

Status of Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Emerging Diseases of Penaeid Shrimps in Viet Nam

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Abstract

Acute hepatopancreatic necrosis disease (AHPND), formerly called early mortality syndrome (EMS), was first reported in 2010 among penaeid shrimps cultivated in the Mekong Delta Region of Viet Nam albeit without any laboratory confirmation. The disease subsequently spread to a wide range of shrimp production areas in the same region (Soc Trang: 1,719 ha; Bac Lieu: 346 ha; and Ca Mau: 3,493 ha), so that the Government of Viet Nam requested for technical assistance from the Food and Agriculture Organization (FAO) of the United Nations in 2011. In 2012, FAO supported Viet Nam through the project TCP/VIE/3304 *Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam*, under which the Department of Animal Health of Viet Nam (DAH) collaborated with the University of Arizona and FAO experts to carry out in-depth studies to identify the etiologic agent of the disease. As a result, unique isolates of *Vibrio parahaemolyticus* was identified as the causative agent of AHPND in 2013. Viet Nam has been vigilant and transparent with regard to aquatic animal diseases through official notifications to the World Organization for Animal Health (OIE) and the Network of Aquaculture Centres in Asia-Pacific (NACA). AHPND outbreaks have no clear temporal pattern with black tiger (*Penaeus monodon*) and whiteleg (*P. vannamei*) shrimps showing similar incidence risk. The disease occurs at any stage of shrimp cultivation, i.e. on average about 35 days after stocking. To date, unwarranted outbreaks of AHPND in major shrimp-producing provinces in Viet Nam have been apparently regulated. Aside from AHPND, white spot disease (WSD) has also been a persistent problem responsible for serious economic losses in many shrimp-producing areas in Viet Nam. To prevent and control the further spread of infectious diseases of shrimps including AHPND and WSD, multiple control measures have been implemented including guidance of farmers to improve production conditions, facilities and biosecurity application, active surveillance of shrimp production areas for early warning, screening of broodstock and postlarvae for any OIE listed diseases, regulation on movement of stocks, and collaboration with regional and international organizations in carrying out in-depth epidemiological studies that will be needed in the formulation of pragmatic and holistic disease interventions.

Introduction

Viet Nam has 28 coastal provinces with more than 3,260 km of coast line and many river systems such as the Red river and Mekong river system. These bodies of water are suitable environments for the aquaculture of various aquatic species including brackishwater shrimps. Having this advantage, the shrimp industry of Viet Nam developed rapidly and has become one of the major shrimp exporters in the world. In 2009, 2010, and 2011, the annual volume of shrimps produced in Viet

Nam were 352,000 metric tons (MT), 469,893 MT, and 240,000 MT, respectively. Additionally, the shrimp products exported by Viet Nam were estimated at USD 1.5 billion, USD 1.9 billion, and USD 2.4 billion, respectively, in 2009, 2010, and 2011 (Mai, 2012).

The Mekong River Delta in Southern Viet Nam has approximately 565,000 ha of shrimp aquaculture areas contributing approximately 95% of the total volume of shrimp produced in the country. These shrimp aquaculture areas include the coastal provinces of Ben Tre, Tien

Giang, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau. The shrimp farms located in these areas have been practicing various types of shrimp culture system including intensive, semi-intensive, extensive, improved extensive, and integrated rice and shrimp culture, depending on shrimp density, type of management employed, and existing facilities and infrastructures in the farm.

While intensification of shrimp aquaculture in Viet Nam over the past several years has led to remarkable improvements in productivity, it has also been associated with disease epidemics involving bacterial, viral, fungal, and parasitic pathogens. Undoubtedly, disease has been one of the biggest constraints on production, development and expansion of Viet Nam's shrimp aquaculture industry. Among the major infectious diseases confronting Viet Nam's shrimp industry, white spot disease (WSD) and very recently, acute hepatopancreatic necrosis disease (AHPND), formerly called early mortality syndrome (EMS), have by far been implicated in serious economic losses.

