Current Status of Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Diseases of Farmed Shrimps in Indonesia

Mukti Sri Hastuti[1] and Desrina[2]*

[1] Directorate for Fish Diseases, Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries, Indonesia

[2]Fisheries Department, Faculty of Fisheries and Marine Sciences. Diponegoro University. Jl. Prof. Soedharto SH. Tembalang, Semarang, Indonesia 50276

**rinadesrina@yahoo.com*

Abstract

Transboundary diseases have been a constant challenge for the aquaculture industry in Indonesia. In spite of this, Indonesian aquaculture has experienced a steady growth since 2010. Early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) is a serious emerging transboundary disease of cultured shrimp that has not been reported in Indonesia. On the contrary, hepatopancreatic microsporidiosis (HPM) was first detected in 2015. Other previously reported transboundary diseases of shrimp and fish include white spot disease (WSD), Taura syndrome and infectious myonecrosis (IMN), and viral nervous necrosis (VNN) and koi herpesvirus (KHV), respectively. These diseases have been included in the surveillance program conducted in 2016. To avert the spread of these transboundary pathogens in the Indonesian aquaculture facilities and natural waters, competent authorities have been tasked to implement stringent control measures including government policy and regulation, active and passive surveillance, and strengthening farmers' and stake holders' awareness of the importance of disease control and health maintenance.

Introduction

Aquaculture has contributed significantly to the Indonesian economy for over 4 decades. Currently, Indonesia is one of top farmed food fish-producing countries in the world (FAO 2014). The Ministry of Marine Affairs and Fisheries (MMAF) of Indonesia has provided supports, among them, through regulations of disease control and sustainable aquaculture, technical assistance for various stakeholders, working closely with experts from universities to achieve the national aquaculture production target. Moreover, rigorous efforts aimed at increasing farmers' awareness on transboundary diseases and best manufacturing practices (BMPs) have been aggressively instituted over the past years. As a result, aquaculture production increased significantly from 6.28 million metric tons (MT) in 2010 to 14.36 million MT in 2014 (Table 1). For 2016, the annual production target has been projected at 19.45 million MT, with penaeid shrimps anticipated to contribute approximately 0.93 million MT. Although seaweed represents the major aquaculture commodity in terms of production, shrimps have been an essential part of the aquaculture industry in Indonesia chiefly because of the price that they command in the international market (Table 2).

Diseases have been the major challenge faced by the aquaculture sector in Indonesia. Over the past 10 years, the Government of Indonesia, through MMAF and various stakeholders, has been exerting tremendous efforts to reduce the impact of transboundary diseases, with adequate results. The most important diseases of cultured penaeids so far documented include white spot disease (WSD) and infectious myonecrosis (IMN) caused by white spot syndrome virus (WSSV) and infectious myonecrosis virus (IMNV),

respectively. To date, acute hepatopancreatic necrosis disease (AHPND), a newly reported emerging disease of cultivated shrimp, has not been encountered in Indonesia. However, hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) was detected in some areas in 2015. Major fish diseases include koi herpesvirus disease caused by koi herpesvirus (KHV) and viral nervous necrosis (VNN) caused by betanodaviruses. This paper reports the current status of newly emerging and previously reported transboundary diseases of aquacultured organisms in Indonesia with particular emphasis on emerging diseases of penaeid shrimps.

Status of newly emerging transboundary diseases of penaeid shrimps

Acute hepatopancreatic necrosis disease (AHPND)

AHPND first occurred in China in 2009 and has since been reported in Viet Nam (2011), Malaysia (2011), Thailand (2012), México (2013), and the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; dela Peña *et al.*, 2015). Indonesia is one of the major shrimp-producing countries in Asia in which AHPND outbreak has not yet been encountered. Competent authorities have

Table 1. Aquaculture Production of Indonesia from 2010 to 2014.

