

Acute Hepatopancreatic Necrosis Disease (AHPND) of Penaeid Shrimps: Global Perspective

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Abstract

The Organization for Economic Cooperation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations Agricultural Outlook 2015-2024 reported that fisheries production worldwide is projected to expand by 19% between the 2012-14 base period and 2024, to reach 191 million metric tons (MT) and the main driver of this increase will be aquaculture, which is expected to reach 96 million MT by 2024, 38% higher than the base period (average 2012-14) level. Among the 7 key uncertainties that affect gains in productivity, the potential of animal disease outbreaks to affect aquaculture production and subsequently domestic and international markets are once again highlighted, although for the first time in this outlook. Another milestone document, the “Blue frontiers: managing the environmental costs of aquaculture” identified a number of fish health issues, including increased risk of the spread of pathogens and diseases with intensification, through increased movement of aquatic animals, inter-regional trade and introduction of new species and new strains, and through the use of trash fish or live feed; concerns on residues and development of drug resistant pathogens brought about by the abuse on the use antimicrobials and other veterinary drugs; limited availability of vaccines; environmental stressors that compromise the immune system; difficulties faced by developing countries in implementing international standards; and the need for legislation, enforcement and capacity building. The issues identified then and now are almost the same.

Addressing animal health issues in aquaculture is very challenging because the sector is highly complex (with a wide range of diversity in terms of species, systems, practices and environment, each presenting different risks), its fluid environment, and the transboundary nature where fish is considered as one of the most traded commodity, aquatic animals require more attention in order to monitor their health: they are not visible except in tank holding conditions; they live in a complex and dynamic environment and feed consumption and mortalities are hidden under water.

This paper looks at the status of a newly emerging disease of cultured shrimp, acute hepatopancreatic necrosis disease (AHPND), which has been recognized as the most important non-viral disease threat to cultured shrimp. In particular, this paper presents the highlights of the International Technical Seminar/Workshop: “EMS/AHPND: Government, Scientist and Farmer Responses” held from 22–24 June 2015 in Panama City, Panama, which was organized under the auspices of an FAO inter-regional project TCP/INT/3502: Reducing and Managing the Risks of AHPND of Cultured Shrimp, being participated by 11 countries, namely: Colombia, Ecuador, Guatemala, Honduras, Mexico, Panama and Peru from Latin America and the Caribbean (LAC) region and India, Iran, the Philippines and Sri Lanka from the Asian region. The Panama EMS/AHPND June 2015 event aimed to provide a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. More than 100 stakeholders from 21 countries representing the government, academe and producer sectors participated in the event. The highlights contain the latest available information at that time (June 2015) about AHPND including the current state of knowledge about the causative agent, the host and geographical distribution, detection methods, risk factors, management and actions of regional and international organizations.

Introduction

The Food and Agriculture Organization (FAO) Global Aquaculture Production statistics database has registered a total of 575 aquatic species and species groups grown in freshwater, seawater and brackishwater. In terms of value, the total food fish value was USD 150 million while crustaceans were valued at USD 31 million (FAO, 2015). Crustaceans are an important source of aquatic food protein. Production (as food and ornamental) and trade are extremely important for developing countries. It provides both economic development and empowerment in terms of contribution to gross domestic product (GDP), consumption, employment, catch value and exports. The crustacean sector generates high value export products which enables producers to buy lower value products in the world market - thus a positive contribution to food security in both producing and exporting countries (Bondad-Reantaso *et al.*, 2012). However, the crustacean sector is facing huge challenges due to disease outbreaks.

The OECD-FAO Agricultural Outlook is a collaborative effort of the Organization for Economic Co-operation and Development and FAO. It brings together the commodity, policy and country expertise of both organizations and input from collaborating member countries to provide an annual assessment of prospects for the coming decade of national, regional and global agricultural commodity markets. The OECD-FAO Agricultural Outlook 2015 for the first time had included fish as one of the commodities assessed. The outlook reported that by 2015, aquaculture is projected to surpass capture fisheries as the most important source of fish for human consumption, and by 2020 should represent about 45% of total fishery production (including non-food uses). Compared to the 2008-2010 period, average capture fish prices are expected to be about 20% higher by 2020 in nominal terms compared with a 50% increase for aquaculture species. Fisheries production worldwide is projected to expand by 19% between the 2012-14 base period and 2024, to reach 191 million MT and the main driver of this increase will be aquaculture, which is expected to reach 96

million MT by 2024, 38% higher than the base period (average 2012-2014) level. The outlook identified a number of key uncertainties affecting productivity gains. These include the following: natural productivity of fish stocks and ecosystem; occurrence of El Niño; fish meal and fish oil outlook; trade policies, and in particular bilateral trade agreements, remain an important factor influencing the dynamics of the world fish markets; availability and accessibility to land and water; financial resources; improvement in technology; and feeds, etc. The outlook reported that in addition, animal disease outbreaks have shown the potential to affect aquaculture production and subsequently domestic and international markets depending on the size and the species involved (OECD/Food and Agriculture Organization of the United Nations, 2015).

