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Education Series

Early Mortality Syndrome (EMS), Acute Hepatopancreatic Necrosis Disease (AHPND), and *Penaeus monodon* Mortality Syndrome (*Pm*MS)

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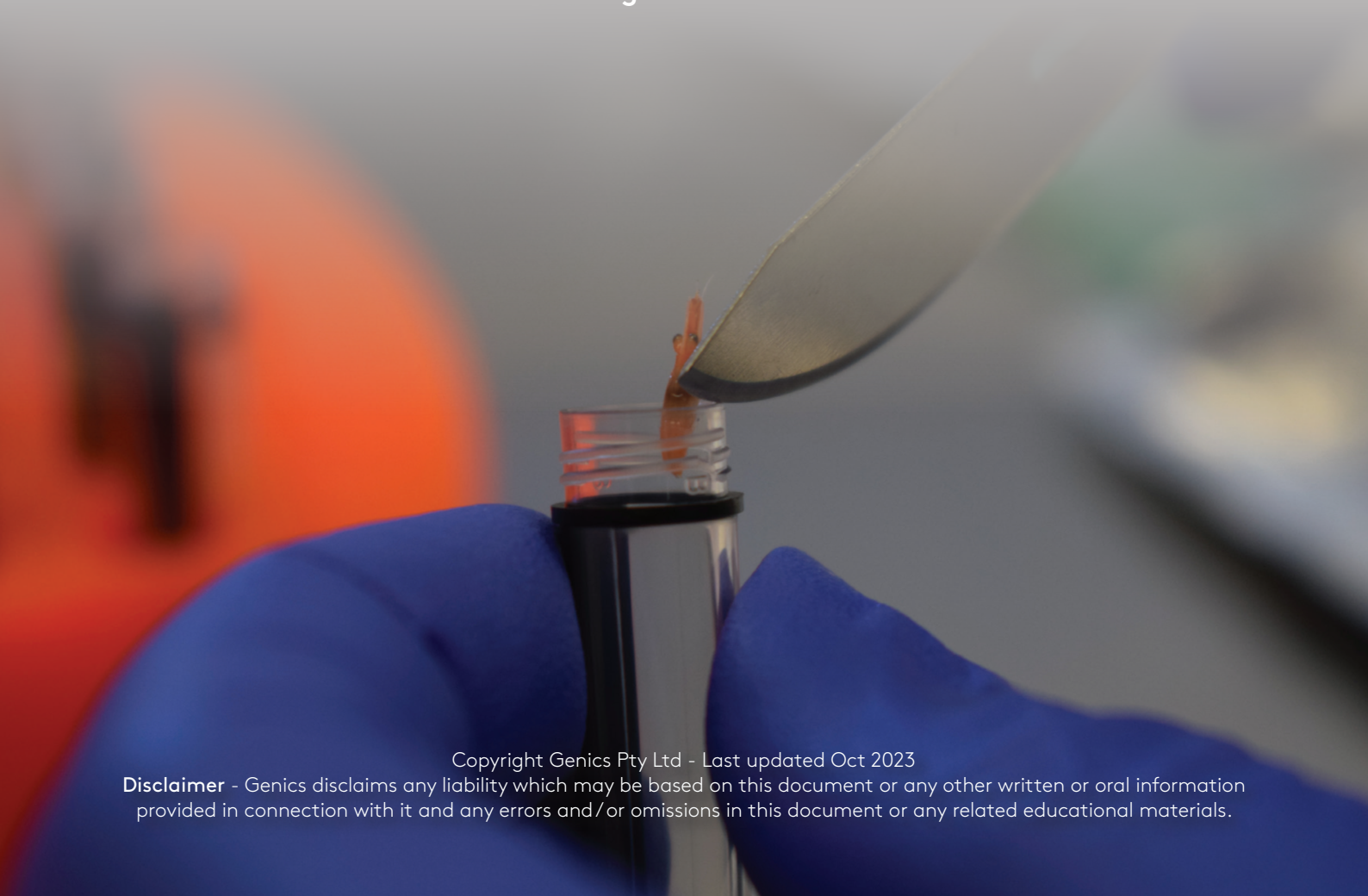

Shrimp get sick too. Early Mortality Syndrome (EMS), Acute Hepatopancreatic Necrosis Disease (AHPND) and *Penaeus monodon* Mortality Syndrome (*Pm*MS) are the result of the presence of toxin producing *Vibrio* species. These toxins, Pir A and B, cause sloughing of the cellular lining of the shrimp stomach and digestive tract as well as damage to the hepatopancreas (HP) tubules. When expressed in *Vibrio parahaemolyticus,* these toxins are notifiable to the OIE as by the case definition they cause EMS or AHPND. *Pm*MS has been found to be due to the toxins expressed in *Vibrio harveyi*.

