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Research progress on Acute Hepatopancreatic Necrosis Disease (AHPND) in Viet Nam

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Abstract

The work by conducted in Vietnam the ShrimpVet Laboratory on early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) of penaeid shrimp is summarized, and includes evaluation of diagnostic methods (histology, bacteriology, polymerase chain reaction), challenge studies to evaluate the efficacy of various products in controlling AHPND in shrimp (e.g. probiotics, acidifiers, immunostimulants, bacteriophages, quorum quenching, feed additives, toxin absorbents, essential oils, herbal extracts), and approaches to field practices (e.g. better selection of PL and PCR tests for PL, nursery phase, polyculture, using "mature" water from fish ponds for stocking and water exchange, sludge removal, water discharge with central drainage, using probiotics to remove excessive organic matter, more water exchange and more reservoir area, avoiding eutrophication and excessive algal bloom, better natural food bloom before stocking, using gut probiotics, including organic acids in feed, and using herbs such as garlic and turmeric). To reduce the risk of AHPND in shrimp farming, a very holistic approach is needed that includes: biosecurity, PL quality, good farming practices, a more diversified microflora in both the shrimp gut and shrimp pond, sustainable farming practices and better environmental management. In short, shrimp farming should be considered as a value chain in which every part of the chain is equally important.

Keywords: AHPND, challenge studies, disease diagnosis, EMS, field practices, Viet Nam

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Introduction

Since the emergence of early mortality syndrome (EMS) in Viet Nam in 2010, these disease outbreaks were further identified as due to a toxin-mediated disease produced by strains of *Vibrio parahaemolyticus* (Tran et al. 2013a, b). The disease was later named acute hepatopancreatic necrosis disease (AHPND), and research and measures for control became more focused on dealing with the bacterial etiology.

Laboratory Research

The ShrimpVet Laboratory, founded in 2014 by Loc Tran and his team in Vietnam, has been involved in research on shrimp diseases, with a strong focus on EMS/AHPND, both in terms of academic studies and field application.

Diagnostics

So far at the ShrimpVet Laboratory, three main methods of disease diagnosis have been applied to screen for and confirm the presence of EMS/AHPND in affected shrimp or suspected materials.

- *Histology:* Based on the cased definitions proposed by Lightner et al. (2012), histology is a principle method used to confirm the presence of pathology in field specimens, as well as infections in infected shrimp in challenge studies.
- *Bacteriology:* So far, the ShrimpVet Laboratory has a collected over 40 EMS/ AHPND-causing bacterial strains (confirmed by both polymerase chain reaction (PCR) and bioassay (Koch's postulate)). Using the API 20 NE strips bio-chemical test, 36 of the 40 strains were identified as *V. parahaemolyticus*, while 4 were not *V. parahaemolyticus* . Among the EMS/AHPND-causing *V. parahaemolyticus*, there was no special biochemical characterization confirmed. Therefore, bacteriology alone is not recommended for confirmation of AHPND.
- *Polymerase Chain Reaction (PCR):* the ShrimpVet Laboratory has applied both AP3 primers released by Dr Tim Flegel (1-step PCR) in detecting the toxin gene of AHPND since 2014. The results so far have shown that 1-step PCR alone is not sensitive enough to detect AHPND, either in fixed samples of postlarvae (PL) or in pond water. The ShrimpVet Laboratory has conducted a series of challenge studies showing that, shrimp start to die when the bacterial load (AHPND *V. parahaemolyticus*) exceeds 10^4 CFU.g^{-1} shrimp. This also means that if the shrimp sample is extracted for DNA (only 2 mg of sample to be extracted), and it is presumed that the sample has 10^4 CFU.g^{-1}, then only be10^2 bacterial DNA genomes will be extracted. That means that this number of copies extracted might not be enough to be detected. Even with samples of moribund shrimp from challenge studies with AHPND, the PCR analysis often gave negative results.

In our clients' PCR samples of alcohol-fixed shrimp that are submitted to the laboratory, we seldom see PCR-positive results, even with parallel samples confirmed with AHPND using histology. However, if the fresh samples are used to culture bacteria in broth media for 4 hr, the PCR results for AHPND can be positive.

With parallel samples tested with AP4 (nested PCR) or real-time PCR, more positive results were obtained. This indicates that with fresh shrimp samples, it is best to have a 4-hr bacterial culture before running the PCR. With fixed samples of shrimp, only nested PCR or real-time PCR is sensitive enough to detect AHPND.