The first outbreak of EMS was reported in Soc Trang province in 2010. Thereafter, EMS continuously occurred and spread to other provinces such as Tien Giang, Ben Tre, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau causing marked reduction in Viet Nam's annual shrimp production in 2011. Accordingly, researchers of the Ministry of Agriculture and Rural Development (MARD) attempted to identify the etiology of the disease and carried out concomitant practical prevention and control strategies to address the pressing problem at that time. MARD also requested assistance from international organizations such as the World Organization for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO) and Network of Aquaculture Centres in Asia-Pacific (NACA). Experts from the FAO's Crisis Management Centre-Animal Health (CMC-AH) made a quick assessment of this unknown disease affecting cultured shrimps in the Mekong Delta provinces of Viet Nam. The findings of the CMC-AH mission based on epidemiological observations and other relevant field data confirmed that outbreaks actually occurred since the early part of 2010 until 2011 causing significantly high mortalities among

cultured black tiger and whiteleg shrimps. Based on the nature of disease spread, it was at that time speculated to be caused by an infectious agent as evidenced by the pattern of disease transmission, i.e. starting from a pond in one location and subsequently spreading to several ponds within the farm, and finally to neighboring farms.

Through the recommendation of the FAO's CMC-AH, FAO extended technical and financial assistance to implement the project TCP/VIE/3304 *Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam* with the primary aim of better understanding the etiology of the disease and identifying a number of risk management measures and key areas for future research (FAO, 2013). The DAH eventually convened and carried out in-depth investigations of the disease outbreaks. Accordingly, a total of 181 diseased shrimp samples from the three main EMS-affected provinces, i.e. Soc Trang, Bac Lieu and Ca Mau, were collected and examined. To speed up the elucidation of the etiology of the disease, the DAH also invited experts from Mahidol University, Thailand and Arizona University, United States of America, in the persons of Dr. T. Flegel and Dr. D. V. Lightner, respectively.

In mid-2011, D.V. Lightner of Arizona State University described the histopathology of both *Penaeus monodon* and *P. vannamei* obtained from many cultivation ponds in Viet Nam that were infected with EMS. The histopathology of infected shrimp was typified by massive sloughing of hepatopancreatic tubule epithelial cells that commenced in the center of the hepatopancreas and progressed outward to the embryonic (E-cell) region. Armed with this information, a more descriptive term for the syndrome, acute hepatopancreatic necrosis syndrome (AHPNS), was proposed and eventually adopted. At that time, AHPNS was still classified as an idiopathic disease because no specific causal agent had been identified yet. Fortunately, the Aquaculture Pathology Laboratory of the University of Arizona isolated the causal agent of AHPNS in pure culture in 2013 (Tran *et al.*, 2013). The causal agent of AHPNS was identified as unique strains of *Vibrio parahaemolyticus* (VP_{AHPND}) that colonized the stomach of shrimp

and produced toxins that consequently caused massive sloughing of hepatopancreatic tubule epithelial cells of the hepatopancreas. When the healthy shrimps were exposed to the isolated VP_{AHPND} via immersion, 100% mortality rate was obtained coupled with the expressions of typical pathognomonic histology of AHPNS thereby fulfilling Koch's postulates (Tran *et al.*, 2013). It was also documented that cell-free broth alone obtained from the VP_{AHPND} cultures could induce the massive sloughing of the hepatopancreatic tubule cells even in the absence of bacterial cells (Tran *et al.*, 2013). Subsequent histological analyses revealed that AHPNS lesions could be experimentally induced *in vitro*, identical to those observed in AHPNS-infected shrimp samples collected from the endemic areas in Viet Nam. Following the identification of the etiologic agent, the disease was eventually named as acute hepatopancreatic necrosis disease (AHPND). Furthermore, this finding spurred the Government of Viet Nam to issue an official notification to the public regarding the etiology of AHPNS. In June 2013, FAO and DAH organized an international workshop to disseminate the outputs of the FAO-funded project.

Aside from AHPND, Viet Nam's shrimp aquaculture industry has been likewise facing serious problems associated with white spot disease (WSD) caused by white spot syndrome virus (WSSV). WSD unequivocally impacted the annual production of penaeid shrimps cultivated in major shrimp producing areas, i.e. from North to South of Viet Nam in 1994 and 1995, respectively. Because AHPND and WSD have been observed to erratically occur particularly during the early period of shrimp cultivation when shrimps are highly susceptible to these diseases, Viet Nam shrimp growers have resorted to practicing pragmatic and effective interventions to control the devastating effects of these diseases.

Status of AHPND and its economic impact

In 2010, unconfirmed outbreaks of EMS were reported in shrimp cultivation areas along Viet Nam's Mekong Delta provinces constituting the main shrimp production region. In the succeeding year, the disease consequently

spread to a wide range of shrimp production areas including the provinces of Ninh Thuận (production area: 16 ha), Soc Trang (1,719 ha), Bac Lieu (346 ha) and Ca Mau (3,493 ha).

