally confirm AHPND. Later several molecular methods were developed to detect AHPND. Detection of

AHPND using molecular method initially targeted pVA1 plasmid as a marker (Lo and Flegel, 2014). However, when pirA and pirB genes encoding binary toxin, PirAB were identified as virulent factors in *V. parahaemolyticus*, efforts were made to develop a PCR-based assay to detect the toxin genes causing AHPND (Han *et al*., 2015b). Since then, pirA and pirB genes have become molecular hallmark of AHPND detection. Both conventional PCR (Dangtip *et al*., 2015; Han *et al*., 2015b; Tinwongger *et al*., 2014) and real-time PCR using *Taq*Man chemistry (Cruz-Flores *et al*., 2019a; Han *et al*., 2015a) and SYBR green dye (Cruz-Flores *et al*., 2019a; Han *et al*., 2015a) were developed for the detection of AHPND. More recently, point-of-care diagnostic methods including a loop-mediated isothermal amplification (LAMP) and a recombinase polymerase amplification (RPA)-based detection methods have been reported for AHPND detection at a pond-site (Koiwai *et al*., 2016; Mai *et al*., 2021). Apart from DNA based detection, polyclonal and monoclonal antibodies against PirA and PirB toxins have been generated to detect AHPND via ELISA and western-blot assays (Mai *et al*., 2020b; Wangman *et al*., 2017).

Hepatopancreatic Microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei*.

Hepatopancreatic microsporidiosis

Unlike many diseases causing large-scale mortalities, retarded growth syndrome receives less attention until the economic impact of this syndrome is reported. For example, in 2002 in Thailand the losses caused by retarded growth syndrome was estimated as much as $3M (Chayaburakul *et al*., 2004). Although there are several microbial agents reported to be associated with retarded growth

