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The Challenge of Shrimp Diseases in Asia

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Aquaculture is one of the fastest growing food-production sectors but the economic impact that parasites and bacterial, fungal and viral diseases have on the industry is highly significant for the many countries that rely heavily on this industry. Research into the diseases that affect penaeid shrimps that are grown in aquaculture systems is therefore vital, writes Bob Carling for TheFishSite.

Two diseases that are currently being actively researched by the Fish Vet Group Asia Ltd., in Thailand, are:

- the bacterial infection, Acute Hepatopancreatic Necrosis Disease (AHPND) – also called Early Mortality Syndrome (EMS);
- the fungal infection, hepatopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei (EHP).

AHPND

Acute Hepatopancreatic Necrosis Disease (AHPND) is a problem of the main countries that farm shrimps – China, Thailand, Vietnam and Malaysia. AHPND can occur in the first 30 days after stocking shrimp into 'grow-out' ponds, which is why AHPND is commonly, but incorrectly, called early mortality syndrome (EMS).

The disease is caused by a bacterium that colonizes the shrimp gastrointestinal tract and produces a toxin that causes tissue destruction and dysfunction of the hepatopancreas, the shrimp digestive organ. The culprit is the bacterium *Vibrio parahaemolyticus* (*Vp*), a common enough bacterium in brackish saltwater which, when ingested, can cause gastrointestinal illness in humans.

The species of shrimp affected are:

- giant tiger shrimp (*Penaeus monodon*)
- whiteleg (or Pacific white) shrimp (*Litopenaeus vannamei*, formerly *Penaeus vannamei*)
- Chinese white shrimp (*Penaeus chinensis*)

Mortality of infected shrimp stock can exceed 70%.

AHPND originated in China in about 2009 and was officially reported in the People's Republic of China and Vietnam in 2010, in Malaysia in 2011, in Thailand in 2012 and in Mexico in 2013 – with the worst affected country being Thailand. However, despite its close proximity to Malaysia, to date there are no records of AHPND in Indonesia – which has resulted in a significant increase in Indonesia's shrimp export market following its outbreak and spread elsewhere.

The AHPND outbreak has cost Thailand an estimated US\$ 5.01 billion to date. In 2011, prior to AHPND, Thailand produced just over 600,000 tons of *L. vannamei* and was the number one exporter of shrimp in the world. But, following the outbreak of AHPND, in 2014 Thailand reported 189,080 tons of *L. vannamei* production, a drop of 65%.

The transportation of live feeds including polychaete worms fed to broodstock shrimp is thought to have resulted in the spread of AHPND across the Asia region. In Malaysia, Thailand and Vietnam live polychaetes have indeed been confirmed as the infection source of *Vp* during AHPND disease outbreaks. The reason why Indonesia has to date managed to prevent AHPND from entry into the country is probably through its embargo on the importation of live shrimp and because it is not importing live polychaetes from China as feed for

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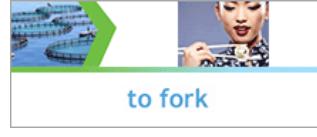
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broodstock shrimp, using domestic live polychaetes instead.

In experiments to search for the way in which the infection is transmitted, it has been transferred by intramuscular injection, by immersion and by feeding infected shrimp to healthy shrimp. It is likely therefore that transmission occurs when healthy shrimp eat AHPND-infected shrimp and by cohabitation.

Prevention, control and mitigation

Measures include:

- improved biosecurity at the farm, zone, national, and regional levels,
- zonal management of production units,
- disease risk assessments, and
- development and implementation of aquatic veterinary health plans.

Unless they are certified by independent third parties as being free from AHPND, the use of live feeds for shrimp broodstock should be discouraged – or, where live feeds are used, these should be sterilised or frozen to reduce the likely transfer of AHPND.

Stocking density can have an effect, as does the use of probiotics. The use of Biofloc technology – enhancing water quality by balancing carbon and nitrogen in the system – has also been reported as helping in reducing the impact of AHPND (see '[Workshop on Biofloc Technology and Shrimp Diseases](#)').

AHPND meets the definition of an emerging disease in the Aquatic Animal Health Code of the World Organisation for Animal Health (OIE). AHPND is listed in the Quarterly Aquatic Animal Disease (QAAD) Reporting Program for Asia and the Pacific, but there is on-going discussion on whether AHPND should be OIE listed, although it is likely to become a notifiable disease pathogen.

EHP

In comparison to AHPND, very little is known about the effects on aquaculture systems by the fungal infection *hepatopancreatic microsporidiosis* (HPM) caused by *Enterocytozoon hepatopenaei* (EHP).

Hepatopancreatic microsporidiosis was first detected in *P. monodon* in Thailand in 2004 and then later in Vietnam. With it now being reported in China and Malaysia, the infection is also reported as being an increasing problem in India, exacerbated by flooding (see '[Flood, disease to hit aquaculture sector in 2015–16](#)').

