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Acute Hepatopancreatic Necrosis Disease (AHPND) in Vietnam

T.L. DANG[1,*], A.T. PHAM[2] and T.V. PHAN[1]

[1]Research Institute for Aquaculture No.1, Bac Ninh, Viet Nam
[2]Directorate of Fisheries, Ha Noi, Viet Nam

Abstract

Acute hepatopancreatic necrosis disease (AHPND) is the most severe disease currently affecting brackish-water shrimp aquaculture in Viet Nam. The disease causes huge losses to shrimp farmers in the Mekong Delta, where more than 70 percent of Vietnamese shrimp production originates. Losses due to AHPND were recorded in about 59 000 ha of farms in the Mekong Delta when the disease first appeared in 2011, and at present, the disease has spread to 294 communes belonging to 86 districts in 25 provinces throughout the country. However, the shrimp industry is now recovering from the disease and is continuing to play an important role in the country's aquaculture sector. This paper reviews the significant work related to AHPND that has been done by Vietnamese scientists under programmes designed by the National Task Force for Shrimp Disease. It includes a history of the disease in Viet Nam, reviews the various research projects that have been conducted and the management actions that have been implemented by governmental authorities, as well as the activities taken by the farmers to recover from the disease.

Keywords: AHPND, disease management and prevention, shrimp disease, Viet Nam

Introduction

A serious shrimp disease outbreak causing high mortalities in giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*P. vannamei*) that was later called acute hepatopancreatic necrosis disease (AHPND) was officially documented in Soc Trang province in southern Viet Nam in April 2011. However, it was believed that the disease had been occurring in some provinces in the Mekong Delta area from the end of 2010. The pattern of spread and the clinical signs of disease were not similar to those associated with any previous shrimp disease outbreak in Viet Nam.

[*]Corresponding author. E-mail address: danglua@ria1.org

Affected shrimp consistently showed abnormal hepatopancreas that appeared either shrunken or swollen, and discoloured. Records at the Department of Animal Health (DAH) of Viet Nam show that the total affected area of shrimp farms in the Mekong Delta was 58 812 ha in 2011, including the provinces of Tra Vinh (6 200 ha), Soc Trang (20 648 ha), Ca Mau (15 000 ha) and Bac Lieu (16 964 ha). In Soc Trang province, about 68.5 percent of the shrimp-producing area was affected and farmers were suffering from production losses. In Bac Lieu and Ca Mau provinces, an estimated 30 000 farming households were affected.

In 2012, the disease spread to 19 provinces throughout the country. The total AHPND-infected area, based on the clinical signs shown by shrimp samples, was 46 093 ha (or 45.7 percent) of the total area under culture. The most-affected province was Soc Trang with 23 371 ha (56.6 percent of the total culture area), followed by Bac Lieu with 16 919 ha (41.9 percent of the total culture area). The affected area in Tra Vinh was 12 224 ha (49.5 percent of the total culture area), while Ben Tre province had 2 237 ha affected (29 percent of the total culture area) (D-Fish 2012). In the year 2017, AHPND has spread to 294 communes belonging to 86 districts in 25 provinces throughout the country (DAH 2017).

Because of the significant impacts of the disease, the Government of Viet Nam took the following actions to respond to this disaster:

- established the National Task Force responsible for prevention and control of shrimp diseases;
- performed an intensive epidemiological survey;
- carried out screening for shrimp pathogens to narrow down the suspected causative agents of AHPND;
- carried out diagnostic investigations done by national and regional laboratories;
- distributed water treatment materials/disinfectants to provinces affected by AHPND outbreaks;
- granted funds for emergency research to be done by national institutions and universities; and
- solicited technical assistance from regional and international organizations.

The DAH, on behalf of the Government of Viet Nam, sent a request for technical assistance to the Food and Agriculture Organization of the United Nations (FAO), and subsequently, a Rapid Deployment Team (RDP) fielded by FAO visited the Mekong Delta provinces in July 2011 to conduct a quick assessment of the then unknown disease. Viet Nam then received support from FAO through project TCP/VIE/3304(E) “Emergency assistance to control the spread of an unknown disease affecting shrimps” (see FAO 2013).