Hall *et al.* (2011), in a milestone publication of WorldFish Center and Conservation International Blue Frontiers¹ made a comprehensive assessment of how the global aquaculture industry uses natural resources and its impacts on the environment. They have identified a number of fish health issues such as:

- increasing risk of the spread of pathogens and diseases with intensification, farms getting larger and more concentrated;
- increasing movement of aquatic animals, inter-regional trade and introduction of new species and new strains increases the risk;
- use of trash fish as another risk factor in pathogen transfer;
- abuse on the use of antimicrobials and other veterinary drugs, concerns on residues and development of drug resistant pathogens;
- availability of vaccines limited for a few species and against few diseases;
- environmental stressors compromising the immune system;
- international standards more widely applied by developed countries; developing countries facing difficulties; and
- legislation, implementation and capacity building needed

¹http://www.conservation.org/publications/documents/BlueFrontiers_aquaculture_report.pdf

Transboundary aquatic animal diseases or TAADS have the following characteristics: highly contagious/transmissible (infectious!); has the potential for very rapid spread irrespective of national borders (no passport!); cause serious socio-economic and possibly health consequences (high risk and high impact!); one of the negative impacts of trade globalization (important pathway!). The World Organization for Animal Health (OIE) lists about 27 aquatic pathogens/diseases which fit established criteria for listed diseases in terms of consequence, spread and diagnosis (important to trade!) (Bondad-Reantaso *et al.*, 2005).

Diseases in aquaculture can be infectious or non-infectious. Infectious diseases can be categorized into three. The first group are diseases that are important to trade (OIE list of diseases), governed by international standards, with a set of criteria to be met to be included in the list, pathogens/diseases of important traded species (e.g. finfish, crustaceans, mollusks), mandatory reporting/notification is required during an outbreak. The second group are diseases that are consistently affecting aquaculture species at the hatchery, nursery and grow-out levels, and may be caused by bacteria, parasites, fungi, and viruses. In addition, the third group are emerging diseases; these can be known diseases which have spread into new geographical areas or affected new susceptible species and diseases of unknown aetiology.

FAO TCP/INT/3502 Reducing and Managing the Risks of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp

The FAO inter-regional project, TCP/INT/3502 'Reducing and Managing the Risks of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp', is being participated by 11 countries, namely: Colombia, Ecuador, Guatemala, Honduras, Mexico, Panama and Peru from Latin America and the Caribbean (LAC) region and India, Iran, Philippines and Sri Lanka from the Asian region. One of the major outcomes expected from this project is enhanced knowledge and strengthened capacities for dealing with AHPND in the Asian

and LAC regions. The conduct of an international technical seminar involving resource experts from the government, academe and producer sectors is one of the mechanisms that will contribute to achieving the above expected outcome. The International Technical Seminar/Workshop: "EMS/AHPND: Government, Scientist and Farmer Responses" was held from 22–24 June 2015 in Panama City, Panama. This technical seminar was implemented under the auspices of the project TCP/INT/3502 and was jointly organized with OIRSA, the Regional International Organization for Plant Protection and Animal Health. The objective of the Panama EMS/AHPND June 2015 event was to provide a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. More than 100 stakeholders from 21 countries representing the government, academe and producer sectors participated in the event. The highlights contain the latest available information at that time (June 2015) about AHPND including the current state of knowledge about the causative agent, the host and geographical distribution, detection methods, risk factors, management and actions of regional and international organizations. The information presented below was based on the 21 technical presentations by resource experts representing the government, academe and producer sectors. Drs. J.R. Arthur and M. Reantaso led the writing of the summary and highlights with comments and contributions received from Drs. C. Lavilla-Pitogo, I. Karunasagar, and V. Alday-Sanz.