It is now probably endemic in Australasia, affecting both *L. vannamei* and *P. monodon* and possibly also *P. japonicus* in Australia (see '[Hepatopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei \(EHP\)](#)').

A warning from NACA to "Asian farmers and hatchery operators after 2009 to monitor *P. vannamei* and *P. monodon* for EHP in broodstock and post larvae (PL) ... were not heeded because of the overwhelming focus on early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND)"(see '[Urgent appeal to control spread of the shrimp microsporidian parasite Enterocytozoon hepatopenaei \(EHP\)](#)').

The authors "feared that lack of interest in EHP would lead to its build up in production systems and that its spread would be masked by EMS/AHPND because it kills shrimp before the negative effects of EHP on growth are apparent".

The Asia Regional Advisory Group on Aquatic Animal Health (ARAGAH) met in Bangkok, Thailand 23–25 November 2015 (see <http://bit.ly/1LZTWD2>) and recommended that *E. hepatopenaei* should be known as the pathogen causing HPM and that NACA member countries should report to NACA on EHP/HPM outbreaks, severity and spread (see '[Hepatopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei \(EHP\)](#)').

What is known so far is that EHP infects only the tubule epithelial cells of the hepatopancreas of the shrimp. The spores are very small ($1.1 \times 0.60\text{--}0.07\mu\text{m}$) and have a polar filament with 4–5 coils. FVG Asia Ltd is working on modified staining techniques which make it much easier to see the spores.

Although previously EHP was only reported as being found in the shrimp hepatopancreas tubule epithelial cells, Fish Vet Group Asia Ltd staff have also found EHP spores in the shrimp intestine as well as the hepatopancreas. These findings suggest that the term 'hepatopancreatic microsporidiosis' is perhaps incorrectly applied.

In 2010, EHP was reported as being associated with 'white faeces syndrome' (WFS) in Vietnam, but further experiments have however failed to show the



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association with WFS, although transmission has been demonstrated. EHP spores are extremely hardy and EHP can be transmitted horizontally between shrimp, particularly in earthen rearing ponds (see <http://bit.ly/1LZTWD2>). As a result EHP infection can spread progressively and is believed to intensify with successive shrimp crops over time.

Prevention, control and mitigation

Detection and screening is done by using nested polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) tests (see [Suebsing, R., Prombun, P., Srisala, J. and Kiatpathomchai, W. \(2013\) Loop-mediated isothermal amplification combined with colorimetric nanogold for detection of the microsporidian Enterocytozoon hepatopenaei in penaeid shrimp. Journal of Applied Microbiology 114\(5\): 1254–1263](#)

Histological analysis for the spores is possible, but is difficult unless performed by expert histopathologists. Live feeds should be avoided and/or freezing/sterilization should be carried out.

Research is currently being prioritised on:

- a better understanding the lifecycle of EHP
- the EHP transmission pathway
- development of real-time PCR testing
- easier histopathological identification of spores
- development and registration of surface disinfectants to control EHP
- development and registration of drug treatment for the treatment of live shrimp infected with EHP
- development of cell lines to culture microsporidia

As of January this year, there is currently no drug to control this infection (see '[Enterocytozoon hepatopenaei \(EHP\) hampers the growth of farmed shrimp](#)').

Shrimp farmers have tried a variety of measures to try and eradicate EHP from hatcheries, nurseries and grow-out farms but with little success. There has been some experimentation with alternative treatments, including coccidiostats used to treat poultry like Monensin, but no research has yet been conducted to ensure the product is safe to use in shrimp production systems.

EHP is very likely to have spread to Mexico in shipments of PL's or broodstock from Asia – legal or otherwise. In a review of Penaeid shrimp introductions, Briggs et al. (2005) ([Briggs, M., Funge-Smith, S., Subasinghe, R. & Phillips, M. \(2005\). Introductions and movement of Penaeus vannamei and Penaeus stylostris in Asia and the Pacific. Food and Agriculture, RAP Fisheries Technical Paper 476, 79pp](#)) have demonstrated that there has been frequent movement of shrimp disease pathogens between Asia and South America as a result of both illegal smuggled movement of shrimp post-larvae and broodstock and legal movement of shrimp.

The NACA appeal (see <http://bit.ly/1LZTWD2>) concluded with a warning about the outbreak of EHP in Mexico. If the outbreak of AHPND in Mexico originated from contaminated *P. vannamei* illegally imported to Mexico from Asia, "given the high prevalence of EHP in Asia, it is quite probable that the imported shrimp would also have been infected with EHP". They called on the "Mexican quarantine authorities [to] check their current and archived DNA samples used to monitor for AHPND bacteria by PCR to also check for the presence of EHP target DNA by PCR. If they find it, it would support the hypothesis that AHPND bacteria were imported from Asia".

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