

Shrimp diseases & mitigation strategies in India

At Aqua India 2024, the session on shrimp diseases and mitigation strategies reviewed some research updates, disease mitigation and diagnostic tools. Accepting the disease situation in India, the panel, led by **Dr D. Ramraj**, Director at Padmanabha Labs, discussed how recent research developments could be applied to the industry. Aqua India 2024, the biennial event of the Society of Aquaculture Professionals (SAP), was held in Chennai from February 15-17.

Earlier this year, SAP conducted its annual Shrimp Crop Review 2023 where in a presentation, Kumaresan A, Director at Sheng Long Biotech India, showed data on the prevalence of diseases in coastal and inland shrimp farms. Inland farms are those in Rajasthan, Punjab, Haryana. Together these states contributed to 10,200 tonnes of vannamei shrimp in 2023. Kumaresan noted that in inland farms, outbreaks of white spot syndrome virus (WSSV) was not reported. Neither were there reports on *Enterocytozoon hepatopenaei* (EHP) or retarded growth. White faeces syndrome (WFS) as well as running mortality syndrome (RMS) was a problem.

Research advances in mitigating major shrimp diseases

To better understand the evolution of viruses in shrimp, APL uses WSSV as a proof-of-concept study. **Dr Hung N. Mai**, Research Scientist at the Aquaculture Pathology Laboratory (APL) University of Arizona, USA, explained the work with the genome reconstruction of WSSV from archived histological tissue. APL has a collection of >110,000 histology tissue blocks dating back to the mid-1970s. The aim is to develop a technology platform to create oral therapeutics to control shrimp diseases. The research on acute hepatopancreatic necrosis disease (AHPND) with *In silico* analysis of PirA/PirB structure and receptors aims to understand molecular pathogenesis in AHPND to develop disease-resistant lines such as an AHPND tolerant *Penaeus vannamei* line.

Hung concluded that identifying the etiology of diseases involving more than one pathogen is difficult, but critical to developing management strategies to mitigate those diseases.

WSSV transmission and mitigation

The question frequently asked is whether evolved strains of WSSV are more virulent and cause increased mortalities. Contrarily, Hung said Chinese researchers showed that an earlier strain, CN01, showed more virulence than the latter strain CN03, which had low virulence. "In nature, when the virus evolves, it needs to be with the host and stay alive. Therefore, it is not to their advantage to kill the host."

Updated research information on WSSV transmission pathways is critical in managing farm operations. **Professor Uma Arumugam**, Head, Department of Health Management, Tamil Nadu Dr J. Jayalalithaa (TNJ) Fisheries University, updated that with new diagnostic essays, researchers continue to identify targets; however, the exact transmission pathway is unclear, partly because there are too many ways this virus can be transmitted.

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*in Vibrio-challenged trial conditions, Orapint 2022

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D Ramraj, left, led the panel discussion with speakers: From second left, Dr Hung N. Mai, Aquaculture Pathology Laboratory (APL), University of Arizona, USA; Dr Lukas Neumann, Skretting. Panellists were Professor Uma Arumugam, Department of Health Management, Tamil Nadu Dr J. Jayalalithaa (TNJ) Fisheries University, second right, and Dr D Ramesh Kumar, Salem Microbes, right. Saurabh Sekhar, Nutreco South Asia, centre right, gave out the tokens of appreciation to the panel.

Disease surveys have pointed out that transmission of WSSV through specific pathogen-free (SPF) post larvae seems unlikely. The horizontal transmission of diseases on farms is of greater magnitude. There is more attention to biosecurity to manage diseases, but it is easy to implement biosecurity on large farms. When farms are small and share resources, a farm that applies good biosecurity is at the mercy of poor biosecurity on neighbouring farms. Hung suggested biosecurity at a community level. There are often higher risks of WSSV outbreaks during the cooler seasons. The panel discussed mitigation steps, such as increasing the health of shrimp with probiotics and functional additives.

In-feed WSSV RNAi solutions

Dr Lukas Neumann, Researcher on Aquatic Animal Health, Skretting, updated on an in-feed WSSV RNAi solution developed by ViAqua. The 2-week trial conducted in Belgium had shrimp injected or fed the double-strand RNAi solution and then challenged with the virus.

The results showed that injecting dsRNAi improved survival and that commercially produced dsRNA gave the same results as the that produced by ViAqua. With the infeed trial, there was a successful proof of concept. Although there was no noticeable dose response, the oral delivery of dsRNAi gave an RPS of up to 64%. Next will be field trials.

Vibrio infections and their mitigation

Dr D Ramesh Kumar, CEO, Salem Microbes, showed data on the microbial load in the hatchery environment, which indicated 2.2×10^6 CFU/mL in Artemia-holding tanks compared to raw seawater containing 1.70×10^4 CFU/mL and outdoor algae tanks with 1.0×10^3 CFU/mL. However, he cautioned about vibrio buildup in algae culture as hatcheries use many types of algae.

"In recent incidents of vibrio related mortality, we are observing these Vibrio species; *Vibrio alginolyticus*, *Vibrio vulnificus*, in addition to *Vibrio parahaemolyticus*. The severity of infection is equally intense in young shrimp at below DOC 30 as well as in shrimp, towards harvest size of 20-30g. This reiterates the caution for farmers to maintain biosecurity as well as maintain low CFU of vibrios in the

pond water through using probiotics or bacteriophages active against Vibrio species," said Ramesh.

Clinical signs of EHP and its mitigation

There is no specific clinical sign. Target organs include the hepatopancreas and midgut, affecting digestion and absorption functions, said Uma. On the screening of post larvae, the panel concurred on the difficulties with accurate PCR detection of EHP. The detection limit is very high, with false negatives at the early stages of detection are not uncommon.

Uma noted that for the past 10-15 years, there has been a reliance on PCR testing, based on an assay which is not specific to EHP. Diagnostic sensitivity has increased with the target on polar tube protein. A team in China and Korea developed a sensitive and specific nested PCR method targeting the EHP β -tubulin gene. Diagnostic sensitivity has increased with the target on polar tube protein.

Intervention strategies for EHP

Skretting has now identified an ideal product with functions to inhibit EHP spore infectivity and proliferation after *in vitro* screening tests of 30+ ingredients. Additionally, the product solidifies digestive functionalities.

Uma discussed the work on using albendazole, a broad-spectrum anthelmintic and antiprotozoal agent against EHP in *vannamei* shrimp. The team at TNJU showed that albendazole is an inhibitor of the β -tubulin polymerization in microsporidians. Efficacy was tested in reducing EHP infection in the shrimp at various dosages of albendazole. They found that a 98% reduction in EHP DNA copies was observed in the shrimp treated with 75 mg/L per day for 24 days.

However, work is required on the interventions in broodstock. Although albendazole is a potential drug to control EHP infection in shrimp, which might help to combat the production losses caused by EHP, Uma commented that they need to assess the safety issue for shrimp and the environment and, before any commercial use, get the approval of regulatory authorities.