No.	Commodities	Production (MT)				
		Year				
		2010	2011	2012	2013	2014
1	Shrimp					
	Tiger shrimp	125,519	126,157	117,888	171,583	131,809
	White shrimp	206,578	246,420	251,763	390,278	442,380
	Freshwater prawn	1,327	1,386	1,721	3,387	1,809
	Others	47,548	27,191	44,331	73,707	63,371
	SUB TOTAL	**380,972**	**401,154**	**415,703**	**638,955**	**639,369**
2	Seaweed					
	Gracilaria	515,581	630,788	776,166	975,211	1,105,529
	Cottonii	3,399,436	4,539,413	5,738,688	8,335,663	8,971,463
	SUB TOTAL	**3,915,017**	**5,170,201**	**6,514,854**	**9,310,874**	**10,076,992**
3	Fish					
	Milkfish	421,757	467,449	518,939	627,333	631,125
	Grouper	10,398	10,580	11,950	13,464	13,346
	Asian Sea bass	5,738	5,236	6,198	6,735	5,447
	Molluscs	58,079	48,449	19,472	29,091	44,394
	Crabs	9,557	8,153	14,330	11,911	13,606
	Common Carp	282,695	332,206	374,366	412,703	434,653
	Tilapia	464,191	567,078	695,063	914,778	999,695
	Catfish	242,811	337,577	441,217	543,774	679,379
	Pangasius	147,888	229,267	347,000	410,883	418,002
	Giant Gourami	56,889	64,252	84,681	94,605	118,776
	Others	281,931	287,360	231,778	285,800	284,348
	SUB TOTAL	**1,981,934**	**2,357,607**	**2,744,996**	**3,351,077**	**3,642,769**
	TOTAL	**6,277,923**	**7,928,962**	**9,675,553**	**13,300,906**	**14,359,129**

declared Indonesia as an AHPND-free country in the region since 2013 based on the decree of the Director General of Fish Quarantine and Inspection Agency No. 130/KEP-BKIPM/2013.

AHPND field observation

The etiological agent of AHPND is a virulent strain of *Vibrio parahaemolyticus* that contains pVPA3-1 plasmid producing PirA and PirB toxins. Because the plasmid contained in *V. parahaemolyticus*-causing AHPND (VP_{AHPND}) is transmissible coupled by the fact that it is commonly found in brackishwater environments, MMAF has been conducting field observations integrated with the surveillance program for WSSV and IMNV in North Sumatra and Lampung provinces since 2013 and 2015, respectively. *P. vannamei* broodstocks, larvae and juveniles, as well as polychaetes (*Nereis* sp.), clams and *Artemia* which are fed to the shrimps are covered by the surveillance for these aforementioned shrimp pathogens.

Pond managers were interviewed for disease occurrence with emphasis on AHPND, i.e. nonspecific mortality during the first 50 days of culture. Specimens for diagnostics consisted of juvenile *P. vannamei* from intensive and traditional ponds. Isolation and detection of AHPND causal agents were conducted according to methods outlined in the OIE manual. Bacterial identification was done using API 20NE (bioMerieux). *V. parahaemolyticus* isolates were tested for the presence of specific plasmid of VP_{AHPND} using the primer set AP2, AP3, AP4, Tox R and VpPir A and VpPir B. Pathological changes in the hepatopancreas

Table 2. Indonesian aquaculture production value.

No.	Commodities	Value in Indonesian Rupiah (Rp) 1,000,000,000				
		Year				
		2010	2011	2012	2013	2014
1	Shrimp					
	Tiger shrimp	7.20	7.24	6.86	6.97	9.09
	White shrimp	8.19	10.24	10.38	17.84	25.87
	Freshwater prawn	0.08	0.04	0.19	0.18	0.09
	Others	1.40	0.60	1.03	1.82	2.04
	SUB TOTAL	**16.86**	**18.11**	**18.46**	**26.80**	**37.09**
2	Seaweed					
	Gracilaria	1.52	0.66	1.31	1.40	1.22
	Cottonii	10.23	10.23	10.28	20.31	23.38
	SUB TOTAL	**11.75**	**10.90**	**11.59**	**21.71**	**24.60**
3	Fish					
	Milkfish	4.89	6.75	8.42	8.97	9.58
	Grouper	2.27	1.16	1.56	1.35	1.46
	Asian Sea bass	0.18	0.19	0.26	0.28	0.21
	Molluscs	0.26	0.18	0.13	0.04	0.20
	Crabs	0.27	0.23	0.45	0.43	0.50
	Common Carp	5.84	6.51	7.26	6.60	9.58
	Tilapia	9.52	9.47	10.70	12.39	18.03
	Catfish	2.75	3.93	5.26	7.26	10.32
	Pangasius	2.48	3.30	4.62	4.38	6.45
	Giant Gourami	1.65	1.51	2.54	2.13	3.32
	Others	4.70	4.31	4.67	3.70	6.36
	SUB TOTAL	**34.81**	**37.54**	**45.87**	**47.52**	**66.01**
	TOTAL	**63.42**	**66.55**	**75.92**	**96.04**	**127.70**