Research Activities

Recognizing the severity of the disease, the Ministry of Agriculture and Rural Development (MARD) gave full support to the study of the causative agent and the development of prevention strategies to mitigate the disease. A National Task Force for Shrimp Disease was established by Decision No. 1254/QD-BNN-TCTS dated May 28, 2012 with the mandate to identify the causative agent and minimize the problem caused by AHPND. The approach applied in this massive study included screening of all possible causes of the mortality, as well as taking a closer look at any farm not affected by the disease. Figure 1 shows how the study on AHPND was approached.

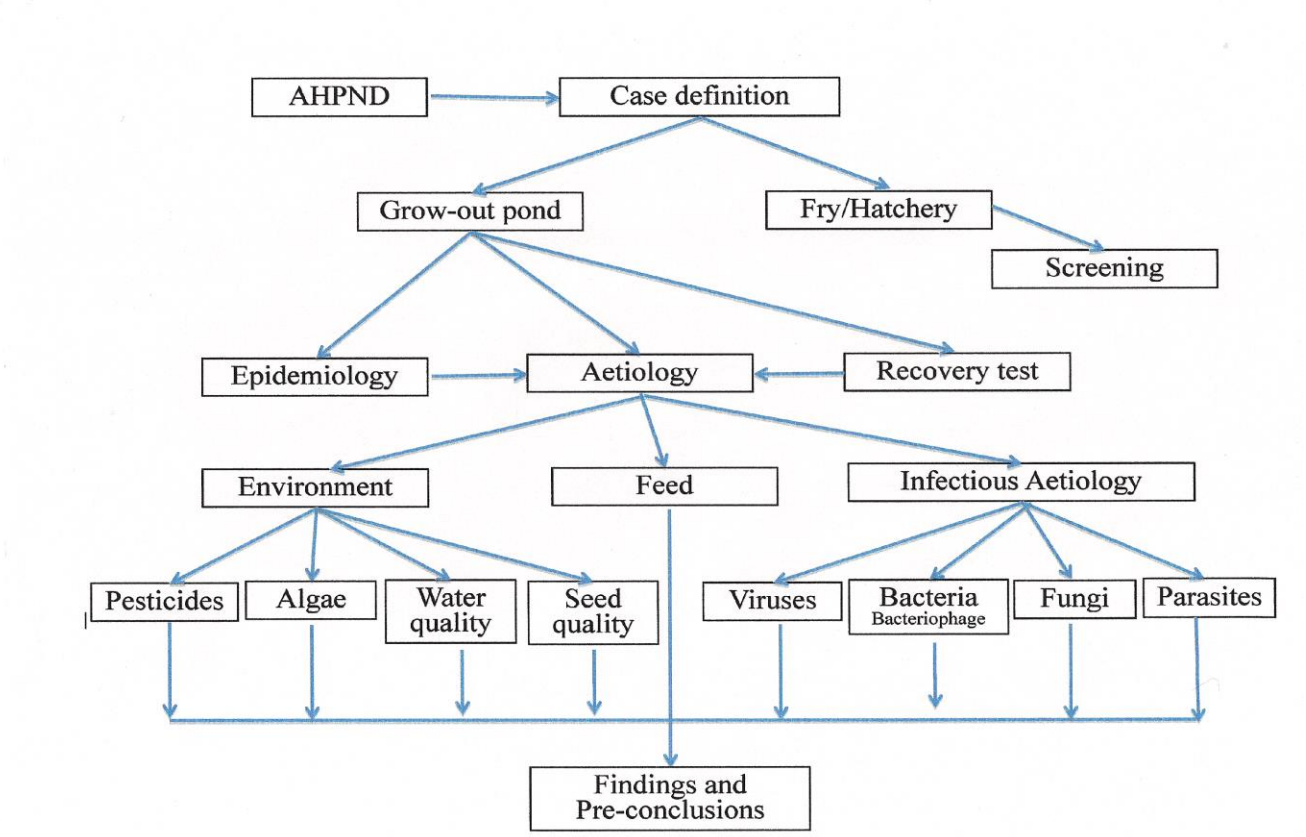

Fig. 1. Approach to the study on acute hepatopancreatic necrosis disease (AHPND)