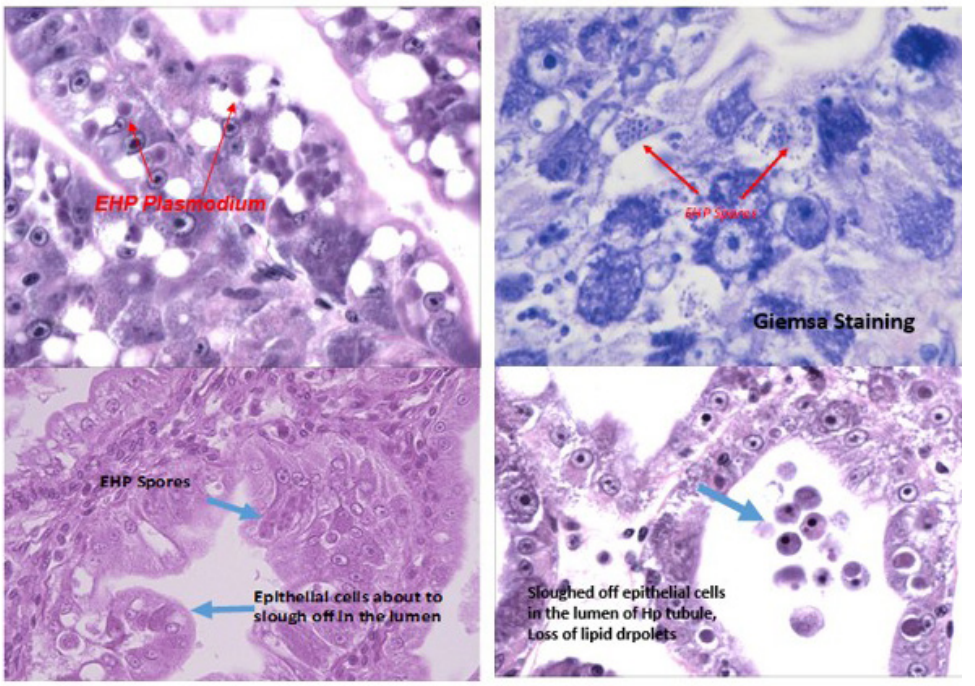

Figure 7. Histopathology of EHP infected Hepatopancrease

syndrome, a microsporidian species called *Enterocytozoon hepatopenaei* (EHP) was found to be involved with retarded growth syndrome in *P. vannamei* shrimp (Chayaburakul *et al*., 2004; Tourtip *et al*., 2009). Microsporidia were first identified in silkworm as a causal agent of Pebrine disease in domesticated silkworm 150 years ago (Han and Weiss, 2017). Microsporidia are intracellular parasite and has now been reported from an wide array of invertebrate and vertebrate species including *Nosema apis* and *Nosema caeranae* in honeybee, *Loma salmonae* in Salmon *Oncorhynchus kisutch* and EHP in shrimp (Fries, 1993; Kent *et al*., 1989; Tourtip *et al*., 2009). Although EHP has been identified since 2009, the potential threat of the parasite was largely ignored due to the impact of AHPND. However, EHP soon emerged as a threat for its negative impact on shrimp production.

Biology and pathogenicity of EHP

EHP was first reported from Thailand in 2009 and found to be associated with retarded growth syndrome *P. vannamei* grow-out ponds. Since then, EHP has been detected in most of retarded growth syndrome pond from other shrimp producing countries including Vietnam, India, Indonesia, Malaysia, China in Asia, and Venezuela in the western hemisphere. EHP is known to infect several shrimp species including *P. monodon*, *P. vannamei*, *P. stylirostris* (Tang *et al*., 2015). EHP was also detected in artemia (Tang *et al*., 2015) although pathogenicity of EHP to artemia has not been demonstrated. It is and presumed to serve as a carrier of the parasite in shrimp hatchery. EHP has been detected in *P. vannamei* cultured in high and low salinities although the severity of infection is higher in high salinity (30 ppt) than low salinity (2 and 15 ppt) (Aranguren *et al*., 2021). Since EHP was detected in a wide range of salinities, it is speculated that there are probably other host species of EHP apart from marine penaeid shrimp species. In laboratory challenge assays, EHP was successfully transmitted through oral administration, reverse gavage and via direct injection of the inoculum into hepatopancreas (Mai *et al*., 2020a). Moreover, EHP has been reported to be associated with *P. vannamei* grow-out ponds displaying white feces syndrome (WFS) (**Figure 8A**). The prevalence of EHP was found to be higher in WFS ponds compared to ponds displaying no WFS (**Figure 8B**) (Aranguren *et al*., 2020a). In addition, using a case-control study, it has been reported that EHP is a risk factor for AHPND since shrimp pre-infected with EHP exhibited higher mortality compared to uninfected shrimp (Aranguren *et al*., 2017).

In the natural environment, EHP exists as mature spores. The life cycle of EHP is not known completely. However, based on information in the published literatures and analogy to the life cycle of other microsporidia where different stages are well known, a tentative life cycle of EHP can be drawn. Most of microsporidian species start infecting host cells by using the extruded polar tube puncturing the plasma membrane of host cells and transfer the content of the spore, the sporoplasm into host cell cytoplasm. Then, the sporoplasm develops into a plasmodium. Once the extrusion precursors (i.e., polar filament and anchoring dish) are generated, the cytoplasm of plasmodium is cleaved to sporoblast. Finally, the sporoblast develops into mature spores which are released and infect other cells (Chaijarasphong *et al*., 2020). EHP spores are unicellular containing coiled polar filaments with the size of 1.1 ± 0.2 µm × 0.6 ± 0.2 µm(Cruz-Flores *et al*., 2019b; Tourtip *et al*., 2009). The spores have shared a common ultrastructure with other microsporidian