In 2012, occurrences of AHPNS did not only further spread to a wide range of shrimp production areas along the Mekong Delta provinces such as Soc Trang (production area: 2,100 ha), Tra Vinh (1,642 ha), Bac Liêu (2,000 ha), Ca Mau (4,007 ha), Ben Tre (133 ha) but also in shrimp production areas located in the middle of Viet Nam including the provinces of Quang Ngai, Binh Dinh, Ninh Thuan, Phu Yen, and Khanh Hoa.

In 2015, AHPND extensively occurred in 23 provinces of Viet Nam covering approximately 9,463 ha of the shrimp cultivation areas (1.4% of the total shrimp cultivation areas) with infected shrimps showing the typical clinical signs of AHPND. Notably, the number of affected areas abruptly increased in 2015. However, the data from 2013 to 2015 revealed that the proportion of AHPND-infected areas represented only <1.5% of the total shrimp cultivation areas (Table 1). Unwarranted outbreaks of AHPND have caused serious economic losses in Viet Nam estimated annually at less than USD 57 million. To date, though AHPND outbreaks have already been apparently regulated, minor occurrences could still be sporadically observed in some shrimp production areas in Viet Nam (Figure 1).

Shrimp species and culture stages affected

Outbreaks of AHPND have been so far documented in cultured black tiger (*P. monodon*) and whiteleg (*P. vannamei*) shrimps in Viet Nam. As shown in Table 1, the proportions of AHPND-infected cultivation areas documented from 2013 to 2015 were significantly different between black tiger and whiteleg shrimp. Whiteleg shrimp appeared to be more susceptible to AHPND than tiger shrimp based on DAH's observations over the past three years (Table 1). On average, AHPND occurred 35 days after the stocking of shrimps in cultivation ponds. However, AHPND has a wide range of occurrence period, i.e. from 0 up to 165 days and a narrower period range of 15 to 60 days post-pond stocking of shrimps (Table 2).

Table 1. Occurrence, prevalence, and impact of acute hepatopancreatic necrosis disease (AHPND) outbreaks on Viet Nam's shrimp productivity.

Year of AHPND occurrence	2013	2014	2015
No. of provinces affected	19	22	23
Total culture area (ha) affected by AHPND	5,875	5,509	9,463
<i>P. monodon</i>	3,300 (56.17%)	2,088 (37.89%)	5,132 (54.5%)
<i>P. vannamei</i>	2,575 (43.83%)	3,421 (62.11%)	4,285 (45.5%)
Prevalence of AHPND in shrimp			
<i>P. monodon</i>	0.53%	0.36%	0.87%
<i>P. vannamei</i>	5.57%	3.63%	5.06%
Total shrimp culture area (ha)	664,783	678,913	677,459
Proportion of culture area affected by AHPND	0.88%	0.81%	1.40%
Estimated losses in million USD due to AHPND	60.63	57.64	97.96