Highlights of the International Technical Seminar/Workshop: "EMS/AHPND: Government, Scientist and Farmer Responses" (22–24 June 2015, Panama City, Panama) under the FAO project TCP/INT/3502

Currently considered the most important non-viral disease threat for cultured shrimp, AHPND is characterized by mass mortality during the first 35 days of culture where affected shrimp show massive sloughing of hepatopancreatic epithelial cells followed by death. This emerging disease, unlike most diseases affecting farmed

penaeid shrimp, is caused by the ingestion of toxins (PirA and PirB) generated by a specific plasmid carried by certain strains of *Vibrio parahaemolyticus*, a bacterium that is ubiquitous in marine and brackishwater environments. The pathogen can thus be present both in cultured shrimp and in the water, sediments and associated organisms of the culture ponds.

The genus *Vibrio* comprises about 30 species of bacteria that generally require sodium chloride supplementation of the medium for growth. *V. parahaemolyticus* occurs naturally in coastal and estuarine environments, in both tropical and temperate parts of the world, and has been isolated from water, sediment, molluscs, crustaceans, finfish and other animals. Environmental conditions such as temperature, salinity, zooplankton, dissolved oxygen and tidal flushing may affect the survival, establishment and growth of this organism. The *V. parahaemolyticus* genome has several clusters of genes that have been acquired by horizontal gene transfer. Some of them (called *tdh* and *trh* gene clusters) are associated with pathogenicity to humans. AHPND-causing strains lack the gene clusters involved in pathogenicity to humans.

The Network of Aquaculture Centres in Asia-Pacific (NACA) Quarterly Aquatic Animal Disease (QAAD) reporting system² includes AHPND in the NACA regional list of disease. A request for the inclusion of AHPND in the List of Notifiable Diseases of the World Organization for Animal Health's (OIE) was submitted in 2014, but the OIE Aquatic Animal Health Standards Commission (AAHSC) did not endorse the listing during its February 2014 meeting since AHPND did not meet all the criteria for disease listing. However, in March 2015, the AAHSC endorsed the listing of AHPND and a final decision to this effect was made during the OIE General Session held in May 2015. Reporting of AHPND to OIE commenced in January 2016.

Current state of knowledge about AHPND

The causative agent was discovered in 2013 as unique isolates of *V. parahaemolyticus* (VP_{AHPND}) that carry a plasmid (pAP1) of approximately 69 kbp. This plasmid contains two genes that

produce toxins (one 12.7 kDa and one 50.1 kDa) that are capable of acting together to cause AHPND. The Pir A/B toxin genes that code for the two toxin proteins that induce AHPND in shrimp have been reported to be similar to PirA/B toxin genes known from *Photobacterium* spp. (Gram-negative, luminescent, rod-shaped bacteria that are members of the Family Enterobacteriaceae). In nature, *Photobacterium* spp., that live in obligate, symbiotic relationship with the entomopathogenic nematode *Heterorhabditis* spp. and a closely-related genera *Heterorhabditis* spp., are parasites of insect larvae. *Heterorhabditis*/*Photobacterium* have a wide geographic distribution and, since the 1980s, have been researched extensively for application in insect control. Fortunately, the VP_{AHPND} isolates characterized so far pose no threat to human health.

Current host and geographic distribution

AHPND first appeared in the People's Republic of China (around 2009 and was called Covert Mortality Disease), and has since been recorded from Viet Nam (2011), Malaysia (2011), Thailand (2012), Mexico (2013 from the scientific literature) and the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; Dabu *et al.*, 2015; dela Peña *et al.*, 2015). It is suspected to be present in, but unreported from other countries in both Asia and Latin America and the Caribbean (LAC). The disease infects mainly whiteleg shrimp (*Penaeus vannamei*), but has also been reported from giant tiger prawn (*P. mondon*) and fleshy prawn (*P. chinensis*).

Current status of detection methods to diagnose AHPND

The presumptive gross signs of AHPND in penaeid shrimp include an empty stomach and midgut, a pale and shrunken hepatopancreas, and mortality within approximately 35 days after stocking of postlarvae (PL). However, similar gross signs may occur with other diseases, thus, confirmation requires histological examination of the hepatopancreas to reveal the unique feature of the acute stage of AHPND, i.e. massive sloughing of cells of the

²http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report

tubule epithelium in the absence of any clear evidence of a causative agent.

