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NO CONFIRMED CASE OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE/ EARLY MORTALITY SYNDROME (AHPND/EMS) IN INDIA

Since 2009, an emerging threat, popularly known as early mortality syndrome (EMS) and scientifically termed as Acute Hepatopancreatic Necrosis Disease (AHPND) has severely affected shrimp farming in many countries in the Southeast Asian region. Though the disease is reported to affect mainly Pacific white shrimps (*Penaeus vannamei*), tiger shrimp (*Penaeus monodon*) and Chinese shrimp (*Penaeus chinenesis*) have also been reported to be susceptible. The disease is mainly characterized by mass mortalities (reaching up to 100% in many cases) during the first 20-35 days of culture (post-stocking in grow-out ponds). This new disease was first reported from China in 2009, followed by Vietnam in 2010, Malaysia in 2011, Thailand in 2012, and Mexico in 2013. Very recently during 2015, this disease has also been reported to be present in the Philippines. The disease has caused severe economic losses throughout the region. Considering the severity of the disease, several meetings coordinated by the Network of Aquaculture Centres in Asia-Pacific (NACA), Food and Agricultural organization (FAO) and others, have been organised involving international shrimp health experts, regional governments and industry to share information on this emerging disease, its occurrence, pathology and diagnosis, and to develop a coordinated regional response to the issue. AHPND has already been considered as a reportable disease in the Network of Aquaculture Centres in Asia Pacific (NACA) Quarterly Aquatic Animal Disease (QAAD) reporting system. AHPND has been also recently listed by the OIE and is reportable since 2016. Central Institute of Brackishwater Aquaculture (CIBA) has been involved in creating awareness on this emerging disease among stakeholders/farmers/ researchers in India by conducting/participating and making presentations. CIBA has also carried out investigations on suspected cases of mass mortalities of shrimp during the past and continuously monitoring the culture practices since then. Findings of CIBA are included in NACA QAAD report through a National Disease Surveillance Programme.

What is AHPND/EMS?

The early mortality syndrome in shrimp has been named based on unusually high mortality within the first 30 days of shrimp grow-out culture, due to a variety of pond management and pathogen related factors. Some of the farm level clinical signs include;

- Onset of clinical signs and mortality starting as early as 10 days post stocking.
- Moribund shrimp sink to bottom.
- Often soft shells and partially full to empty guts.
- Hepatopancreas (HP) often appears pale to whitish due to loss of pigment.
- Significantly emaciated HP (shrunken, small, swollen or discoloured).
- HP does not squash easily between thumb and finger.
- Sometimes black spots or streaks within the HP may be visible.

HP is the main target in AHPND and therefore the pond-level observations have to be further confirmed as AHPND by laboratory investigations involving characteristic pathology of HP such as necrosis of B, F, R cells and sloughing of cells into the lumen. Though histo-pathological investigations are the gold standard for the confirmation and presence of AHPND, recently detection of the causative agent of AHPND by molecular methods such as PCR has been developed.

Causative agent of AHPND/EMS is a strain of *Vibrio parahaemolyticus*

Initially, the causative pathogen/factor for EMS remained un-identified despite intensive efforts by many leading scientists. Therefore, it was suspected to be either due to biotic or abiotic agents. Experiments failed to demonstrate that the disease was caused either by a virus or a toxin. Towards the end of 2012, Dr. D. V. Lightner's group from University of Arizona (Tran *et al*., 2013, *Dis. Aquat. Org*., 105(1): 45-55), conducted several experiments in Vietnam, and demonstrated that the disease was infectious using fresh tissue samples from the infected animals. Their repeated experiments proved that the disease and the typical pathology was caused by a bacterial pathogen belonging to Vibrio group and identified the species to a special strain of *Vibrio parahaemolyticus* (VP_{AHPND}). A plasmid (pAP1) of about 69 kb present in this specific strain contains two genes that produce toxins (Pir A/B) and cause massive damage to the hepatopancreatic cells.

What CIBA has been doing?

During Oct 2012 to Jan 2013, mass mortality that occurred in nineteen vannamei farms and two monodon farms (in Kalpakkam, Kattur and Nagapattinam area of Tamil Nadu, Nellore, Gudur and Bhimavaram areas in Andhra Pradesh) were investigated. These mortalities of shrimp occurred during 20-72 days post stocking and the shrimps were harvested by 47-72 days. Affected shrimp had partially filled gut and the hepatopancreas was normal in most cases, although few had some signs of melanisation. In majority of such cases, the mortality was due to WSSV infection.