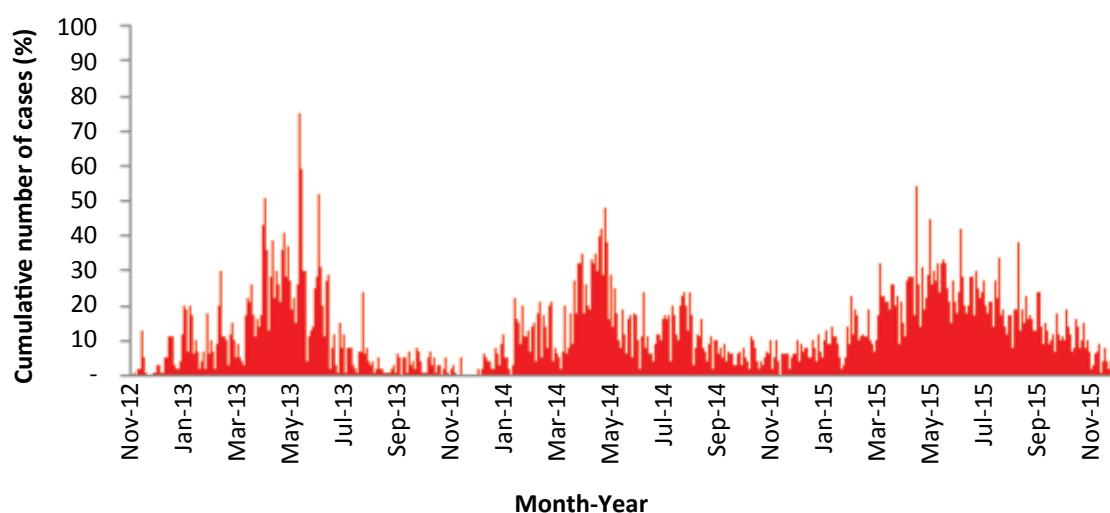


Figure 1. Temporal distribution of acute hepatopancreatic necrosis disease (AHPND) outbreaks in Viet Nam documented from 2013 to 2015.

Prevention and control of AHPND

To prevent and control the unwarranted outbreaks of AHPND, stringent biosecurity measures in both hatcheries and shrimp production farms have been strictly practiced in Viet Nam. New approaches or techniques and standards for shrimp culture such as those stipulated in the Viet Nam Good Aquaculture Practice (VietGAP) and Global Good Aquaculture Practice (GlobeGAP) have been implemented in all shrimp production areas. Active surveillance program for AHPND risk analysis has been also carried out with financial assistance extended by private farmers, local government,

and the central government. For instance, the active surveillance conducted on 8 major shrimp production provinces namely Quang Ninh, Nam Dinh, Ha Tinh, Ninh Thuan, Binh Thuan, Soc Trang and Ben Tre was conducted in 2015. Active surveillance has been so far routinely carried out in all shrimp hatcheries to ensure that postlarvae are negative for shrimp pathogens before they are permitted to be transported to other shrimp cultivation areas. Every month, at least 5 shrimp postlarvae samples are obtained from small-scale hatcheries and tested for infectious diseases especially those listed in the OIE. In the case

Table 2. Incidence of acute hepatopancreatic necrosis disease (AHPND) outbreaks during the different culture stages of shrimp in grow-out ponds.

Days of culture in grow-out ponds after stocking	Year					
	2013		2014		2015	
	No. of cases of AHPND outbreaks	(%)	No. of cases of AHPND outbreaks	(%)	No. of cases of AHPND outbreaks	(%)
0-15	21	0.90	8	0.26	17	0.40
15-30	349	15.01	645	20.83	642	15.23
30-45	599	25.76	1,664	53.75	2,205	52.33
45-60	931	40.04	602	19.44	957	22.71
60-75	354	15.23	135	4.36	281	6.67
75-90	59	2.54	26	0.84	61	1.45
90-105	9	0.39	16	0.52	44	1.04
105-120	3	0.13	-	-	4	0.09
135-150	-	-	-	-	1	0.02
150-165	-	-	-	-	2	0.05
Total	2,325	100	3,096	100	4,214	100

of large-scale hatcheries, the same number of samples are taken for disease testing every week.

From January to June 2015, the DAH carried out an intensive (active) surveillance of AHPND, WSD, and hepatopancreatic microsporidiosis (HPM) in 60 hatcheries, i.e. 30 hatcheries from Ninh Thuan province plus another 30 from Binh Thuan province. In each of these randomly selected hatcheries, animal (shrimp postlarvae with mean body weight of 1.5 g) and environmental samples (water and waste) were taken and tested for the aforesaid diseases. Likewise, the DAH carried out an active surveillance of WSD, AHPND and EHP in 210 grow-out farms located in the northern (Quang Ninh, Nam Dinh), central (Ha Tinh) and southern (Ben Tre and Soc Trang) provinces. In each of these selected provinces, 30 farms were selected randomly using the designed multiple criteria approach. Similarly, specimens composed of shrimp postlarvae, water, and waste were collected and subjected to disease diagnosis. Moreover, the DAH has also implemented a passive surveillance system using a standardized reporting mechanism, i.e. from the farm level up to the national level.

One of the national strategies that has perhaps played a pivotal role in averting the further

spread of AHPND among cultivated shrimps in Viet Nam was the enhancement of farmers' capacity in dealing with AHPND through a series of pertinent training courses organized by the staff of the DAH. In 2015 alone, a total of 1,500 shrimp farmers underwent the training. The training covered lectures and hands-on exercises related to shrimp health management including diagnostic methods for AHPND and corresponding good aquaculture practices, among others. To strengthen farmers' knowledge gained in the training course and ascertain that prevention and control approaches have been effectively carried out in the field, personnel of the DAH further extended field technical assistance to shrimp farmers in a collaborative manner with the local veterinary agencies.