Case Definition of AHPND

For the purpose of monitoring and early detection of the disease, as well as to assist in accurate reporting, a case definition of AHPND was developed at both the animal and pond levels. This was officially documented on June 21, 2012 (Document No. 970/TY-TS). The case definition is as follows:

- Susceptible host species: *P. monodon* and *P. vannamei.*
- Susceptible host stages: All grow-out stages are potentially susceptible, but mortality mainly occurs during 10–45 days poststocking.

- Clinical signs at the pond level:

    - At the early stage of the disease, the clinical signs are unclear. Shrimp exhibit slow growth, stop eating, show corkscrew swimming behaviour, and moribund shrimp sink to die at the bottom of the pond.
    - During a disease outbreak, diseased shrimp often have soft shells and abnormal hepatopancreas (HP) which becomes shrunken or swollen and has pale to white discolouration.

- Clinical signs at the animal level (based on Lightner et al. 2012):

    - Affected shrimp show acute progressive degeneration of the HP accompanied initially by decrease of R (Restzellen), B (Basenzellen), F (Fibrillenzellen) and E (Embyonalzellen) cells;
    - Lack of mitotic activity in E cells;
    - Proximal-to-distal dysfunction of R, B, F cells;
    - Prominent karyomegaly (enlarged nuclei), with rounding and sloughing of cells into the HP tubule lumens; and
    - Bacterial infection during advanced/terminal stages of the disease.

Table 1. Detection of acute hepatopancreatic necrosis disease (AHPND) in shrimp farms in northern Viet Nam in 2012.

Location of shrimp farm	No. samples	No. shrimp ponds	Histo-pathology findings	Pathogen isolation[1]			
				Bacteria	Bacterio-phage	Viruses	Parasites
Nghe An	321	25	AHPND	*Vibrio parahaemolyticus* *V. vulnificus* *V. cholerae* *V. harveyi* *V. ordalii* *V. mimicus*	–	IHHNV (1/25 ponds)	–
Ha Tinh	28	2	AHPND	*V. parahaemolyticus* *Photobacterium damselae*	–	–	–
Quang Ninh	20	2	AHPND	*V. alginolyticus* *V. vulnificus* *V. harveyi* *V. ordalii* *V. rotiferianus* *V. mytilis* *V. fischeri*	–	–	–
Hai Phong	146	7	AHPND	*V. parahaemolyticus* *V. vulnificus* *V. harveyi* *V. mimicus* *V. cholerae*	–	HPV, WSSV (2/7 ponds)	–

[1] – = not detected, HPV = hepatopancreatic parvovirus, IHHNV = infectious hypodermal and haematopoietic necrosis virus, WSSV = white-spot syndrome virus.

Epidemiology of AHPND

To determine if AHPND was already present in northern Viet Nam, diseased shrimp showing clinical signs of AHPND were collected from Nghe An, Ha Tinh, Quang Ninh and Hai Phong provinces, the main shrimp culture areas in the north of the country. Samples were processed for histopathological analysis and for pathogen isolation (Phan et al. 2012). Histopathological analysis confirmed that AHPND has spread to the northern part of Viet Nam (Table 1), indicating a nation-wide occurrence.

Monitoring of shrimp postlarvae (PL) for AHPND was also done by collecting more than 300 PL samples (about 50 PL/sample) from northern, central and southern Viet Nam for analysis based on the case definition. Results showed that AHPND was present in PL in Viet Nam (Table 2).

Table 2. Detection of acute hepatopancreatic necrosis disease (AHPND) in postlarvae obtained from various hatcheries in Viet Nam in 2012.

Location of hatchery	Number of PL samples	AHPND detection[1]	Bacterial isolation	Reference
Northern	6	–	*Vibrio* spp.	Phan et al. 2012
Central	> 200	–	*Vibrio* spp.	Vo 2012
Southern	113	3	*Vibrio* spp.	Dang 2012

[1] – = not detected.

