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Acute Hepatopancreatic Necrosis Disease (AHPND): An Emerging Threat to the Shrimp Aquaculture Industry

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Abstract

Acute Hepatopancreatic Necrosis Disease (AHPND) is an emerging shrimp disease caused primarily by certain virulent *Vibrio parahaemolyticus* strains possessing a plasmid with a gene that encodes for Pir A and Pir B toxins. AHPND affects many shrimp species, notably *Penaeus monodon* (giant tiger prawn) and *Litopenaeus vannamei* (whiteleg shrimp) in their post-larval stages within 30-35 days after stocking and can cause mass mortalities up to 100%. AHPND causes devastating economic losses of about billions of dollars annually to the shrimp aquaculture industry. The pathogen primarily targets gut-associated organs and tissue and therefore causes clinical symptoms such as pale and shrunken hepatopancreas, an empty stomach, and an empty gut. Proper diagnosis and treatment methods are necessary to control and prevent further outbreaks. This article highlights the history of AHPND, its causative agent, vulnerable shrimp species, signs and symptoms, diagnosis and detection methods, and control and prevention methods of AHPND.

1. Introduction

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium widely disseminated in marine environments worldwide. A specific strain of this bacterium causes Acute Hepatopancreatic Necrosis Disease (AHPND), which is formerly known as Early Mortality Syndrome (EMS). It typically affects shrimp post-larvae within 30–35 days after stocking and can cause mass mortalities of up to 100 percent (Leobert et al., 2015). Reports regarding the disease reveal greater susceptibility in *Penaeus monodon* (giant tiger prawn) and *Litopenaeus vannamei* (whiteleg shrimp). However, other shrimp species such as *Penaeus chinensis* (fleshy prawn) and *Penaeus japonicus* (kuruma prawn) are also known to be affected (OIE, 2019). It is an emerging disease that has caused significant economic losses to the shrimp aquaculture industry ever since the first outbreak in China in 2009. Thereafter, it has been reported in many other countries, especially in Southeast Asian countries.

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2. History of Pathogenesis

In 2009, a newly emerging disease in shrimp was reported in China and was initially named 'early mortality syndrome' or 'EMS' (more descriptively called acute hepatopancreatic necrosis syndrome or AHPNS). The disease continued to spread as an epidemic in other southeast Asian countries like Vietnam (2010), Malaysia (2011), Thailand (2012), Philippines (2013), and Mexico (2013). Since its first emergence, the causative agent of AHPNS remained unknown. However, in 2013, a group from the University of Arizona identified that the etiologic agents of EMS/AHPND were specific virulent strains of *V. parahaemolyticus* (Tran et al., 2013).

3. AHPND Causing *V. parahaemolyticus* Strains

The primary causative agent of AHPND is *V. parahaemolyticus*, a Gram-negative, curved, rod-shaped, halophilic bacterium that has acquired plasmids encoding the deadly binary toxins Pir A/Pir B, which cause the infected shrimp to die quickly. These specific virulent *V. parahaemolyticus* (*Vp* AHPND) strains contain a ~70-kbp plasmid containing genes encoding homologues of the *Photorhabdus* insect-related (Pir) binary toxin, Pir A and Pir B (Yang et al., 2014; Lee et al., 2015). The Pir A and Pir B toxins are the main virulence factors associated with the AHPND causing *V. parahaemolyticus* strains. The plasmid inside *Vp* AHPND is known as pVA1, and its size may vary slightly. The capacity of *Vp* AHPND strains to cause AHPND is eliminated when pVA1 is removed (or "cured"). A cluster of genes linked to conjugative transfer is also found on the plasmid pVA1, which depicts its potential to transfer to other bacteria. Some studies have shown that certain other *Vibrio* species like *V. owensii* (Liu et al., 2015), *V. campbellii* (Han et al., 2017), and a strain close to *V. harveyi* (Kondo et al., 2015) are known to cause AHPND. The presence of pVA1-like plasmids in these *Vibrio* species suggests that the toxin genes can be passed to other *Vibrio* species (Kondo et al., 2015) via horizontal gene transfer. Thus, the ability of these toxin genes to mobilize through recombination events, transposition, conjugation, and plasmid uptake during infection period concerns the future of the shrimp aquaculture industry as there is a significant risk of conversion from non-pathogenic to pathogenic strain, which would have a favourable impact on the spread of AHPND (Restrepo et al., 2018).

4. Vulnerable Species (Penaeid Shrimp)

The most susceptible shrimp species to AHPND are *Penaeus monodon* (giant tiger prawn) and *Litopenaeus vannamei* (whiteleg shrimp). Other shrimp species such as *Penaeus chinensis* (fleshy prawn) and *Penaeus japonicus* (kuruma prawn) are also susceptible. However, the complete evidence to fulfill the criteria for listing as susceptible to AHPND is not yet specified. Gut-associated tissues and organs are the primary targets for AHPND causing *V. parahaemolyticus* strains. Mortalities occur within 30–35 days and as early as ten days of stocking shrimp ponds with post-larvae (PL) or juveniles (Nunan et al., 2014; Soto-Rodriguez et al., 2015). However, mortality as late as 46-96 days after stocking has been reported during a disease outbreak in the Philippines (Leobert et al., 2015).

5. Signs and Symptoms

The onset of clinical signs and mortality can start as early as ten days post-stocking (OIE, 2019). Gross symptoms of the disease include lethargy, slow growth, soft shells, an empty stomach and midgut, and a pale to white atrophied hepatopancreas (HP) (Figure 1), black spots or streaks visible within the HP (due to melanized tubules), with death at the bottom of the pond. Behaviour changes, such as sluggishness, swimming spirally, and reduced feeding, are also observed (Zorriehzahra and Banaederakhshan, 2015).

