# Regional Response on AHPND and Other Emerging Shrimp Diseases in the Asia-Pacific

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## Abstract

Transboundary aquatic animal diseases are among the major concerns for establishing biosecurity measures and strengthening of aquatic animal health (AAH) management capacity (including emergency preparedness) in the region. In aquaculture, biosecurity and AAH management entails protection of fish or shellfish from infectious agents (viral, bacterial, fungal or parasitic) as well as prevention of disease spread from one area to another. Several transboundary aquatic animal diseases have swept the region over the past 25 years causing massive economic and social losses. These include spread and outbreaks of epizootic ulcerative syndrome (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (e.g. white spot disease [WSD], infectious haematopoietic necrosis [IHHN]). The spread of these transboundary diseases clearly demonstrates the vulnerability of the aquaculture industry to disease emergence where impacts have been aggravated by the lack of effective preparedness and response when diseases emerge.

Recently, outbreaks of acute hepatopancreatic necrosis disease (AHPND), popularly known as early mortality syndrome (EMS), among cultured shrimps were reported in China and Viet Nam (2010), Malaysia (2011), Thailand (2012), Mexico (2013) and the Philippines (2014). There have been reports of its spread in South American countries but limited report is available in this regard. This disease caused significant losses in the production of *Penaeus monodon* and *P. vannamei* in the affected countries. NACA's regional response to this disease during its initial outbreak in Viet Nam, Thailand and Malaysia signified that improved control on transboundary diseases and emergency preparedness are still needed in the region. In collaboration with international organizations (OIE, FAO), NACA has implemented awareness programs, efficient information dissemination, and emergency regional expert consultation to address this disease problem. OIE and FAO also deployed experts to assess the disease and identify the pathogen involved. All of these efforts, together with subsequent studies on prevention and disease management, have paved the way in preventing further spread of this disease to other shrimp-producing countries so far. However, the risk is still very high that this disease will spread, as transboundary movement of live shrimps within and outside the region is inevitable. In addition, other emerging diseases are now affecting production of major cultured shrimps in the region. These include hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) with confirmed reports from China, Viet Nam, Thailand, Malaysia and Indonesia (unconfirmed reports from India), and viral covert mortality disease (VCMD) which was reported to be affecting cultured shrimps in China.

By and large, outbreaks of damaging aquatic animal diseases are likely to continue and the potential consequences are likely to increase with the expansion (intensification) of aquaculture systems and introduction of new species for culture. Consequently, the risks associated with emerging and transboundary diseases are shared – shared water bodies and epidemiological links through trade (especially live movement) – thus, a collaborative approach in dealing with these diseases is therefore warranted and necessary.

## Introduction

Aquaculture is one of the important sectors in the economies of the ASEAN Member States (AMS). However, majority of aquaculture farms are small-scale and most often lack the necessary facilities to comply with or not well informed of the product standards imposed by concerned authorities, especially for international trade. Although some AMS (e.g. Thailand, Philippines, Malaysia, Indonesia and Viet Nam) have made significant progress in disease surveillance and aquatic animal health certification for biosecurity purposes, which is required for exported aquaculture products (both live and processed), other less-developed countries in the region have made very limited progress in this regard.

It is, therefore, necessary for these countries to develop and implement national as well as regional biosecurity frameworks to minimize the impacts of aquatic animal diseases, prevent the introduction and spread of infectious diseases, and to produce high quality and safe aquaculture products. The continuous globalization in the aquaculture trade has increased the potential of disease introduction and spread to new areas. Important aquatic animal diseases have been reported to spread among major aquaculture-producing and -exporting countries, and can usually cause serious economic losses, as well as some social and ecological consequences. The movement of live aquatic biota (plants and animals), their products and the water they are in has the potential to transfer pathogens from one country or region to another where the pathogens may not currently exist. In shrimps as example, most major disease outbreaks were associated with the movement of live animals (broodstock, nauplii and postlarvae) when the patterns of disease spread were analyzed. Transboundary diseases are among the major concerns for establishing biosecurity measures and strengthening of aquatic animal health management capacity in the region. Many aquatic animal diseases, once established, are often difficult to treat or to eliminate.

Several transboundary aquatic animal diseases have swept the region over the past 25 years causing massive economic and social losses. These include spread and outbreaks of Infection with *Aphanomyces invadans* (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (white spot disease [WSD], white tail disease [WTD], yellow head disease [YHD]) (Rogers *et al.*, 2011). More recently, infectious myonecrosis (IMN) and acute hepatopancreatic necrosis disease (AHPND) are seriously affecting shrimp aquaculture in Indonesia (IMN; Senapin *et al.*, 2007), Malaysia, Philippines, Thailand and Viet Nam (AHPND; Flegel, 2012; Leaño and Mohan, 2012; Dabu *et al.*, 2015). The spread of these transboundary aquatic animal diseases clearly demonstrates the vulnerability of the aquaculture industry, as well as the wild fish populations, to disease emergence where impacts have been exacerbated by the lack of effective preparedness and response when diseases emerge. It is also necessary that aquafarmers, especially small-scale, be properly informed and educated on the current market standards being imposed by most importing countries, so that they can produce aquaculture products that can be considered safe for trading and consumption based on these standards on aquatic animal health and food safety.

