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ACUTE HEPATOPANCREATIC NECROSIS DISEASE: A NEW EMERGING THREAT IN THE SHRIMP INDUSTRY - A REVIEW

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ABSTRACT

Acute hepatopancreatic necrosis disease (AHPND) is considered to be a new emerging shrimp disease currently affecting brackish water shrimp aquaculture in Southeast Asia including Viet Nam. It was first recorded in shrimp farms in China in 2009 and then in Vietnam in 2010, Malaysia in 2011, Thailand in 2012, and Mexico in 2013. It has been found that AHPND is caused by unique bacterial strains belonging to the *Vibrio* group. AHPND-causing strains have been found to be carried in a plasmid harboring virulent Pir A/B genes that code for the two toxin proteins inducing AHPND in shrimp. This paper will update all research progress on AHPND, including epidemiology and risk factors, etiology, disease diagnosis, disease prevention, and management. This review will serve as a useful introduction for researchers who are currently unfamiliar with AHPND and will hopefully encourage readers to participate in the research efforts to reduce AHPND's impact on shrimp aquaculture.

Keywords: Acute hepatopancreatic necrosis disease -AHPND, shrimp, *Vibrio parahaemolyticus*, *Vibrio*.

Bệnh hoại tử gan tụy cấp: Mối đe doạ mới đối với nghề nuôi tôm công nghiệp

TÓM TẮT

Bệnh hoại tử gan tuỵ cấp (AHPND) đã và đang được xem là một bệnh tôm nguy hiểm mới xuất hiện gần đây ảnh hưởng đến nghề nuôi tôm nước lợ ở khu vực Đông Nam Á trong đó có Việt Nam. Bệnh được ghi nhận đầu tiên tại các trang trại nuôi tôm ở Trung Quốc vào năm 2009, sau đó lần lượt được phát hiện thấy ở Việt Nam và Malaysia năm 2011, Thái Lan năm 2012 và Mexico năm 2013. Các nhà khoa học đã phát hiện ra rằng AHPND bị gây ra bởi các chủng vi khuẩn thuộc nhóm *Vibrio*. Các chủng vi khuẩn gây bệnh AHPND có chứa Plasmid mang các gen độc lực Pir A/B mã hoá cho các protein gây ra hiện tượng hoại tử cấp ở gan tuỵ tôm. Bài viết này sẽ cập nhật tất cả các thông tin về AHPND, bao gồm các thông tin về dịch tễ học và các yếu tố nguy cơ, tác nhân gây bệnh, chẩn đoán bệnh, và biện pháp phòng trị bệnh. Đây sẽ là nguồn thông tin hữu ích cho các nhà nghiên cứu quan tâm đến AHPND cũng như khuyến khích các nhà khoa học tiếp tục nghiên cứu để góp phần ngăn ngừa, giảm thiểu ảnh hưởng của AHPND đối với nghề nuôi tôm nước lợ.

Từ khoá: AHPND, Bệnh hoại tử gan tuỵ cấp, tôm, *Vibrio, Vibrio parahaemolyticus.*

1. INTRODUCTION

According to FAO estimates, to feed the world in 2050, agricultural output must increase by over 70 percent (www.fao.org). Among these agriculture outputs, aquaculture production has taken over as a major supply factor. Aquaculture, especially shrimp culture, makes valuable contributions to the local, national, and regional economies through goods and services sold in domestic and export markets.

As a result of the rapid changes of shrimp culture worldwide in terms of increasing both farming areas and culture densities, newly emerged diseases and occurrences of other diseases have increased year to year. The shrimp farming industry has been suffering from many serious infectious diseases such as white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV), etc. Since 2009, and a new emerging disease known as Early Mortality Syndrome (EMS), descriptively called as Acute Hepatopancreatic Necrosis Disease (AHPND), has been a major issue of concern for economic losses in the shrimp farming industry (Leano and Mohan, 2012; FAO, 2013).