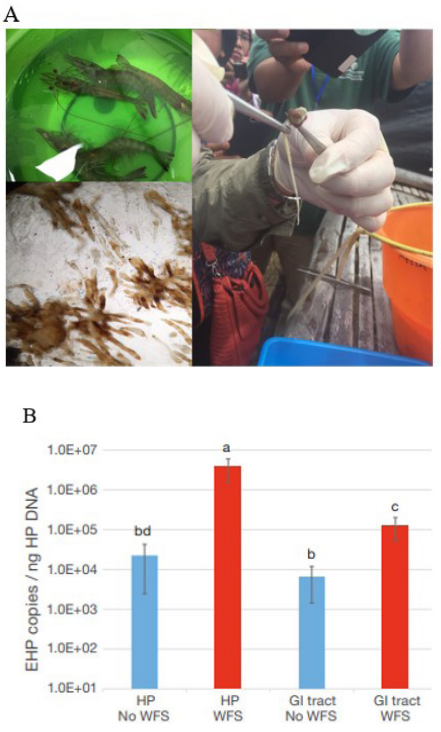

Figure 8. The association of EHP and White feces syndrome (WFS). (A) Clinical sign of WFS. (B) EHP quantification in Hepatopancreases (HP) and GI tracts from different types of samples

spores in containing a plasma membrane covered by two electron-dense layers, and a nucleus surrounded by polar tube coils (**Figure 6**). Histopathology of EHP-infected hepatopancreas reveals epithelial cell sloughing into lumen of hepatopancreatic tubules, and spores and plasmodium could be seen in the detached cells in the lumen (**Figure 7**). A draft genome sequence of EHP has been published and a putative spore wall protein (SPW) gene has been identified although its function remains unknow. It is presumed that, like other microsporidia, SWP of EHP enables spore to maintain its shape and withstand environmental stress. Since SWP represents the outer layer of spore, it may be involved in some critical steps in EHP pathogenesis including adherence to host cells and spore extrusion.

Diagnosis

The current standard for detection of EHP include histopathology, *in situ* hybridization (ISH), and PCR. The use of gross clinical signs is not typically very reliable due to the EHP infection requiring around 30 to 45 days to cause slow growth syndrome. Initially, H&E histology was developed to detect EHP. The H&E of EHP infected HP is characterized as the presence of EHP life stages and mature spores in the tubule epithelial cells and the sloughing off of epithelial cells (Aranguren *et al*., 2017). However, the sensitivity of H&E method does not meet the requirement in EHP prevention strategy. Moreover, the result of histopathology needs to be confirmed by *in situ* hybridization (ISH). The DIG-labelled 18SrRNA gene probe is used in *in situ* hybridization assay to detect EHP. *In situ* hybridization enables to determine the severity of EHP infection because all life stages of EHP from meront to mature spore can be detected even with a low magnification (Tang *et al*., 2015). However, ISH is a technically challenge method, takes long time and is not feasible to use as a routine method to detect EHP in laboratory where resources are limited.

Molecular detection methods were developed based on EHP 18S rRNA or SSU rRNA gene sequence (Tang *et al*., 2015) (**Figure 7** and **Figure 9**). Using PCR EHP can be successfully detected in different samples including *P. vannamei* tissue, feces, artemia and contaminated water (**Figure 9A**). However, PCR assay for EHP detection has been problematic as it gives false positive results with the samples collected from environment. It was shown that the annealing site of primer developed by Tangprasittipap and colleagues (Tangprasittipap *et al*., 2013) has 66.7 %–90 % identity to that of other microsporidia resulting in false positive result. Subsequently, Jaroenlak *et al*.,

(2016) and Han *et al*., (2018) developed nested PCR based on the sequence of spore wall protein and □-tubulin genes. The specificities of the later methods are higher than method where primers are designed based on SSU rRNA sequence.

Apart from conventional PCR, *Taq*Man-probe based qPCR for EHP quantification also has been developed (Liu *et al*., 2018). The primers and probes used in real-time PCR assay are designed based on SSU rRNA gene sequence. There is a need to further improve the specificity and sensitivity of the currently available real-time PCR for EHP quantification considering occasional spurious results are obtained while using feed and feed ingredients for EHP screening (Dhar *et al*., unpublished).