## AHPND and its spread

AHPND is a new disease causing unusually heavy mortality in cultured shrimps at approximately 30-45 days of culture. It was first reported in 2009 (officially in 2010) in China as a novel disease of unknown aetiology in shrimps and was initially named early mortality syndrome (EMS). The disease has spread to Viet Nam (2010), Malaysia (2011), Thailand (2012) (Flegel, 2012; Leaño and Mohan, 2012; Joshi *et al.*, 2014), Mexico (2013) (Nunan *et al.*, 2014; Gomez-Gil *et al.*, 2014), and the Philippines (2014) (NACA-FAO 2015; Dabu *et al.*, 2015; dela Peña *et al.*, 2015).

Clinical signs observed include slow growth, corkscrew swimming, loose shells, as well as pale coloration. Affected shrimp also consistently show an abnormal hepatopancreas (HP) (shrunken and discolored). The key diagnostic features needed for confirmation is the medial sloughing off of HP cells as seen in histological sections of the affected shrimp's HP. Other histopathological features of AHPND in both *P. monodon* and *P. vannamei*, which appear to be limited to the HP, are the following (Prachumwat *et al.*, 2012; Tran *et al.*, 2013):

- Lack of mitotic activity in generative E cells of the HP;
- Dysfunction of central hepatopancreatic B, F and R cells;
- Prominent karyomegaly and massive sloughing of central HP tubule epithelial cells;
- Terminal stages including massive intertubular hemocytic aggregation followed by secondary bacterial infections.

The disease is caused by a highly virulent strain of *Vibrio parahaemolyticus* (Tran *et al.*, 2013), carrying plasmids containing the genes that code for the toxins pirA and pirB (Han *et al.*, 2015; Lee *et al.*, 2015). Recently, genome sequencing has identified a non-*V. parahaemolyticus* associated with diseased shrimp in Viet Nam that also contain the same toxin plasmid, and indicated that it was *Vibrio harveyi* (Kondo *et al.*, 2015). A strain of *V. owensii* causing serious AHPND in shrimp, was also found to contain the plasmid similar to that of *V. parahaemolyticus*, indicating that the plasmid plays an important role in shrimp AHPND (Liu *et al.*, 2015).

## NACA's regional response on EMS/ AHPNS/AHPND

In April 2011, Viet Nam, which has been suffering from the devastating effect of AHPND (then known as EMS) for more than a year, officially sought assistance from regional and international organizations including the Network of Aquaculture Centres in Asia-Pacific (NACA), the World Organization for Animal Health (OIE) and the Food and Agriculture Organisation of the United Nations (FAO). OIE and FAO immediately sent emergency missions to Viet Nam, to investigate and make a quick assessment of the disease problem. NACA fully coordinated and collaborated with both OIE and FAO in the implementation of subsequent projects to solve the disease problem. In November 2011, the NACA's Asia Regional Advisory Group on Aquatic Animal Health discussed the issue during its 10th Meeting held in Mangalore, India. The disease was referred to by Prof. Timothy Flegel as acute hepatopancreatic degenerative necrosis syndrome (AHDNS). Recognizing the importance/threat of AHDNS to the shrimp industry in the region, the Advisory Group gave the following recommendations to NACA during the meeting (NACA, 2011):

- Make a request to Dr. Donald V. Lightner (Arizona State University) to prepare the case definition of the disease. NACA will then prepare the corresponding Disease Card for circulation to NACA member countries and uploading in the NACA website.
- Encourage member governments to report any occurrence/outbreak of AHDNS through immediate reporting and/or the QAAD Reporting System.

On the other hand, initial findings of the different emergency missions in Viet Nam suggested an infectious etiology based on how the disease has spread. Other agents, however, were not discounted including environmental causes (e.g. toxins, pesticides, etc.). In May 2012, a formal Circular and a Disease Advisory were released by NACA and widely disseminated to Competent Authorities (CA), partner institutions and other relevant organizations in the Asia-Pacific region and the world. The Advisory was also published in key publications for wider dissemination of information regarding this emerging shrimp disease problem (Leaño and Mohan, 2012). NACA also made all the necessary efforts to look for donors which can possibly fund an emergency regional consultation to discuss the current status of the disease.