To aid in the identification of reservoirs and potential transmission routes, two interim polymerase chain reaction (PCR) detection methods based on primers designated as AP1 and AP2 were introduced at the NACA website⁵ in December 2013 and later updated. The AP2 primer turned out to be the better primer with about 3 percent false-positive results. Despite this weakness, the method was used successfully to reveal a high prevalence of VP_{AHPND} in live broodstock feeds (e.g. polychaetes and bivalves), in pond-reared broodstock, and in PLs used to stock shrimp farms. Testing in Thailand also provided evidence that specific-pathogen-free (SPF) stocks that had tested free of VP_{AHPND} later became positive after use for PL production in some local shrimp hatcheries, providing clear evidence of biosecurity failures.

To overcome the problem of false-positive PCR test results, an improved PCR detection method (AP3) was developed based on the discovery of the two AHPND toxins and on use of the gene sequence of the smaller 12.7 kDa toxin. The AP3 method, which was released at the NACA website in June 2014, gave no false-positive or false-negative results with the 104 bacterial isolates tested. Since the AP1 to AP3 methods for VP_{AHPND} detection were one-step PCR detection methods and could not be successfully modified into nested-PCR methods, samples with low pathogen loads had to be subjected to an enrichment step by culture in broth medium for 4 hr before separation of bacterial cells to prepare the DNA template for the PCR assays.

To overcome problems with samples that could not be subjected to the enrichment step (e.g. samples preserved in alcohol or archived DNA samples), a nested-PCR method (AP4) was developed and announced at the NACA Website on 20 February 2015. It targeted the whole sequence of the 12.7 kDa toxin gene and 70 percent of the large toxin gene, and it gave 100 percent positive and negative predictive values for the same 104 isolates used to validate the AP3 method. However, it had 100 times higher detection sensitivity (down to 100 fg template DNA).

⁵www.enaca.org

By cooperation between Centex Shrimp and the Sakarindrwirote University in Bangkok, antibodies have been produced against heterologously expressed AHPND toxins and used for detection by enzyme-linked immunosorbent assay (ELISA). This will allow for quantification of the toxins in feeds and the environment and for more convenient laboratory testing for therapeutic measures and resistant shrimp stocks.

Risk factors

The most important risk factors for the international spread of AHPND are:

- movement of live shrimp from a geographic region where AHPND is prevalent to an unaffected region for aquaculture (AHPND is thought to have been transmitted to Mexico from Asia by this route).
- the importation of live animals (e.g. polychaetes, clams) as feeds for shrimp broodstock (polychaetes imported from P.R. China may have been the major route for introduction of AHPND to Thailand).

Other potential but as yet unconfirmed routes of disease transfer are by:

- crabs, crayfish and other crustaceans
- predatory birds and mammals
- attachment of flocs to zooplankton that are carried long distances by ocean currents
- attachment on crustaceans and in ships' ballast waters
- via untreated wastes from infected shrimp in processing plants
- via use of infected shrimp

Environmental factors that are believed to promote infection by VP_{AHPND} in shrimp ponds include:

- high concentration of nutrients in pond water by addition of fertilizers, molasses, etc.
- high water temperature, salinity (>5 ppt) and pH (>7)
- low water turnover coupled with low planktonic biodiversity

- presence of soluble nutrients (feed), unconsumed pelleted feed, shrimp carcasses, leading to accumulation of organic-rich sediment

Most cases of VP_{AHPND} have shown co-infection with other shrimp pathogens, for example, monodon baculovirus (MBV), white spot syndrome virus (WSSV), hepatopancreatic parvovirus (HPV), *Enterocytozoon hepatopenaei* (EHP) and unidentified gregrarine-like entities.

Disease management

Several innovations in shrimp management have been targeted at reducing the number of VP_{AHPND} in the shrimp and its environment by promoting bacterial diversity and control high numbers of pathogenic VP_{AHPND}. This have been achieved by disinfection of water, use of reservoirs to microbially mature water, use of probiotics, clean feeds and screening of broodstock and PL.

Other effective management measures are primarily at the farm-level. These include:

- ensuring good farm biosecurity and best management practices (BMPs)
 - beginning with PL derived from broodstock verified to be free of AHPND (i.e. PL derived from SPF or high health broodstock)
 - avoidance of overfeeding as uneaten pellets are substrate for AHPND bacteria to grow
 - removing sediment as often as possible as it also serves as substrate
 - ensuring that all facilities and equipment are properly disinfected before stocking of PL (e.g. implementing cyclical dry-out and clean-up routines after every production cycle, involving careful cleaning and disinfection of all facilities, including the insides of air lines, pipes, water pumps and air pumps)
 - ensuring that live and treated feeds are free of infection (e.g. by sterilization of frozen material via gamma irradiation or pasteurization)