Studies on the Causative Agent of AHPND

To determine the causative agent of AHPND, studies were conducted to analyze shrimp pond water (temperature, pH, DO, salinity, NH_4-N, NH_3, NO_2-N, NO_3-N, PO_4-P, chemical oxygen demand (COD), H_2S and Fe) and sediment quality (pH, percentages of total N, P and C). Also analyzed were toxic algae in pond water, pesticides used in shrimp ponds and feed quality, as well infectious organisms associated with AHPND-affected shrimp. Analysis of water and sediment quality of ponds with AHPND-infected shrimp showed that parameters were within normal levels except for high concentrations of NH_4-N (2.5 $mg.L^{-1}$) and PO_4-P (0.4 $mg.L^{-1}$) in pond water and high levels of total N (0.96 percent) and P (476.50 $mg.kg^{-1}$) in pond sediments, indicating high organic loads in some of the AHPND-infected ponds (Phan et al. 2012).

A total of 44 water samples were collected from AHPND-infected shrimp ponds and AHPND-uninfected ponds located in Nghe An, Ha Tinh, Quang Ninh and Hai Phong provinces and analyzed for toxic algae. Results showed that toxic algae belonging to the Dinophyta and Cyanobacteria were present in water sampled from both AHPND-infected and uninfected shrimp ponds, but in very low prevalences and densities (Table 3). These indicate that toxic algae were not the cause of AHPND.

Table 3. Toxic algae in AHPND-infected and uninfected shrimp ponds.

Toxic algae	Prevalence	Density (cells.L^{-1})
Dinophyta		
Dinophysis caudata	1/44	8
Prorocentrum minimum	3/44	10 – 62
Ceratium fucar	5/44	3 – 25
Cyanobacteria		
Oscillatoria limosa	17/44	2 – 7 292
Oscillatoria sp.	6/44	3 – 60
Annabaena cf. *spiroides*	1/44	57 500
Microcystis flos-aquae	1/44	1 300
Microcystis sp.	7/44	2 – 2 250
M. aeruginosa	6/44	6 – 65 625

For pesticide analysis, a total of 42 water samples and 23 sediment samples were collected from both AHPND-infected and uninfected shrimp ponds and analyzed by spectral scanning method using liquid chromatography mass spectrometry. The data (Table 4) showed that pesticides were present in both infected and uninfected ponds, indicating that pesticides were not the cause of AHPND.

Table 4. Analysis of pesticides in water and sediments in shrimp ponds.

Pesticide	Water (n = 42)		Sediment (n = 23)	
	Concentration (mg.L^{-1})	Prevalence	Concentration (mg.L^{-1})	Prevalence
Deltamethrin	0.001 – 0.070	23/42	0.035 – 0.097	11/23
Permethrin	0.001	1/42	0.010 – 0.063	2/23
Cypermethrin	0.001	1/42	–	0
Fenitrothion	0.001 – 0.012	14/42	0.028 – 2 316	14/23
Chlopyrifos	0.0002	1/42	–	0
Hexaconazole	0.001 – 0.074	13/42	0.020 – 0.134	09/23
Fipronil	0.001 – 0.065	16/42	0.024 – 2 597	6/23
Abamectin	0.003	1/42	0.197 – 0.839	3/23
Carbaryl	–[1]	0	0.030	1/23

[1] – = not detected.

For feed analysis, two commercial shrimp feeds used in AHPND-infected shrimp ponds were collected from Nghe An Province for pesticide residue analysis by spectral scanning method using liquid chromatography mass spectrometry.

The data showed that pesticide residues are not found in feeds, but preservatives such as ethoxyquin and butylated hydroxyl toluene (BHT) are present within allowable limits (Table 5). These results indicate that contamination of feeds with pesticides may not have a role in the occurrence of AHPND.

Table 5. Analysis of feed used for shrimp infected with acute hepatopancreatic necrosis disease (AHPND).