1 USD = 13,070 Rp

and intestines of shrimps were examined by histopathology. In addition, selected *V. parahaemolyticus* strains were subjected to 16S rRNA sequencing. The sequence data obtained from these strains were compared with the sequence data in the National Center for Biotechnology Information (NCBI) database. In addition, seventeen specimens composed of *P. vannamei* juveniles obtained from shrimp ponds in North Sumatra with white feces disease (WFD) were likewise tested in 2013. Isolation and subsequent identification were done as described above. Other *Vibrio* species including *V. vulnificus* and *V. furnissii* were also isolated in some shrimps examined. Although histopathological examinations of some shrimp samples exhibited sloughing of the hepatopancreatic tubules, they were however positive for Cowdry type A inclusion bodies indicative of WSSV infection. Fortunately, all shrimps collected during the active surveillance conducted from 2013 to 2015 were by far negative for AHPND by AP2, AP3 and AP4 PCR methods (Table 3).

White feces disease (WFD)

White feces disease outbreaks occurred in some shrimp cultivation areas in Indonesia including East Java, DI Yogyakarta, North Sumatra and Lampung in 2014. *Vibrio parahaemolyticus* obtained from 19 samples with WFD across Indonesia were examined by 16S rRNA sequencing. As a result, *V. parahaemolyticus* strains that are closely related to VP_{AHPND} were found to be present in Indonesia but fortunately they turned out to be AHPND-negative by PCR for both the plasmid and toxin, clearly indicating that AHPND is still absent in Indonesia,

Hepatopancreatic microsporidiosis (HPM)

Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) was detected in Indonesia in 2015. EHP-positive shrimp was found in East Java (Banyuwangi and Situbondo), Lampung (Rawa Jitu), and Bali (Nagara,). However, at this stage the prevalence of HPM in Indonesia has yet to be determined because a systematic survey for EHP has not been conducted yet. So far, no overt clinical signs have been noted in any of the shrimp samples examined. In September 2015, farmers in Situbondo, East Java, reported concerns regarding the slow growth of their shrimps. At that time, shrimps cultivated in some ponds exhibited white feces disease. However the association between these two factors, i.e. slow growth and white feces disease, still needs to be thoroughly investigated.

EHP was detected in the hepatopancreas and intestines of *P. vannamei* samples by PCR. However, the source and route of infection remains unclear. Control measures that are currently being undertaken involve rigorous

Table 3. Results of surveillance and monitoring for transboundary diseases of penaeid shrimps in Indonesia from 2013 to 2015.

No.	Location	Disease			
		WSSV	IMNV	AHPND	HPM
1	North Sumatra	+	+	-	ND*
2	Lampung	+	+	-	+
3	Banten	+	+	-	ND
4	West Java	+	+	-	ND
5	Central Java	+	-	-	ND
6	East Java	+	+	-	+
7	West Kalimantan	+	+	-	ND
8	East/North Kalimantan	+	+	-	ND
9	South Sulawesi	+	-	-	ND
10	Bali	+	+	-	+
11	West Nusa Tenggara	+	-	-	ND

**Not done*

biosecurity and BMPs, lowering stocking density from 150 to 80 larvae/m^2, proper pond preparation, screening of broodstocks and larvae for EHP by PCR method, and using probiotics. HPM has been included in the surveillance program in 2016.