- modifications to farm and pond designs to allow better biosecurity (e.g. use of smaller-sized ponds with plastic liners that can be fully drained, dried and disinfected between culture cycles)
- using an increased number of reservoirs and water filtration to eliminate fish and other disease carriers
- using water of a salinity of 5 ppt for growing shrimp
- using water drawn from a deep well for growing shrimp
- avoiding heavy chlorination pre-treatment of water
- avoiding traditional fertilization schedules with commonly used products, especially if these strategies have been used previously and were found to not reduce AHPND losses
- avoiding stocking ponds during the high-temperature season
- applying “designer” pre- or probiotic preparations (if available)
- applying “designer” phages that specifically target the VP_{AHPND} (if available)
- Management of culture systems to delay infections where AHPND is present in the culture environment by, e.g.:
 - stocking larger-size PL
 - co-culture of shrimp with finfish (e.g. tilapia) or use of water from tilapia pond
 - use of appropriately designed grow-out systems which mitigate the environmental conditions that support high densities of VP_{AHPND} (i.e. central drainage)
 - stocking at appropriate density according to farm capacity
 - monitoring of shrimp health and removal of infected animals
 - if diseased shrimp are found, conducting laboratory analyses to aid decision making

The international spread of AHPND can be prevented or at least, reduced, by moving only live penaeid shrimp broodstock or PL that have been tested free from AHPND by use of

the AP4 test. Another important measure is the use of fresh feeds that are free from infectious agents. This could involve the use of treatment methods to ensure any bacteria present in the feeds are destroyed (e.g. by heating) or by the development of specific-pathogen-free (SPF) lines of polychaetes and clams for use in shrimp culture.

Actions of International and Regional Organizations

- **Network of Aquaculture Centres in Asia-Pacific:** NACA has listed AHPND as reportable by NACA member countries to its Quarterly Aquatic Animal Disease Reporting System. NACA has also prepared a AHPND Disease Card (updated June 2014) (<http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf>) and routinely provides new information on AHPND on its website (www.enaca.org).
- **Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA, the Regional International Organization for Plant Protection and Animal Health):** OIRSA began actions related to AHPND in 2013 with an official notice through its website, complemented by virtual lectures to all member countries, with the aim of preventing the entry of this emerging disease into the region. A proposed “Regional Epidemiological Surveillance Program for EMS (RESPE)” was then put forward, in order to have a tool that includes suggestions for the development and establishment of AHPND surveillance.
- **World Organization for Animal Health (OIE):** AHPND has been on the OIE Aquatic Animals Health Standards Commission agenda since its emergence in 2010. In December 2013, the OIE developed an OIE Technical Fact Sheet on AHPND that was available on the OIE website. The information provided in this Fact Sheet reflected the epidemiological observations and research information available at that time on aetiology, epidemiology, diagnosis and prevention and control measures. In May 2015, after several years of discussion, OIE Member Countries agreed that AHPND met the OIE criteria for listing an OIE listed disease (as per Chapter 1.2. of the Aquatic Code) and adopted its listing as an OIE listed aquatic animal disease (in Chapter 1.3. of the Aquatic Code). Consequently, as of 1 January 2016 OIE Member Countries must report to the OIE the presence or absence of this disease in their country. The objective of listing a disease is to support Member Countries’ efforts to prevent transboundary spread of important diseases of aquatic animals through transparent and consistent reporting. New chapters on AHPND to be included in the Aquatic Code and Manual are currently under development and have been circulated to Member Countries for comments. Consequently, the factsheet has been removed from the OIE website.
- **Food and Agriculture Organization of the United Nations (FAO):** The FAO initiated work on understanding “early mortality syndrome” via the project TCP/VIE/3304 “Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam.” The project produced a better understanding of the cause of the disease and identified a number of risk management measures and key areas for future research (FAO, 2013). FAO is currently funding an inter-regional TCP project TCP/INT/3502 “Reducing and Managing the Risk of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp” aimed at providing a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. The project’s activities in 2015 include the holding of back-to-back major interregional meetings in Panama City: (i) the International Technical Seminar/Workshop “EMS/AHPND: Government, Scientist and Farmer Responses”, 22–24 June 2015 (Panama EMS/AHPND June 2015) and

(ii) the First Inter-regional Workshop on EMS/AHPND Risk Management and Risk Reduction Strategies at National and Regional Levels, 25–27 June 2015. A second interregional seminar/workshop under this TCP will be held in Asia in 2016 (Bangkok EMS/AHPND June 2016).

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