In November 2014, the National Steering Committee for the Prevention and Control of diseases in brackishwater shrimps was established to advise the Ministry of Agriculture and Rural Development in developing and carrying out practical disease intervention and control of cultured shrimps in Viet Nam. With the protocol for prevention and control strategies in place, the program was successfully implemented in each of the shrimp-producing provinces. This was realized by enhancing the capacities of provincial veterinary personnel

in conducting accurate disease diagnosis. Relative to this, the provincial veterinary laboratory facilities were accordingly improved and application of novel techniques for shrimp disease diagnosis was instituted. On June 19, 2015, Viet Nam's Law on Animal Health was approved by the National Assembly. The Law supersedes the Veterinary Ordinance 18/2004/PL-UBTVQH10, and will be effectively implemented starting July 1, 2016. The new law covers a wide range of animal health related areas including prevention, control, and surveillance of animal diseases, management of animal slaughtering and processing, import quarantine inspection of animal and animal products, and the management of veterinary drugs. By and large, shrimp farmers in Viet Nam have been encouraged to use biological and chemical products that are approved for preventing and treating diseases of cultured penaeids. Because AHPND is caused by VP_{AHPND} , farmers used antibiotics during the height of AHPND outbreaks in Viet Nam. However, the application of antibiotics in hatcheries and grow-out ponds via medicated feed did not completely prevent AHPND outbreaks. Additionally, in compliance with the regulations on antibiotic residue and food safety, shrimp farmers were instructed to strictly follow the correct dosage of antibiotic administration and concomitant withdrawal period.

Status of white spot disease (WSD) in cultured shrimps

In 2015, WSD occurred in 23 provinces covering a total area of approximately 5,396 ha (0.79% of the total of cultivation area) in Viet Nam. The number of infected area decreased sharply compared to the data generated in 2013 and 2014, i.e. the proportion of infected to the total cultivation area was less than 4% (Table 3). While WSD occurred annually from January to September in 2013, 2014 and 2015, serious outbreaks however so far recorded usually commenced in March and seemingly peaked in May (Figure 2).

Table 3 clearly shows that the proportion of WSD-infected cultivation areas were significantly different between the black tiger and whiteleg shrimps. Whiteleg shrimp was seemingly more susceptible to WSD than black tiger shrimp (> 3.9 times) throughout the course of our 3-year investigation. This could be attributed to the fact that the black tiger shrimp is a local species, hence they could easily adapt to the local environment whereas the whiteleg shrimp was imported from other countries. On average, shrimps could be infected by WSD at 35 days after stocking in grow-out ponds. It should be noted however, that WSD has a wide range of occurrence period, i.e. from 0 up to

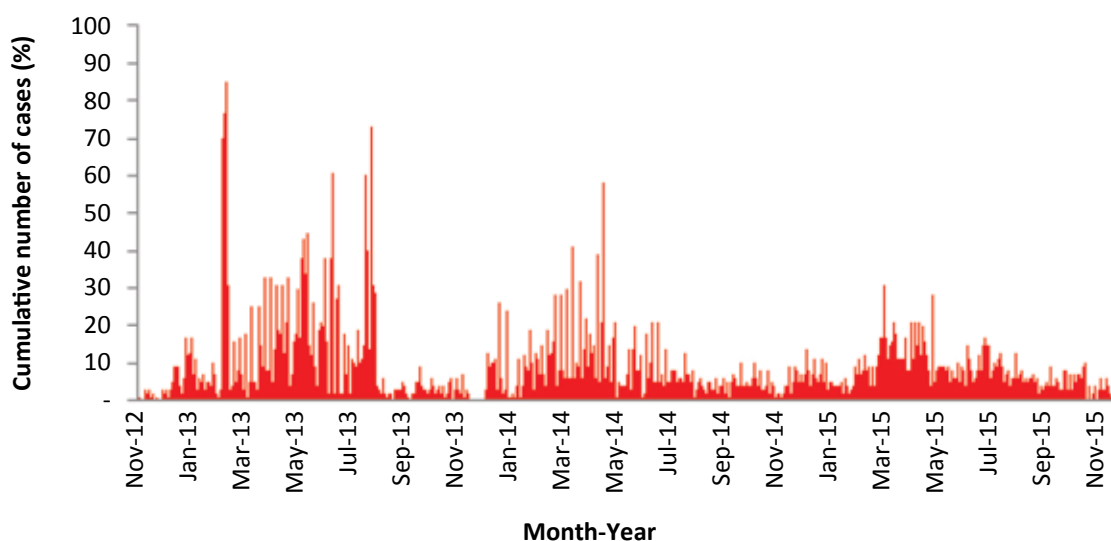


Figure 2. Temporal distribution of white spot disease (WSD) outbreaks in Viet Nam documented from 2013 to 2015.