The aim of this paper is to review the current information on AHPND, including epidemiology, etiology, as well as disease diagnosis, and control measures in hatcheries and shrimp farms.

2. EPIDEMIOLOGY AND RISK FACTORS

2.1. Geographical distribution of AHPND

AHPND caused mass mortality in China (2009) initially, and then in Vietnam (2010), followed by Malaysia (2011), Thailand (2012) (FAO, 2013), and Mexico (2013) (Fig. 1) (De Schryver *et al.,* 2014; Zorriehzahra and Banaederakhshan, 2015). Recently, the presence of AHPND has also been confirmed in the Philippines in 2015 (de la Pena *et al.,* 2015; Dabu *et al.*, 2017), Latin America in 2016 (Han, 2017), and Korea in 2016 (according to information from National Institute of Fisheries Science in Korea, unpublished data).

In China, shrimp farming in Hainan, Guangdong, Fujian, and Guangxi provinces suffered during the first half of 2011 with almost 80% losses. In Malaysia, the outbreaks of AHPND resulted in a significant drop in *P. vannamei* production, from 70,000 million tonnes in 2010 to 40,000 million tonnes in 2011 (Leano and Mohan, 2012). In Thailand, related to the impact of AHPND, shrimp production dropped from an all-time high of approximately 600,000 metric tons in 2011 to less than 200,000 in 2014 (Thitamadee *et al.*, 2016). In Mexico, the disease caused about 118 million USD economic losses in part of this country (De schryver *et al.*, 2014).

In Vietnam, AHPND affected almost all shrimp production areas throughout the country with a total affected shrimp area of around 28,000 hectares in the year 2012. According to DAH (Department of Animal Health) reports, although the shrimp culture areas affected by AHPND decreased significantly from 2012 to 2016, the affected culture locations increased from 192 communes to 299 communes, respectively (DAH reports, 2014; 2015; 2016; Fig. 2).

Fig. 1. Geographical distribution of AHPND

Source: Zorriehzahra and Banaederakhshan, 2015

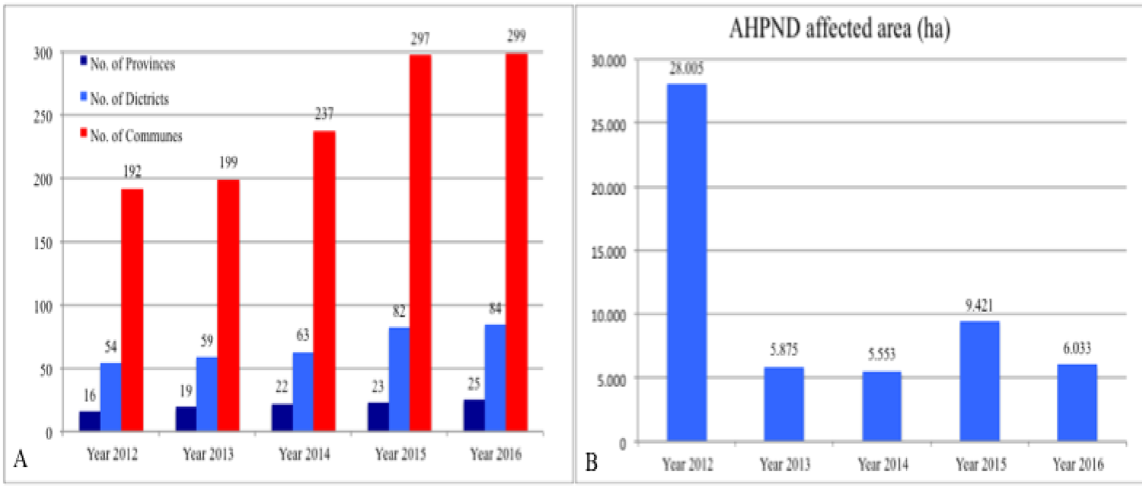

Fig. 2. Spread of AHPND in Vietnam throughout years

Note: A. Affected location; B. Affected area (ha)
Source: DAH reports 2014; 2015; 2016

2.2. Susceptible species

Important cultivated shrimp, including black tiger shrimp (*Penaeus monodon*) and white leg shrimp (*P. vannamei*), have been reported to be susceptible to AHPND. The disease has also been reported in *P. chinensis* in Southeast Asia while other shrimp species are resistant or less susceptible to AHPND (OIE, 2013). The main susceptibility stage of shrimp is during the early cultivation period approximately 35 days after stocking (Hong *et al.*, 2016).