Subsequently, targeted surveillance for AHPND/EMS was carried out in 35 more farms of Tamil Nadu and Andhra Pradesh. Again, a majority (66%) of these samples were infected with WSSV and a fraction (22%) by mixed infection with both WSSV and IHHNV. Though, HP from some of these samples showed pathological changes, they were not characteristic of AHPND/EMS. This programme is now being routinely carried out in different farms of Tamil Nadu and Andhra Pradesh and all other maritime states.

A National Surveillance Programme on Aquatic Animal Diseases (NSPAAD) funded by National Fisheries Development Board (NFDB) and co-ordinated by National Bureau of Fish Genetic Resources (NBFGR) has been initiated since 2013. CIBA along with a few other fishery institutes are continuously undertaking targeted surveillance for the detection of AHPND/EMS. So far samples of over 130 farms in various coastal districts especially Andhra Pradesh and Tamil Nadu, with history of early mortalities have been investigated using up-to-date protocols of molecular diagnostics (AP4 of Dangtip *et al*., 2015), histopathology, isolation of *V. parahaemolyticus* and *in vivo* challenge studies.

All these samples were tested by PCR, initially with primers (AP1 and AP2) subsequently by AP3 and most recently by AP4 developed by Prof. T. W. Flegel. So far, all samples were found to be negative. A total of 106 *V. parahaemolyticus* isolates were tested till recently, and none of these isolates tested positive by PCR. 36 of these isolates were also tested by POCKIT system (Gene Reach Tech, Taiwan) and none of these tested positive.

Measures learnt from other countries for prevention and control of AHPND/EMS

1. ***No larvae/broodstock from affected regions***: Broodstocks and larvae may carry AHPND/EMS bacterium. Therefore, it will be wise not to import live animals from such infected area either for hatchery or for culture practice.

2. ***Following strict biosecurity measures****:* Now it is known that AHPND/EMS is caused by an infectious agent, particularly by a specific strain of a bacterium that is commonly found in the shrimp culture environment. Therefore it is advocated for the adoption of strict biosecurity measures to prevent this pathogen entering the culture system. Some of these common practices include bird fencing, avoidance of use of common water body, use of reservoir ponds, etc.

3. ***Adoption of closed re-circulatory systems or zero water exchange practice***: Reports from some farmers of affected countries indicate that shrimps grown in closed re-circulatory system have reduced instances of AHPND/EMS infection. The closed system probably helps to avoid contamination with the surrounding water body.

4. ***Including nursery phase to stock larger size shrimp****:*A majority of the reports show that shrimp are more susceptible to AHPND/EMS during the early days after stocking. Some of the experimental trial proved that larger size shrimps are more resistant to this disease. Therefore, it will be wise to grow shrimps initially to larger sizes in nursery ponds and then transfer to culture ponds.

5. ***Culturing Tiger shrimp as an alternative species*** *:* Some of the very recent reports indicate tiger shrimp to be comparatively resistant to AHPND. Switching over to tiger shrimp culture may therefore help in preventing this disease.

6. ***Co-culture of tilapia and shrimp or culture with tilapia induced green water***: Ponds where tilapia was grown initially and then shrimp or both tilapia and shrimp were grown together in same pond, AHPND problem was found to reduce considerably. Even the green water generated from tilapia pond and used for shrimp culture was also found to be useful in controlling AHPND/EMS.

7. ***Culture of Shrimp in low saline areas:*** The causative organism of AHPND/EMS prefers higher salt concentration for the growth. Accordingly, some of the observations indicate that AHPND/EMS is less prevalent when the water salinity is

<5 ppt. Therefore, switching over culture practices to low salinity areas may help avoid EMS/AHPNS.

8. ***Using biofloc technology in shrimp culture***: Shrimp culture ponds where biofloc was induced, fewer incidences of AHPND/EMS have been reported. The same has also been proved in laboratory condition where EMS/AHPND bacteria could be eliminated in biofloc system. Therefore, biofloc system appears to be useful in preventing AHPND/EMS outbreak.

9. ***Effective feed management***: Many of the farmers indicate that reducing the feed quantity during the infection stage and slowly increasing subsequently helps prevent AHPND/EMS. This is particularly important during the initial stage of infection. The unused feed is speculated to be a good source for the growth of these bacteria.