Table 3. Impact of white spot disease (WSD) outbreaks on Viet Nam's shrimp productivity.

Year of WSD occurrence	2013	2014	2015
No. of provinces affected	28	23	23
Total culture area (ha) affected by WSD	12,352	23,850	5,369
<i>P. monodon</i>	7,677 (62.16%)	14,660 (61.47%)	3,447 (64.19%)
<i>P. vannamei</i>	4,674 (37.84%)	9,190 (38.53%)	1,923 (35.81%)
Prevalence of WSD in shrimp			
<i>P. monodon</i>	1.24%	2.51%	0.58%
<i>P. vannamei</i>	10.12%	9.76%	2.27%
Total shrimp culture area (ha)	664,783	678,913	677,459
Proportion of culture area affected by WSD	1.85%	3.51%	0.79%
Estimated losses in million USD due to WSD	127.47	249.54	55.58

Table 4. Incidence of white spot disease (WSD) outbreaks during the different culture stages of shrimp in grow-out ponds.

Days of culture in grow-out ponds after stocking	Year					
	2013		2014		2015	
	No. of cases of WSD outbreaks	(%)	No. of cases of WSD outbreaks	(%)	No. of cases of WSD outbreaks	(%)
0-15	18	1.03	5	0.31	7	0.33
15-30	294	16.83	345	21.63	362	17.05
30-45	499	28.56	854	53.54	1019	48.00
45-60	635	36.35	267	16.74	543	25.58
60-75	280	16.03	84	5.27	121	5.70
75-90	15	0.86	20	1.25	42	1.98
90-105	3	0.17	17	1.07	17	0.80
105-120	1	0.06	3	0.19	5	0.24
135-150	2	0.11	-	-	5	0.24
150-165	-	-	-	-	1	0.05
210-225	-	-	-	-	1	0.05
Total	1,747	100	1,595	100	2,123	100

225 days after stocking. Moreover, Vietnamese farmers have otherwise experienced a narrower period of WSD occurrence, i.e. ranging from 15 to 60 days after stocking (Table 4).

WSD-infected shrimp exhibit lethargy, empty or little food in gut, swimming on the surface water layer, pale shell color and typical white spots (0.2-0.5 cm) on the head and the tail cover. Viral genome-based detection methods including PCR and loop-mediated isothermal amplification (LAMP) have been employed as confirmatory tests for the detection of WSD in asymptomatic and diseased shrimps. To prevent and control WSD outbreaks among

cultured penaeids, similar strategies used for AHPND have been likewise practiced in Viet Nam.

Way forward

Occurrence of AHPND and WSD has by far been documented in almost all stages of shrimp cultivation with higher frequency of occurrence being observed among the months of March to July in Viet Nam. Whiteleg and black tiger shrimps could be both infected with AHPND and WSD with the former species appearing to be seemingly more susceptible to these diseases. To date, hepatopancreatic microsporidiosis

(HPM) caused by *Enterocytozoon hepatopenaei* (EHP) has not been detected in cultured penaeids in Viet Nam based on the results of the active surveillance conducted in 2015 and 2016, respectively. Viet Nam will continue its vigilance and transparency with regard to aquatic animal diseases through official notifications to the OIE and NACA. Also, the DAH of Viet Nam will strictly continue implementing its comprehensive policy system on disease prevention and control in cultured shrimps. Thus, to efficiently prevent and control the entry and further spread of newly emerging and persistent infectious diseases of cultured penaeids including AHPND and WSD, respectively, multiple control measures will be continually implemented including the guidance of farmers to improve production conditions, facilities and biosecurity application, active surveillance of shrimp production areas for early warning, screening of broodstock and postlarvae for any OIE listed diseases, regulation on movement of stocks, and collaboration with regional and international organizations in carrying out in-depth epidemiological studies that will be needed in the formulation of pragmatic and holistic disease interventions.

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