Challenge Studies

The ShrimpVet Laboratory has done several AHPND challenge studies to evaluate the efficacy a variety of different products in controlling AHPND in shrimp. Probiotics, acidifiers, immunostimulants, bacteriophages, quorum quenching, feed additives, toxin absorbents, essential oils, herbal extracts etc. have been tested.

Our studies indicate that products that have a direct effect on the bacterial population in the shrimp gut seem to work best. Several "designed" probiotics that have been selected from strains that can inhibit the growth of vibrios have been checked for their effect against AHPND. Many challenge studies have shown that several gut probiotics can improve the survival rate above that of the positive control (i.e. shrimp challenged with AHPND-causing *Vibrio parahaemolyticus*), survival rates being increased from 20 to 30 % to 60 to 70 %. Some acidifiers mixed with proper dosages in feed also conferred quite good tolerance to AHPND in the challenge studies. Many other challenge studies with other substances also provided some promising results.

Field Practice

Since the disease has been identified as directly related to the pathogenic bacterium, farmers are paying much more attention to keeping bacteria under control. Several approaches have been applied in the field including: better selection of PL and PCR tests for PL, nursery phase, polyculture, using "mature" water from fish ponds for stocking and water exchange, sludge removal, water discharge with central drainage, using probiotics to remove excessive organic matter, more water exchange and more reservoir area, avoiding eutrophication and excessive algal bloom, better natural food bloom before stocking, using gut probiotics, including organic acids in feed, and using herbs such as garlic and turmeric. Checking for AHPND quality using PCR has become more common in both hatchery and farm practices. Samples analyzed from hatcheries include broodstock faecal matter; live feeds such as bloodworm, squid and oyster; PL and nauplii. So far, we have had a significant number of samples of bloodworm and broodstock faeces testing positive. This indicates that bloodworm can be an important source of AHPND contamination and that finding a replacement for this particular live feed that is caught locally near the faming areas is an urgent need.

Applying a nursery phase in order to improve the health of juvenile shrimp, increase their size before stocking, and shorten the grow-out phase has become a common practice. The nursery phase usually happens in a small, controlled environment such as concrete tanks, fiberglass tanks or plastic-lined ponds. The culture period for this phase usually varies from two to four weeks. Then the shrimp will be transferred to the grow-out ponds. The clear-water system (with a lot of water exchange), recirculation system, or biofloc system is then applied to control the water quality during culture.

Polyculture is also a common practice that has been proven to be quite effective in reducing AHPND (Tran et al. 2014). In Viet Nam, polyculture with tilapia is very common, and includes the mixing of tilapia with shrimp, separating fish in hapas, using water from and exchanging water with tilapia ponds, and tilapia-shrimp crop rotations. The effects of tilapia can include: cleaning up of the pond bottom; consumption of dead shrimp, stopping disease transmission via cannibalism; encouraging beneficial bacteria and algae in the pond; and treating the waste released. By having a more diverse natural microflora in the shrimp pond, the harmful pathogenic bacteria seem to have less likelihood to bloom and cause shrimp mortalities. Measures to control conditions that favour fast-proliferating bacteria like *Vibrio* spp. are the main approach. Removing the pond sediments and sludge and having a more precise feeding programme are also important parts of controlling the excessive nutrients that potentially favour vibrios.

In general, in order to reduce the risk of AHPND in shrimp farming, we must have a very holistic approach that including: biosecurity, PL quality, good farming practices, a more diversified microflora in both the shrimp gut and shrimp pond, sustainable farming practices and better environmental management. In short, shrimp farming should be considered as a value chain in which every part of the chain is equally important.

References

Lightner, D.V., R.M. Redman, C.R. Pantoja, B.L. Noble and L.H. Tran. 2012. Early mortality syndrome affects shrimp in Asia. Global Aquaculture Advocate Jan/Feb 2012: 40.

Tran, L.H, K.M. Fitzsimmons and D.V. Lightner. 2014. Tilapia could enhance water conditions, help control EMS in shrimp ponds. Global Aquaculture Advocate Jan/Feb 2014:26–28.

Tran, L., L. Nunan, R.M. Redman, L.L. Mohney, C.R. Pantoja, F. Fitzsimmons and D.V. Lightner. 2013a. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105:45−55

Tran, L., L. Nunan, R.M. Redman, K. Fitzsimmons and D.V. Lightner. 2013b. EMS/AHPNS: infectious disease caused by bacteria. Global Aquaculture Advocate July/Aug 2013:18 –19.