2.3. Transmission mode

Based on experimental and natural observations, AHPND could be transmitted by oral routes and cohabitation (OIE, 2013). Affected water, affected cultured shrimp, affected PLs, and broodstocks have been considered as materials transferring AHPND pathogens.

It is important to consider that AHPND is caused by unique bacterium strains belonging to the *Vibrio* group and carries virulence genes. *Vibrio* has been consistently identified as one of the dominant bacteria in the natural intestinal flora of penaeid shrimp and is widespread throughout the shrimp aquaculture industry. Moreover, it is known that the virulence genes causing AHPND are located on the plasmid and thus are easily spread between *Vibrio* strains via horizontal mode because the plasmids can transfer genetic material from bacteria to bacteria, leading to a virulence switch and the production of toxins (Corteel, 2016). Taken together, it seems that the spread of pathogens causing AHPND is quite easy in shrimp ponds.

2.4. Factors contributing to the spread of AHPND

According to FAO (2013), Zorriehzahra and Banaederakhshan (2015), Corteel (2016), and Nguyen *et al.* (2016), the following conditions have been considered as risk factors contributing to the incidence and prevalence of AHPND:

(1) High salinity water (Salinity > 5)

(2) High pH water (pH > 7)

(3) Temperature fluctuations

(4) Low oxygen conditions

(5) High amount of *Vibrio* spp. in the water

(6) Sludge/organic substrate in pond bottoms

(7) High stocking density

(8) Lack of reservoir ponds and water treatment

(9) Pond preparation irregularly

(10) Stress for shrimp

(11) Feed mismanagement

(12) Sensitivity of species

(13) Transfer of virulence genes caused acute necrosis of HP cells between *Vibrio* species

(14) Poor/affected shrimp stock

3. ETIOLOGY

After the first occurrence and spread of AHPND in Southeast Asia, initial studies focused on the recognition of pathogens. Several parameters such as cypermethrin, environmental pollution, parasites, viruses, harmful algae, and probiotics were tested for their critical roles on disease spread (FAO, 2013; Hong *et al.*, 2016). However, in early 2013, the causative agent of AHPND was identified as a unique strain of *Vibrio parahaemolyticus* (Tran *et al.*, 2013).

By using multiple whole genome alignments among AHPND-causing and non-AHPND-causing bacterial strains, the unique AHPND-causing strains (VP_{AHPND}) were found to be carried in a plasmid (pVA1) harboring virulent Pir toxin genes, including PirA and PirB genes (Kondo *et al.*, 2014; Lee *et al.*, 2014; Lightner, 2014; Lo *et al.*, 2014; Tinwongger *et al.*, 2014; Yang *et al.*, 2014; Han, 2016). The Pir A/B toxin genes that code for the two toxin proteins (12.7 kDa and 50.1 kDa) that induce AHPND in shrimp have been reported to be similar to the Pir A/B toxin gene known from *Photorhabdus* spp., members of the family *Enterobacteriaceae*. The plasmid pVA1 also carries a cluster of genes related to conjugative transfer, hence, this plasmid may potentially be able to transfer not only among *V. parahaemolyticus* strains but also to different bacterial species (Dong *et al.*, 2017). Fortunately, VP_{AHPND} isolates characterized so far pose no threat to human health (FAO, 2016).