The Australian Department of Agriculture, Fishery and Forestry (now known as Australian Department of Agriculture) provided NACA the necessary funds to organize the emergency regional consultation, which was held in August 2012 in Bangkok, Thailand. The primary objectives of the consultation were to:

- bring together global experts, national participants representing CAs and lead research institutions, regional and international organizations and the private sector;
- facilitate networking and information sharing for better understanding and dealing with EMS;
- document the current state of knowledge on EMS and lessons learned in dealing with disease emergencies at national/ regional levels; and
- agree on a regional action plan for dealing with future aquatic disease emergencies in the region.

The consultation was attended by 17 global shrimp health experts and 65 representatives from NACA member countries, lead research institutions in the region, other regional and international organizations, national universities and private sectors. The proceedings of the consultation was published in a Technical Report which includes the latest updates and review of status of EMS, case definition at animal and pond level, R&D directions, and recommendations for preventive measures at both national and regional levels (NACA, 2012a).

In September 2012, in collaboration with Prof. Donald V. Lightner and Prof. Timothy Flegel, NACA released the Disease Card for AHPNS (NACA, 2012b), which contains relevant updates and information about the syndrome (prior to the identification of the causative pathogen) which was widely disseminated to relevant authorities and institutions in the region. This was for the purpose of harmonizing research effort and supporting disease surveillance and disease outbreak investigations. The NACA AG, during its 11th meeting held in Bangkok, Thailand, also recommended the inclusion of AHPNS in the Quarterly Aquatic Animal Disease (QAAD) Reporting in the Asia-Pacific, commencing in the January-March 2013 reporting period (NACA-FAO, 2013). This was mainly for the purpose of gaining more information and updates on the occurrence and spread of AHPNS in the region.

After the first Regional Consultation, many reports have circulated among the stakeholders in the shrimp industry on the identification of causative agent of the disease, without any scientific bases to prove such claims. Thus, in March 2013, NACA released a second Disease Advisory to address the many circulating false and baseless speculations on the effects, spread and causative agent of AHPNS (NACA, 2013a). After the release of the second Advisory, the team of Dr. Lightner finally identified the causative agent of the disease as a virulent strain of *V. parahaemolyticus*. This was reported in a paper in the journal Diseases of Aquatic Organisms (Tran *et al.*, 2013). With this development and the recommendations made from the FAO TCP in Viet Nam (FAO, 2013), the NACA AG officially renamed the disease to AHPND and recommended to revise the Disease Card to include the identification of the pathogen, bioassay procedures, rapid diagnostic test, and other recent information about the disease (NACA, 2013b). The name in the QAAD list was also changed accordingly, effective the January-March 2014 reporting period.

Currently, the disease is being reported (through QAAD reporting) from China, Philippines, Thailand and Viet Nam. Malaysia's recent reports are questionable, as they have reported the non-occurrence of the disease since the 3rd quarter of 2014, although there are still reports of outbreaks in the country.

On diagnostics, several AHPND detection methods including PCR-based protocols have been developed. The use of AP1 to AP4 primers for PCR-based detection was first announced at the NACA website starting 2013. Primers AP3 and AP4 target the AHPND toxin genes pirA and pirB (Sirikharin *et al.*, 2015; Dangtip *et al.*, 2015), and these primers are released for free, together with positive control plasmids, courtesy of Prof. Timothy Flegel's research

team. Confirmatory diagnosis of the disease is still based on histopathology of HP and infection experiment/bioassay (if possible).

AHPND is now included in the OIE list of reportable diseases after its endorsement in May 2015 during the OIE General Session. The Chapters for both the OIE Aquatic Animal Health Code and Manual are being prepared for endorsement during the GS in May 2016. Official reporting to OIE commenced in January 2016.

As there is no control measure presently available for AHPND, several preventive measures are recommended one of which is the use of clean broodstock and PLs which has been made possible by screening using the different PCR detection methods that have been developed. Additionally, the use of healthy broodstock and production of healthy and strong PLs were recommended to prevent infection, while improved environmental and feeding management as well as efficient biosecurity measures will prevent outbreaks and spread of the disease.

## Other emerging shrimp diseases

### *Heptopancreatic microsporidiosis caused by* Enterocytozoon hepatopenaei *(HPM-EHP)*

Hepatopancreatic microsporidiosis was first seen way back in 2001 in the HP of *P. modonon* (Chayaburakul *et al.*, 2004) and the pathogen identified (*E. hepatopenaei*; EHP) in 2009 (Tourtip *et al.*, 2009). It was discovered in slow growing shrimp but was not statistically associated with slow growth at that time (Sritunyalucksana *et al.*, 2014) Recently, EHP was found in shrimps with white feces syndrome, but was confirmed to be not the causative agent of the disease (Tangprasittipap *et al.*, 2013). However, it was also reported by Tangprasittipap *et al.* (2013) that EHP could infect exotic *P. vannamei* imported for cultivation in Asia and that it could be transmitted directly from shrimp to shrimp via the oral route.