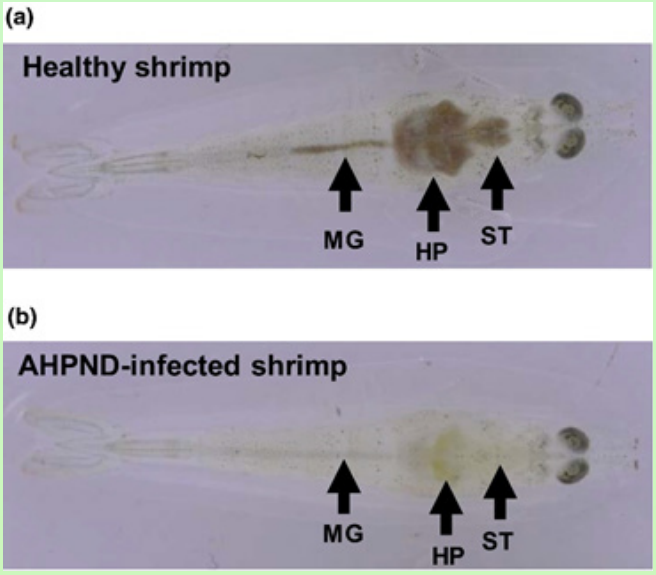

Figure 1: Gross clinical signs of acute hepatopancreatic necrosis disease (AHPND) infection in *Litopenaeus vannamei*. The normally brown midgut (MG), hepatopancreas (HP) and stomach (ST) that as seen in (a) healthy shrimp all turn pale in (b) AHPND-infected shrimp (Kumar et al., 2020)

6. Diagnosis and Detection Methods

The gross clinical signs and behavioural changes, as stated above, can be used as a preliminary diagnostic method to examine affected shrimps. However, these symptoms can only be used to make a preliminary diagnosis; a histological examination is needed to validate the diagnosis. Sloughing and massive rounding of hepatopancreatic tubule epithelial cells are the key and special histological characteristics of AHPND in the early to middle stages of the disease, with no detectable causative pathogen (Figure 2) (Tran et al., 2013). These characteristics are crucial for diagnosis, so ten or more shrimp specimens should be obtained and examined from any suspected pond to ensure that at least one specimen is in this stage of the disease.

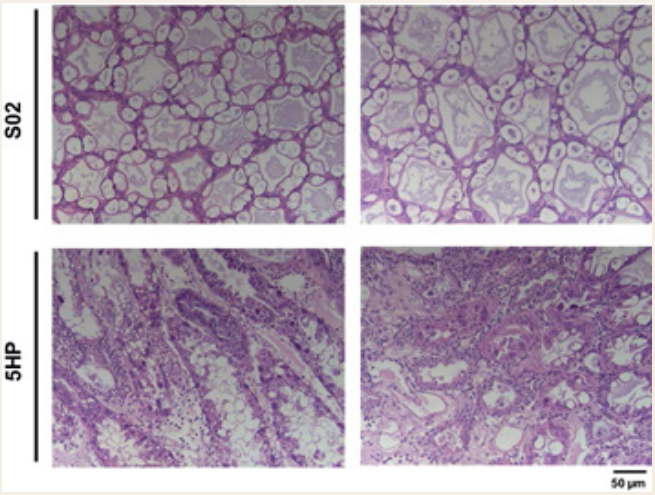

Figure 2: Histology of AHPND infected shrimp hepatopancreas. Haematoxylin- and eosin-stained hepatopancreas from shrimps infected with non-AHPND-causing (S02) and AHPND-causing (5HP) *V. parahaemolyticus*. The S02-infected group shows normal tubules in hepatopancreas, while the 5HP-infected hepatopancreas shows the characteristic signs of AHPND, with sloughed epithelial cells, necrosis and infiltration of hemocytes. Scale bar: 50 µm. (Kumar et al., 2020)

Other than histological methods, molecular approaches can be used as a diagnostic tool in examining AHPND-suspected samples. Polymerase chain reaction (PCR) and Loop-meditated isothermal amplification (LAMP) method are the most frequently used molecular methods to detect AHPND. The PCR methods used for the detection of AHPND targets Vp_{AHPND} toxin genes, and are sensitive and specific. Using the AP3 primer, which targets the Pir A *V. parahaemolyticus*, is the most promising new PCR approach for detecting AHPND because it has high sensitivity and specificity (Soto-Rodriguez et al., 2015). LAMP is another reliable and convenient method which generates results that can be easily interpreted suitably for detecting the early onset of AHPND (Kongrueng et al., 2015).

7. Control and Prevention

AHPND can be prevented by following good sanitary and biosecurity practices, such as improvement of hatchery sanitary conditions and post-larvae (PL) screening; proper broodstock management, use of high-quality PL and effective shrimp farm management, such as strict feeding rate regulation, reasonable stocking density, and so on (NACA, 2012). Antibiotics can be used to treat outbreaks caused by *Vibrio* species; however, antibiotic resistance and the potential spread of the drug in the environment are major concerns. Other potential approaches to control and prevent AHPND include phage therapy, the use of probiotics, and immune priming (Santos et al., 2020). Immune priming is a two-step vaccine procedure in which pathogens are first introduced into the host's system, then a secondary vaccination or infection with the same pathogen is conducted.

8. Conclusion

AHPND can be controlled and prevented by following good management practices. However, more research is needed to find a complete cure to control and prevent further outbreaks. Potential prevention and treatment methods such as phage therapy, use of probiotics, and immune priming have shown promising results. However, more studies are required to establish the methods and find the most optimum dosage and schedule for treatment.

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