Afterward, several studies presented that another species of *Vibrio*, non-*V. parahaemolyticus*, can also cause AHPND in shrimp (LinThong *et al.*, 2014; Dang *et al.*, 2016). Recently, a *V. harveyi* strain was also identified as a causative agent of AHPND in shrimp cultured in Vietnam (Kondo *et al.*, 2015) along with *V. sinaloensis* (85% homology) isolated from East Malaysia shrimps (LinThong *et al.*, 2014) and *V. campbellii* isolated from China (Dong *et al.*, 2017) and Latin American shrimp farms (Han, 2017). All of these strains were identified to harbor Pir genes and cause AHPND in shrimp (LinThong *et al.*, 2014; Kondo *et al.*, 2015; Dang *et al.*, 2016; Dong *et al.*, 2017; Han, 2017).

4. DISEASE DIAGNOSIS

4.1. Clinical signs

AHPND caused mass mortality (up to 100%) in shrimp at 20-45 days after stocking of post-larvae. The most important clinical symptoms consist of lethargy, corkscrew swimming, and pale coloration as well as empty or interrupted gut. Infected shrimp constantly reveal an abnormal hepatopancreas (HP) such as shrunken or swollen and discolored (Fig. 3) (Leano and Mohan, 2012; OIE, 2013; Dang *et al.*, 2016). Specific clinical signs such as soft and dark shell, wasting, anorexia, and discoloration of the HP are the major clinical signs of AHPND (Zorriehzahra and Banaederakhshan, 2015).

4.2. Histopathology

Hepatopancreas (HP) is the main infected organ in this disease. The major lesions should be observed in the HP during acute progressive degeneration with initial decreases of R, B, and F-cells followed by an obvious reduction of mitotic activity in E-cells. The development of lesions is noticed from proximal to distal with dysfunction of R, B, F, and finally E-cells, with affected HP tubule mucosal cells presenting prominent enlarged nuclei, rounding, and sloughing into the HP tubules (Fig. 4) (Leano and Mohan, 2012; Lightner *et al.*, 2012).

4.3. Molecular Biotechnology

With the purpose of controling of AHPND, researchers have attempted to develop methods for early diagnosis of this disease, eventually leading to the construction of PCR and LAMP kits for rapid, sensitive, and inexpensive detection of the disease from several research groups.