10. ***PCR testing of larvae before stocking***: AP-4 protocol and POCKIT system (Gene Reach Tech, Taiwan) are available for PCR diagnosis of AHPND. Shrimp larvae should be tested for AHPND/EMS before transporting from hatcheries. When found positive, the larvae should be discarded/destroyed appropriately and the hatchery should also be disinfected.

11. ***Targeted surveillance for AHPND/EMS***: The culture ponds should be monitored regularly, particularly during the early periods. Sufficient numbers of specimens should be tested to declare a pond as negative. If found positive, the pond water should be disinfected within the pond after recovering the stock. Similarly, the treated water should only be discharged after proper deactivation of the disinfectant.

12. ***Development of AHPND/EMS resistant shrimps by selective breeding***: It appears that some of the trials carried out in Thailand have provided indication on the possibility of developing EMS/AHPND resistant shrimp strains/lines. These strains were shown to provide as high as 60% improvement in survival upon challenge. Another possibility of producing fast growing PL by selective breeding may help to avoid the disease as the shrimps can grow to larger size within a smaller period of time.

How farmers could help?

Considering the great extent of economic loss that AHPND/EMS is likely to cause, shrimp farmers in India should cooperate by consulting with the agencies such as fisheries research institutes and government officials by promptly reporting any mass mortality and sending samples to enable investigation of the problem and to ascertain the actual cause. Farmer groups may approach CIBA for the preservatives for sending samples of shrimp from such mortalities and for the proper procedures to be adopted. They are advised to send such samples to CIBA for histopathological investigations and virus screening of known viruses. Dead and frozen samples cannot be processed for these investigations and morbid shrimp samples suitably preserved need to be used.

At this stage what is required is to identify the cause of mass mortalities of shrimp that farmers are facing. To prove that the shrimps are actually having EMS/AHPND, a systematic investigation involving histopathology, bacterial isolation, infection of healthy shrimps with the isolate to reproduce the disease and PCR test should be carried out. Incomplete diagnosis will unnecessarily create confusion regarding the status of the disease. In case mass mortality is observed within 35 days of stocking, farmers are advised to contact:

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Research on brackishwater aquatic animal health and environment was initiated at the Central Institute of Brackishwater Aquaculture since 1990. Since then it has grown in terms of expertise, manpower and infrastructure. The Aquatic Animal Health and Environment Division or the AAHED in short, has scientists with all relevant specialities and expertise in Microbiology, Virology, Pathology, Parasitology, Biotechnology, Molecular Diagnostics, Soil and water Chemistry, Environment and Aquaculture. The AAHED has well established laboratory facilities for carrying out cutting edge research in molecular biology in addition to aquatic animal health and environment management including diagnostics, prophylactics and health management in brackishwater aquaculture. The advanced facilities have been developed with funding support from ICAR, National Agricultural Research Project (NARP), World Bank, National Agricultural Technology Project (NATP), Department of Biotechnology and National Fisheries Development Board with dedicated efforts of scientists. A well designed wet lab is also in place for carrying out live aquatic animal experiments and evaluating Koch's and River's postulates.

The AAHED, CIBA has the mandate to carry out research on (a) economically impacting diseases of brackishwater culture species and develop technologies for rapid diagnosis, prophylaxis and control; (b) brackishwater environment and develop mitigatory measures as required; and (c) provide technical and policy support to the Government on matters pertaining to aquatic animal health and environment management to improve productivity.

The AAHED of CIBA was the first to commercialise a white spot syndrome diagnostic kit to a premier Biotechnology company in the year 2002. The AAHED also produced kit for diagnosis of white tail disease in scampi in the year 2004. AAHED has the expertise and capacity to carry out all the proposed levels of Diagnostics of OIE listed Brackishwater pathogens and has been serving as a National Referral Laboratory.

The environment section of AAHED has the expertise to look into all aspects of abiotic parameters. Novel methods have been developed for the bioremediation and environmental monitoring of the brackishwater rearing systems, including hatcheries and farms. The unit also has expertise in climate related matters and has developed climate smart solutions for brackishwater farming systems. The section has also capacity for the environmental impact assessment and carrying capacity assessment of source waters for optimisation of brackishwater aquaculture development.

AAHED, CIBA has published over 60 research publications in peer reviewed national and international journals, produced 15 Ph. Ds, who are currently employed in key positions in various Institutions in India and abroad.

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