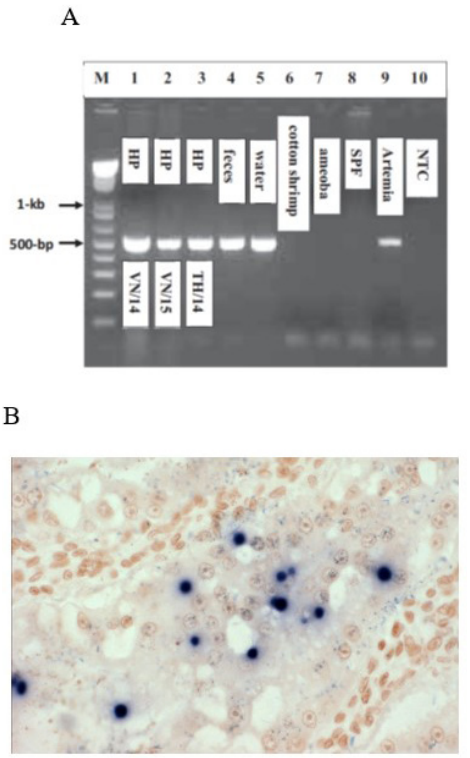

Figure 9. EHP detection by PCR based on 18S rRNA gene. (A) EHP detection in several samples by PCR. (B) EHP detection by in situ hybridization method. Dark blue spots indicated positive reactions

EHP can be transmitted via contaminated water, and it is presumed that EHP can also be transmitted from broodstock to offspring. Therefore, broodstock screening is recommended to prevent EHP spread in the hatchery. Sacrificing broodstock is a very expensive proposition. Recently, Cruz-Flores *et al*. (2019b) developed an invasive but non-lethal method for sampling hepatopancreas for EHP detection (**Figure 10**). Using hepatopancreas biopsy tissue, EHP was detected by real-time PCR and transmission electron microscopy. Since often EHP is not uniformly present throughout the hepatopancreas tissue, it remains to be determined if EHP detected by conventional PCR detection method using an aliquot of entire hepatopancreas homogenate is comparable to tissue biopsy to determine the feasibility of hepatopancreas biopsy as a means of EHP screening of EHP.

Management of AHPND and HPM

It is well known that shrimp diseases like AHPND and HPM need to be controlled in hatchery and grow-out pond levels. Considering the lack of a therapeutic, bio-security remains as a most efficient approach to control AHPND causing *Vibrio* sp. and EHP causing. At a hatchery level, broodstock should be routinely screened for *Vibrio spp*. containing *pir*A/*pir*B genes and EHP. Similarly, before introducing new stock to enhance genetic diversity in a captive breeding program, the new stock should be screened while in a quarantine facility prior. In addition, EHP can be introduced in a hatchery via contaminated feed such as live polychaetes. Thus, live polychaetes and potentially other live feed should be screened for EHP and AHPND prior to feeding broodstock. Recently, Mai *et al*., (2020a) and Munkongwongsiri *et al*., (2021) showed EHP can be inactivated by either freezing at -20 ^{0}C or boiling at

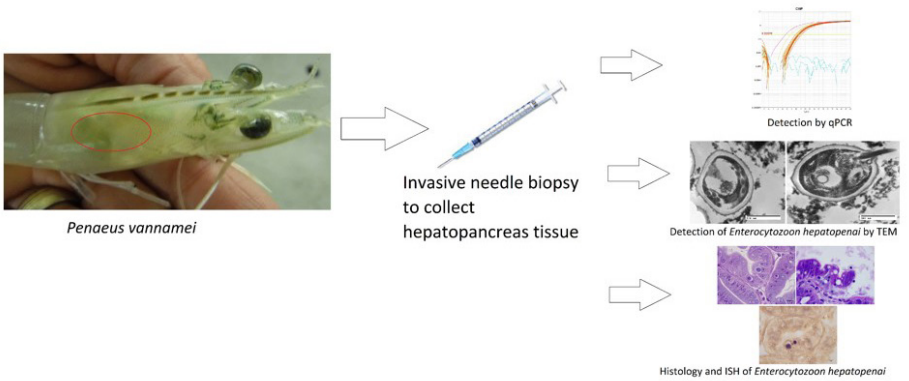

Figure 10. A schematic overview of EHP detection using invasive non-lethal sampling method. (Cruz-Flores et al., 2019)