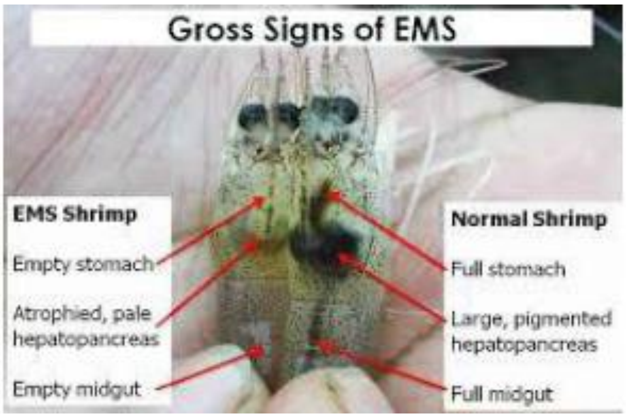

Fig. 3. Gross signs of *P. vannamei* affected by AHPND in Vietnam

Source: DV Lightner, 2012

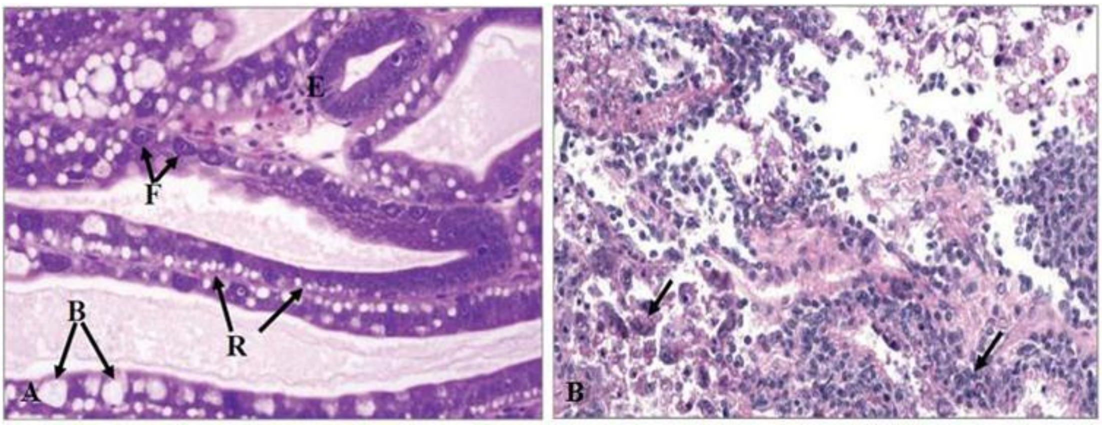

Fig. 4. AHPND-affected HP at acute phase showing acute sloughing of tubular epithelial cells and haemocyte infiltration (Fig. 4B), compared with normal HP with intact tubules and distinct F, B, and R cells (Fig. 4A)

Source: FAO, 2013

Firstly, a PCR test kit was developed by Taiwan and Thailand research groups for AHPND diagnosis based on 2 primer sets called AP1 and AP2 (Shrimp News, 2014). After that, a new and improved PCR method (called AHPND detection version 3) using AP3 replaced the AP1 and AP2 method and a two-tube nested PCR (called AHPND detection version 4) using AP4 was developed for AHPND detection (Sirikharin *et al*., 2014; Dangtip *et al*., 2015). The sequences of AP1, AP2, AP3, and AP4 primers are given in Table 1. Also, a set of primers applying toxin Pir genes for detection of AHPND was designed by a Japanese research group (Tinwongger *et al*., 2014; Table 1).

Recently, a duplex PCR assay that serves to diagnose AHPND and, further, to distinguish among pathogenic AHPND strains collected from various geographic regions was developed by an American research group. For this method, two pairs of primers, MX-345F/R (or Asia-382F/R) and VpPirA-284F/R, are added to a single tube during PCR (Han, 2016). The VpPirA-284F and VpPirA-284R primers (Table 1) allow this test to indicate the presence of the toxin gene located in the AHPND pathogenic bacteria while the MX-345F/R or Asia-382F/R allow the test to distinguish among types of AHPND bacterial strains.

Table 1. Information of primers used for detection of AHPND

Name of primers	Primer sequence	PCR product (bp)	Reference/Source
AP1	F: CCTTGGGTGTGCTTAGAGGATG R: GCAAACTATCGCGCAGAACACC	700	Flegel and Lo (2014)
AP2	F: TCACCCGAATGCTCGCTTGTGG R: CGTCGCTACTGTCTAGCTGAAG	700	Flegel and Lo (2014)
AP3	F: ATGAGTAACAATATAAAACATGAAAC R: GTGGTAATAGATTGTACAGAA	336	Sirikharin *et al.* (2014)
AP4.1	F: ATGAGTAACAATATAAAACATGAAAC R: ACGATTTCGACGTTCCCCAA	1269	Dangtip *et al.* (2015)
Ap4.2	F: TTGAGAATACGGGACGTGGG R: GTTAGTCATGTGAGCACCTTC	230	
TUMSAT-Vp3	F: GTGTTGCATAATTTTGTGCA R: TTGTACAGAAACCACGACT	360	Tinwongger *et al.* (2014)
Toxin	F: GTGGAAATGGTGAACTTGCG R: TACGAGCATTGTTAGGGGTTA	630	Japanese researchers
VpPirA-284	F: TGACTATTCTCACGATTGGACTG R: CACGACTAGCGCCATTGTTA	284	Han (2016)

In addition, a commercial diagnosis test kit called IQ Plus™ EMS/AHPND kit (GeneReach Biotechnology Corp) was designed to use in disease diagnosis. A loop-mediated isothermal amplification (LAMP) assay combined with colorimetric nanogold (AuNP) was developed for detection of AHPND disease with a total assay time of approximately 50 minutes. This LAMP assay was 100-times more sensitive than the 1-step PCR detection method (Suebsing *et al.*, 2014).