These infectious diseases are found in farmed *Penaeus vannamei* and *P. monodon*. They are highly contagious and may cause high mortality rates in semi-intensive or intensive shrimp farming systems. Mortality can be observed as early as 10 days post-stocking and 100% mortality can be reached on days 30-35 after stocking. These infectious diseases become problematic when the salinity, temperature and suspended/bottom organic matter concentrations are conducive for toxin carrying pathogenic bacterial strains to proliferate. They mainly colonize organic matter particles.

It has been proposed that as these organic matter particles are fully colonized by pathogenic bacteria, toxin expression occurs. High toxin concentrations are found in organic matter particles. Shrimp ingest these particles, absorbing the toxins which can lead to a rapidly occurring acute disease outbreak. It has also been proposed that the bacteria colonize the shrimp stomach where they form a biofilm with subsequent toxin production.

The overall lethal impact on farmed shrimp has been higher in Asian countries (>80% mortality) than in Latin America (20-40% mortality) and Australia. The occurrence and severity of the outbreaks has been associated with environmental conditions and the presence of conditions that allow for toxin production. There does not appear to be any role of localized genetic changes in the etiologic agents.

Causative agents of EMS or AHPND and *Pm*MS. It was originally reported that EMS/AHPND disease was caused by a specific highly virulent strain of *Vibrio parahaemolyticus* (*Vp*AHPND) that contains a ~70-kbp plasmid with genes that encode for homologues of the *Photorhabdus* insect-related (Pir) binary toxin (Pir A and Pir B). The ingestion of these PirAB-like toxins by *P. vannamei* and *P. monodon* (and probably *P. chinensis* as well) can damage the stomach and HP epithelial tubule cells, under hatchery and farm production environments. These toxins are located on the pVA plasmid, the primary virulence factor. Removal or "curing" the pVA plasmid abolishes the AHPND-causing ability of *Vp*AHPND strains.

It is now known that other non-*Vibrio parahaemolyticus* species (having been found to be present in first EMS/AHPND etiology report in 2010) are also likely causative agents of this disease as well. They include *V. campbellii*, *V. harveyi*, *V. owensii*, and *V. punensis*. In Australia, PirAB-like toxins have been determined to be the cause of the *P. monodon* Mortality Syndrome (*Pm*MS).

Some of the strains of these distinct *Vibrio* species have been found to express different variants of PirAB-like toxins. These act as binary proteins encoding by the pirA and pirB genes. It is necessary to have both proteins for toxicity in shrimp.

Shrimp species susceptible to EMS/AHPND/PmMS disease include *P. vannamei, P. monodon,* and *P. chinensis*. The screening and detection of EMS/AHPND/PmMS in shrimp broodstock feed is critical, as it will enable farmers to only feed high quality virus free polychaete feed to shrimp and avoid infection of postlarvae by vertical transmission from feed to shrimp.

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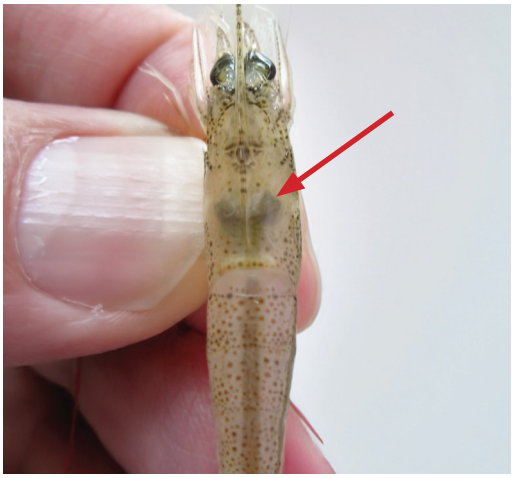

Clinical signs of EMS/AHPND and *Pm*MS. The appearance of clinical signs and mass mortality can occur as early as 10 days post-stocking. Clinical signs include a pale-to-white hepatopancreas, significant atrophy of the hepatopancreas tubules, soft shell, empty or partially empty gut and black spots visible within the hepatopancreas (melanized tubules). Unlike in healthy shrimp, the hepatopancreas cannot be easily squashed between the thumb and forefinger.

Two disease phases are often observed during an outbreak. Initially, the acute phase with massive and progressive hepatopancreas tubule degeneration from the proximal to distal tip, with marked sloughing of tubule epithelial cells into the hepatopancreas tubule lumen and posterior stomach, with no presence of bacteria. This is often followed by a terminal phase which is marked by severe intra-tubular haemocytic infiltration (inflammation) and massive secondary bacterial infections associated with necrotic hepatopancreas tubule cells.