Action plans

Indonesia's competent authorities shall strictly implement pertinent regulations to maintain its AHPND-free status. Action plans in place to prevent the introduction of AHPND and other transboundary diseases of penaeid shrimps and fish into the Indonesian waters are enumerated below. These action plans have been rigorously disseminated to 12 major shrimp production areas, i.e. North Sumatera, Lampung, Banten, West Java, Central Java, DI. Yogyakarta, East Java, West Kalimantan, East Kalimantan, South Sulawesi, Bali, and Nusa Tenggara Barat through public-private partnership consultations, meetings and fora.

(1) Government regulations issued by the Ministry of Marine Affairs and Fisheries (MMAF) on the prevention of AHPND introduction to Indonesia:

   (a) MMAF Decree declaring the prohibition of shrimp and polychaete importation from or transit in countries with confirmed case of AHPND outbreak;

   (b) MMAF Decree on the institution of EMS/ AHPND Task Force;

   (c) Director General (DG) of Aquaculture's Circulation Letter on early mortality syndrome (EMS) prevention; and

   (d) DG of Aquaculture Decree pronouncing the prohibition of probiotic importation from or transit in countries with confirmed case of AHPND outbreak.

(2) Ongoing active and passive surveillance on AHPND, HPM, WSD, IMN in major shrimp production areas and hatcheries in Indonesia. Passive surveillance has been successfully implemented by working closely with the stakeholders, shrimp culture companies, and shrimp farmer associations such as the Shrimp Club Indonesia. Specimens for pathogen detection include shrimps of various stages, broodstocks, and natural food for broodstocks and larvae (e.g. polychaetes, *Artemia*, clams, etc.).

(3) Strengthening of farmers and shrimp hatchery operators' knowledge on transboundary disease prevention and control through strict adherence to biosecurity protocols and BMPs, and judicial use of chemicals and antibiotics in aquaculture.

(4) Eradication of AHPND-infected shrimp population if deemed required and necessary by competent authorities to prevent the spread of AHPND.

Updates on previously reported transboundary diseases of penaeid shrimps

White spot disease (WSD) and infectious myonecrosis (IMN)

Over the last 5 years, two major transboundary disease outbreaks of penaeid shrimps have been reported in Indonesia: white spot disease and infectious myonecrosis caused by WSSV and IMNV, respectively. Although shrimp production has started to recover from the devastating impact of WSD since 2002, WSD has remained as one of the most devastating diseases of farmed shrimp in Indonesia. The surveillance program will be continued until 2017. This is in line with our ultimate goal of declaring certain areas in Indonesia as WSSV- and IMNV-free.

Active surveillance for WSSV and IMNV was conducted following the method of Cameron (2002) and manual for aquaculture disease surveillance of MMAF. Active surveillance has been so far conducted in 11 provinces constituting the major shrimp farming areas in Indonesia. In average, 150-200 shrimp samples were obtained from 150 ponds (10% of the farms per area) per sampling. The surveillance was conducted twice a year when the cultured shrimps were at their most susceptible stage (30-40 days of culture). In addition, pond management and farmers knowledge and awareness about the impacts of WSD, IMN, AHPND and other transboundary diseases

of penaeid shrimps in general was likewise assessed using a questionnaire.

The results of the surveillance showed that WSSV and IMNV were present in the sampling areas examined (Table 3). The prevalence of WSSV-positive shrimp varied among the sampling areas and time of specimen collection. Interestingly, the presence of WSSV in collected samples did not always correlate with WSD outbreak. In general, the prevalence of IMNV in the sampling areas examined was lower than that of WSSV.