75 ^{0}C for 1 minute. Therefore, due to lack of formulated feed, feeding live polychaetes to broodstock should be avoided and instead freezing or boiling polychaetes is recommended. In grow-out ponds, the post-larvae (PL) should be screened for the presence of *pir*A/*pir*B toxin genes and EHP prior to stocking. In addition, *pir*A/*pir*B and EHP can be detected in water and sediment thus reducing the buildup of organic waste by water exchange/ treatment is recommended to reduce the risk of AHPND and HPM. There is a need to develop AHPND and HPM resistant/ tolerant shrimp to prevent the further spread of these diseases. In endemic area, to minimize the losses due to these diseases stocking of ponds with tolerant/ resistant lines are needed. In nature, host–pathogen interaction is considered as a highly dynamic process, and when the interaction is tipped, disease outbreak occurs (**Figure 11**). Understanding the biological and environmental factors that modulate this balance is critical to avoid disease outbreaks.

Future direction in AHPND and HPM research

It has now over a decade that shrimp aquaculture has been affected by AHPND and HPM. For the past decade, considerable efforts have been made to understanding the biology of these diseases and developing tools to diagnose these diseases and preventing their global spread. While these progresses have been instrumental in thwarting the negative impact of these diseases, there are still major gaps in understanding the pathogenesis of these diseases. For example, the receptors of AHPND toxin have not been fully elucidated. Identifying these receptors will contribute to our understanding of disease development at a molecular and cellular levels. This may also aid in developing therapeutics to control AHPND since use of antibiotics to control AHPND is not a feasible option due to the development of antimicrobial resistance. methods to the end of AHPND outbreaks through AHPND toxin receptors blocking. However, controlling AHPND by utilizing AHPND receptors might be challenging because AHPND toxin may utilize more than receptor, and those receptors may play important roles in basic cellular processes. Understanding the molecular and cellular basis of AHPND is certainly a fertile area of future research. Recently, we have identified a genetic line of *P. vannamei* shrimp that shows resistance to AHPND (Aranguren *et al*., 2020c). Such a line could be a valuable genetic resource to identify marker(s) for AHPND resistance/

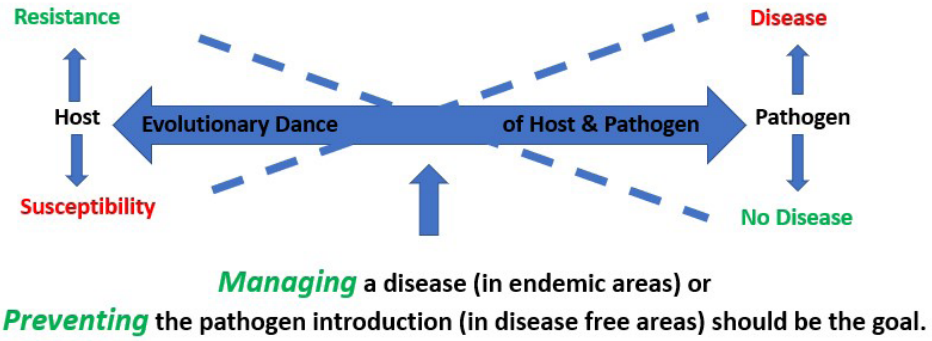

Figure 11. A schematic overview of host-pathogen evolution in shrimp diseases

susceptibility. Similarly, the role of EHP in white feces syndrome has not been demonstrated unequivocally. Therefore, identifying microbiological factors such as involvement of bacteria or environmental factors needs to be determined. Availability of an experimental model to reproduce white feces syndrome at a laboratory level is urgently needed to screen genetic lines that are resistant to HPM and whit feces syndrome.

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