It is also postulated that there can be a non-acute phase whereby animals may have some pathology but nothing to suggest that it is impacting the shrimp negatively. For example, postlarvae in a hatchery where there are low levels of toxins present may show some damage to the HP and some affected animals may go on to develop the acute phase in the presence of higher levels of toxin expression. Depending on the degree of damage some shrimp are also thought to recover. Abundant water exchange, feeding amount optimizing and bottom siphoning twice a day, may also contribute to stopping postlarvae mortality (in tanks or raceways) and sick population recovery, when disease is diagnosed when first clinical signs appear.

Early detection using Shrimp MultiPath™ testing can give farmers up to 10 days' notice before clinical signs appear prior to mass mortality subsequently occurring. This is a critical period that can be used to slow or stop the spread of the disease and maximize production outputs. Early detection empowers the implementation of prompt mitigation strategies. These can include reduction of the primary substrate that the bacteria colonize; suspended and bottom organic matter via syphoning, water exchange and/or bacteria for bioremediation. Stress reduction via increased aeration, reducing feed inputs, increasing biosecurity around infected pond(s) (e.g. managing of affected ponds last in daily routines, separate nets and equipment, physical barriers put in place, inform adjacent farmers of the infection, etc.), and be the first to harvest when commercial size has been reached. Disease mitigation plans should include pathogen exclusion programs. The Shrimp **Multi**Path™ is used to confirm when broodstock or postlarvae are infected with the toxin carrying species/strains. This data can be used to eliminate infected broodstock and/or postlarvae batches from production systems before stocking ponds with infected organisms.

Target life-history stages for accurate early detection include late postlarvae stages (both at hatchery and/or at farm raceways and nursery ponds) and juveniles; mortality in subadults has been reported in the Philippines on days 46-96 after stocking.

Target organs for sensitive Shrimp **Multi**Path™ detection are gut-associated tissues and organs including the hepatopancreas and the stomach. Although not as sensitive, feces can also be tested for the presence of the PirAB toxin genes non-lethally, which is useful if a farmer is testing valuable broodstock. In this instance, fecal strands are collected and incubated in broth (TSB + NaCl), and the bacterial pellet + feces is subsequently tested.

Sampling and preservation of tissues for Shrimp **Multi**Path™ should be done in labelled vials and/or tubes that seal. The fixative should be 70% laboratory grade ethanol. Tissue samples should be 2 to 5 mm^2 in size. Sampling equipment must be sterilized between samples. Holding dead shrimp at room temperature for around 6 hours will increase bacteria counts in tissues and may be useful for enriching for the presence of the pathogen to facilitate detection by PCR. Incubation of suspicious stomach-hepatopancreas samples in peptone broth at 30°C for 24 hours followed by centrifugation, can also enhance the PCR detection. In addition, there is the option to enrich hatchery tank water or to take a scraping of the biofilm for enrichment. Caution should be applied to using using incubation to increase detection sensitivity when target is low in abundance as other bacteria may grow and outcompete to result in false negative PCR data.

Sampling numbers and health management plans should be established with your health expert who will take into account factors such as nauplii/postlarvae source, climate, farm size and location, company structure, market channels for sale of product, etc. Pooling samples for EMS/AHPND testing to maximize value for money with PCR testing is routinely done, although it can reduce the sensitivity of the test resulting in false negatives.

Longer term solutions to disease caused by *Vibrio* spp. that express such toxins include breeding for tolerance and resistance. Good sanitary and biosecurity practices have been found to avoid and/or control the disease. These include, among other things, reduction in stress, improvement of hatchery sanitary conditions, frequent postlarvae PCR-screening, adequate broodstock management, use of high-quality postlarvae and good shrimp farm management like strict feeding rate control, reduction of organic matter in tanks and ponds, and appropriate stocking density. Early pathogen detection and risk mitigation through the use of Shrimp **Multi**Path™ is an important tool for lessening potential *Vibrio* spp. outbreaks.

It is worth noting that according to the WOAH (World Organization of Animal Health), infected products can be treated at 100°C for 1 minute to inactivate the pathogen.

Contact Genics at info@genics.com if you would like to discuss these options for your operation or visit www.genics.com for further details. Watch the instructional video on Shrimp **Multi**Path™ target organ dissection below.

Learn how to dissect your shrimp for testing

Visit our new Educational page here to learn how to:

- Sterilize your equipment before sampling
- Selecting the correct ethanol for tissue preservation
- Identifying and sampling shrimp target organs for SMP testing

Questions?

info@genics.com
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EMS

Did you know?

Shrimp rarely harbour only one pathogen and farmers often don't know which ones they are. This is a significant economic risk for farmers. **Genics has solved this problem** with Shrimp **Multi**Path™. It's the ultimate early warning system for farmers, **detecting 16 pathogens in a single test** that is unparalleled in today's industry for its sensitivity and accuracy.