To date, all the kits/methods mentioned-above can be used to screen for AHPND bacteria in environmental samples, broodstock feeds, feces from broodstock, post larvae before stocking shrimp ponds, and suspect shrimp under cultivation to help to reduce the probability of AHPND outbreaks.

5. DISEASE PREVENTION AND MANAGEMENT

Because AHPND is caused by a bacterium, not a virus, the use of effective antibiotics against bacteria are available but have some limitations such as antibiotic resistance and public health problems. On the other hand, antibiotic usage also kills the useful bacteria in the pond that can increase disease potency. Therefore, disease prevention and health management are the best ways for disease control.

Based on the current knowledge of AHPND transmission, the contributions of risk factors to the spread of the disease and the common use of *Vibrio* in shrimp aquaculture systems, a holistic management approach is really necessary in order to successfully minimize the damage bacteria inflict on cultured shrimp. According to Han (2016), a holistic approach to AHPND management is mentioned as follows: Firstly, basic good practices have to be established in the management of shrimp culture systems to provide an optimal and stable environment. Secondly, the shrimp's health can be reinforced by optimizing nutrition and supportive supplements for the immune system. Thirdly, the presence of virulent bacteria will be reduced and opportunistic bacteria will be prevented from getting a chance to overwhelm the shrimp.

5.1. Notes for shrimp hatcheries (Zorriehzahra and Banaederakhshan, 2015; Corteel, 2016; Hong *et al.*, 2016)

Use specific pathogen free (SPF) broodtocks.

Disinfect nauplii and materials used in the hatcheries as well as improve the formulated diets for broodstocks to eliminate the risk of pathogen transfer via natural feeds.

Pay attention to the health, water, and feed management for high quality and healthy shrimp post larvae production as the first way to disease control.

Check post larvae health and test responsible organs for the presence of *Vibrio* and other organisms, and ensure their quality control.

Continually control different parts of the hatchery and shrimp larvae for the detection of *Vibrio* bacteria, especially *V. parahaemolyticus*, in order to decrease the bacterial load and prevent disease.

5.2. Notes for shrimp farms (Zorriehzahra and Banaederakhshan, 2015; Corteel, 2016; Hong *et al.*, 2016)

Prepare ponds carefully;

Stock healthy and high quality post larvae; avoid a high stocking density;

Use water storage ponds (reservoirs);

Decrease or control pH; reduce and keep water salinity low;

Use high quality and safe food and immune stimulant material for improving shrimp immunity;

Monitor total *Vibrio* during culture period;

Utilize the Biofloc techniques to significantly increase the survival rate of infected farms;

Use a poly culture system (shrimp and tilapia or marine fish) to prevent disease and/or increase the survival rate in affected systems for the reasons as follows:

+ Zooplanktons use from the bacteria as feed and decrease the bacterial load in the water and fish feed on the zooplanktons decrease the bacterial total count indirectly;

+ The blue-green algae population decreases due to fish feed and after that decrease, decreases the bacterial population;

+ The antibacterial effects of fish mucosa decrease the density of bacteria in the ponds.

6. FUTURE PERSPECTIVES

AHPND has been a serious challenge for the shrimp farming industry, not only in Viet Nam, but also worldwide, because the causative agents (*V. parahaemolyticus* and non-*V. parahaemolyticus*) are common inhabitants of coastal and estuarine environments all over the world and are often found naturally associated with shrimp aquaculture systems. The transfer of plasmids carrying toxin genes between bacteria is facilitated by the aquatic environment. Therefore, innovative farm management and appropriate biosecurity are necessary to alleviate the AHPND crisis to ensure sustainable shrimp production.

Applying disinfectant during pond preparation will reduce the risk of horizontal transfer. Management of sludge on pond bottoms is another important strategy, since organic matter that accumulates on pond bottoms can also serve as a substrate for *Vibrio* spp., including *V. parahaemolyticus*.

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