Impact of infectious diseases on aquaculture species

Disease outbreaks in hatcheries and grow-out ponds have remarkably reduced aquaculture production and productivity due to mortality, slow growth, low feed conversion and efficiency. In addition, contamination of aquaculture environments with infectious pathogens, inefficiency in the use of fishery resources, decrease in product quality which could result in loss of potential market, decrease in price and revenue due to the slowing down of aquaculture business intensification and expansion, and unsustainability have likewise resulted in reduced aquaculture production and productivity. Economic losses due to fish diseases consequently gave rise to unpaid bank debts, unemployment, poverty, and decline on investments by feed industries and processing plants among others. Based on the result of the epidemiological analysis undertaken by MMAF and universities in 2011, economic losses due to infectious and non-infectious diseases of major aquaculture species in Indonesia could be billion rupiah (13,070 Indonesian rupiah = 1 USD) per annum. It is also worth noting that despite stringent efforts employed to prevent and control diseases of major aquaculture species through several approaches enumerated in the action plans above, losses ascribed to infectious diseases still appeared to be economically significant.

The estimated annual financial losses were based on the epidemiological data of persistently devastating diseases of major aquaculture commodities in Indonesia (Table

Table 4. Estimated annual financial losses due to aquatic diseases.

Culture environment	Disease	Main commodity affected	Mortality range (%)	Loss estimation/ year (million rupiah)
Freshwater	Koi herpesvirus (KHV)	Gold fish and koi	30-80	50,000
	Motile aeromonad Septicaemia (MAS)	All types of freshwater fish	20-100	400,000
	Streptococcosis	Tilapia	20-50	15,000
	Mycobacteriosis	Gouramy	10-50	7,500
	Ichthyophthiriosis	All types of freshwater fish	25-100	50,000
	Others	All types of freshwater fish	10-100	150,000
SUB TOTAL				**672,500**
Brackishwater	White spot diseases (WSD)	Tiger and white shrimps	30-100	2,500,000
	Infectious myonecrosis (IMN)	Tiger and white shrimps	10-60	1,250,000
	Vibriosis	Tiger and white shrimps	10-40	100,000
	Others	Tiger and white shrimps	10-25	50,000
SUB TOTAL				**3,900,000**
Marine	Viral nervous necrosis (VNN)	Grouper	10-100	250,000
		Seabass	30-70	150,000
	Vibriosis	Grouper and seabass	10-80	150,000
	Ice-ice	Seaweed	10-30	50,000
	Others	Cultured marine fish	10-50	100,000
SUB TOTAL				**700,000**
TOTAL				**5,272,500**

1 USD = 13,070 Rp

4). The economic losses have been estimated at 5.2 trillion Indonesian rupiah.

Way forward

The surveillance program on WSSV, IMNV, and other transboundary pathogens which was instituted in 2013 will be continued until 2017. Notably, MMAF has been actively supporting the development of vaccines against viral diseases of fish (KHV disease and VNN) and shrimps (WSSV infection) and other equally important diseases implicated in serious mortalities of freshwater fish species such as aeromonad septicemia, streptococcal infection, and mycobacteriosis, among others. Field trials of the VNN and bacterial vaccines in marine and freshwater fish species, respectively, will be conducted in 2016. Laboratory data pertinent to the management of IMN and WSD in penaeid shrimps, i.e. through water quality manipulation, have already been generated. However, its practical application in the field has yet to be evaluated. In addition, investigation of the antimicrobial potential of local herbs in treating bacterial diseases of shrimp and fish are being likewise undertaken.

To keep abreast with the impacts of emerging and persistent disease problems on economically important aquaculture species, our future researches will focus on (1) the effect of chemicals and antibiotics not only on cultivated aquatic species but as well as in non-target resident organisms in the pond such as polychaetes, snails, and crab; (2) long-term effects and sustainability of using indigenous and imported probiotics particularly in ponds equipped with plastic liners; (3) establishment of efficient and economically sound approaches geared at increasing productions of SPF shrimp seeds; (4) domestication and diversification of new species; (5) development of effective and economically viable vaccines for viral and bacterial diseases of fish and crustaceans; and (6) elucidation of the mechanisms behind the behaviour of infectious disease agents in asymptomatic (carriers) hosts and vectors. The outputs of these researches will be pivotal in the formulation of pragmatic and economically viable strategies that will propel the institution of a more holistic and sustainable aquaculture sector.

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