Feed Brand	Hexaconazole	Abamectin	Emamectinbenzoate	Ethoxyquin	Butylated Hydroxyl Toluene
			(mg.kg^{-1})		
NA - HH	–[1]	–	–	4 690	2 868
NA - QL	–	–	–	4 045	1 752

[1] – = not detected.

In terms of infectious aetiology, Table 1 shows that no bacteriophages or parasites were isolated from AHPND-diseased shrimp, but diseased shrimp were highly infected by *Vibrio* spp., mainly *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus* and *V. alginolyticus*. Some shrimp were co-infected with viruses such as hepatopancreatic parvovirus (HPV), white-spot syndrome virus (WSSV) and infectious hypodermal and haematopoietic necrosis virus (IHHNV). At present, there are at least two *Vibrio* species confirmed by bioassays and molecular biology to cause AHPND in shrimp in Viet Nam: *V. parahaemolyticus* (Tran et al. 2013) and *V. harveyi* (Kondo et al. 2015; Dang et al. 2016).

Recovery from AHPND

According to the report prepared by the Department of Animal Health (DAH 2015), although AHPND had spread to 19 provinces in 2012 and to up to 22 provinces in 2015 and 25 provinces in 2017, the area affected has significantly decreased from 28 005 ha in 2012 to 9 284 ha in 2015 and 6 793 ha in 2017. The data, which were calculated based on histopathological and molecular biological analysis, indicate that AHPND still occurs in Viet Nam, but also that it seems to be managed, as reflected in the reduction in the affected area. So what has been done in Viet Nam in order to recover from the "AHPND disaster"? Based on current knowledge of AHPND and the outcomes of the research projects, several actions have been taken by the Government of Viet Nam and relevant stakeholders, including the shrimp farmers to reduce or control AHPND outbreaks.

Firstly, at the provincial and national levels, some technological programmes have been applied to shrimp culture, such as VietGAP (Vietnamese Good Aquaculture Practices) and biofloc technology (BFT). The VietGAP standard is a normative good aquaculture practice that is based on four basic criteria in order to ensure safety from epizootics, environmental safety, social security and product origin. Applying VietGAP, 100 percent of shrimp seed was tested and guaranteed for quality by authorities. Farmers worked in groups, complied with a cooperative production calendar and implemented shrimp farming based on environmental protection.

They did not discharge effluents and sludge directly to the enviroment, and applied biosecure shrimp farming by using probiotics to improve the culture environment. Thus, VietGAP standards helped in controlling diseases and made shrimp farming more sustainable. BFT is considered as an ecosystem management technology in which water exchange is limited and organic subtrates are allowed to accumulate. The microorganisms (biofloc) in the BFT system are developed by balancing carbon and nitrogen in the water, resulting in maintenance of water quality and control of bacterial infections in the ponds. Secondly, monitoring and surveillance programmes for shrimp farms have also been put in place. According to DAH information, 45 of 63 provinces have implemented programmes for the prevention and control of aquatic animal diseases, including shrimp disease. Surveillance results are reported monthly to DAH, and the directors present the results to the Minister of MARD during monthly meetings. Thirdly, the government has also funded research projects on aquatic animal diseases and assisted in the establishment of diagnostic laboratories at the provincial and district levels.

At the farm level, farmers have applied practical solutions to avoid disease, such as:

- screening PL for disease before stocking to ensure high quality;
- applying suitable stocking density;
- daily monitoring of environmental parameters (e.g. temperature, pH, salinity);
- monitoring and control of total *Vibrio* by applying probiotics;
- control of feeding, especially during the first month of stocking, by feeding shrimp several times per day in small rations; and
- applying polyculture system and/or rotational farming with shrimp-tilapia or shrimp-marine fish.

Some farmers also believe that application of a nursery system to grow larger PL before stocking into ponds can help to control AHPND outbreaks.

Future Perspectives

The AHPND crisis has been a serious challenge for the shrimp farming industry, not only in Viet Nam, but also worldwide, because the causative agents (*Vibrio parahaemolyticus* and *V. harveyi*) 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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