HPM-EHP shows no clear gross signs for diagnosis, but when severe growth retardation is observed among cultured shrimps, then it can be suspected as a possible cause. To confirm diagnosis, histology, wet mount microscopic observation and/or molecular methods are required (NACA, 2015a). The disease causes retarded growth when copies above 103/ng total HP DNA is observed (Liu *et al.*, 2016). No significant mortality can be observed among shrimps affected by the disease.

HPM-EHP is widespread in China, Malaysia, Thailand and Viet Nam, and probably India (Sritunyalucksana *et al.*, 2014). In response to the growing concerns on the effect and spread of this disease, a Disease Card was prepared and published (NACA, 2015a), while the NACA AG agreed and recommended its listing in QAAD Reporting (NACA, 2015b), starting at the first quarter of 2016.

### *Viral covert mortality disease (VCMD)*

VCMD is caused by covert mortality nodavirus (CMNV), which has caused serious losses among cultured shrimps in China since 2009 (Zhang *et al.*, 2014). Clinical signs include hepatopancreatic atrophy and necrosis, empty stomach and guts, soft shell, slow growth, and whitish muscle in the abdomen (Zhang, 2014; Huang, 2012). Affected shrimps usually die at the pond bottom, and mortality can be observed daily during 30-80 days of culture, peaking during 60-80 days of culture with cumulative mortality of up to 80% (Zhang *et al.*, 2014). The increase in mortality is usually accompanied by increase of nitrite-nitrogen and high temperature above 28°C.

Diagnosis of the disease include histopathological observation (Zhang *et al.*, 2014) of affected shrimps showing separated HP tubules with haemocytic infiltration, nuclear karyomegaly and eosinophilic intracellular inclusions in the tubular epithelium of HP. In some cases, swollen nucleoli could be found in hepatopancreocytes. The muscle fibres composing the whitish muscle lesion have muscle fragmentation tending towards coagulative muscular lysis and myonecrosis. Multifocal myonecrosis in the striated muscle is accompanied by haemocytic infiltration and karyopyknosis. Other diagnostic methods include transmission electron microscopy (Zhang *et al.*, 2014), fluorescence *in situ*

hybridization, nested RT-PCR, RT-LAMP (Zhang *et al.*, in press), and by highly sensitive detection kits that have been developed.

Surveillance of the disease using molecular diagnostic methods revealed that the disease is widespread in shrimp culture provinces of China. In 2014, about 18% of the >300 samples collected from 10 coastal provinces in China were positive for CMNV. The virus was detected not only in *P. vannamei*, but also in *P. chinensis*, *P. japonicus*, *Macrobrachium rosenbergii* and the swimming crab *Portunus trituberculatus* (Huang, 2015). Positives in samples of different life stages, including nauplii, postlarva, juveniles, and broodstock, were also detected. Samples positive for the virus from other countries in Asia and Americas were also detected.

As agreed and recommended by the NACA AG, a disease card for VCMD is now being prepared. The disease card will contain information about the disease including clinical signs, different methodologies for diagnosis, as well as list of experts. It will then be considered for inclusion in the QAAD Reporting to encourage surveillance and reporting.

## Conclusion

The initial outbreaks of AHPND in major shrimp-producing countries in the ASEAN has proven the importance of information dissemination and awareness programs for both affected and non-affected countries, which has prevented the further spread of the disease. So far, the disease did not spread to other shrimp-producing countries, as relevant biosecurity measures were put into place to prevent entry of the disease (e.g. Indonesia). Dealing with disease emergencies like AHPND will require emergency funds to immediately assess the status and impacts of the disease. FAO and OIE emergency missions to Viet Nam were undertaken a few months after Viet Nam sought assistance. However, regional action was only initiated after more than one year (through an emergency regional consultation organized by NACA) due to lack of funds to undertake such important activity. Moreover, coordinated and collaborative works are necessary in order to establish the nature of the disease (e.g. epidemiology, identification of the pathogen, diagnostics), as well as in the formulation of preventive measures and disease management strategies.

Outbreaks of important and emerging aquatic animal diseases will likely continue with the continuous expansion or intensification of aquaculture systems and introduction of new species for culture. The risks associated with these emerging and transboundary diseases are shared, as countries in the region have shared water bodies and epidemiological links through trade and transboundary movement of live aquatic animals. Therefore, a collaborative approach in dealing with these diseases is warranted and highly necessary for better aquatic animal health management at both national and regional levels.

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