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Education Series

Enterocytozoon hepatopenaei (EHP) or "Hepatopancreatic Microsporidosis"

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EHP effected shrimp image supplied by Celia Lavilla-Pitogo. Shrimp exhibit low growth rates and wide size distribution.

Shrimp get sick too. Hepatopancreatic microsporidiosis caused by the microsporidian *Enterocytozoon hepatopenaei* **(EHP)**, is an infectious shrimp microsporidian affecting hepatopancreas tubule cells of Penaied shrimp species *Penaeus monodon*, *Penaeus (Litopenaeus) vannamei* and *P. stylirostris*. It´s also likely to affect *P. japonicus*. When observed in *P. vannamei,* EHP is suspected to be associated with 'white faeces disease' as an eventual concurrant health condition. Notably, there are likely other additional causative agents of white faeces disease. Importantly, EHP is not yet a reportable OIE disease.

Because EHP's etiological agent is a microsporidian, and doesn´t require other hosts for transmission (other than shrimp), it's likely to be a highly contagious disease via horizontal transmission (cannibalism). Although mortality has not been documented as a direct result of EHP, concurrant infection with EMS/AHPND, WSSV or vibrio bacteria may result in pond mortality. It is suspected EMS/AHPND and WSSV are more likely than EHP to cause health problems in densely populated aquatic animal production environments. Furthermore, infection with EHP may be a significant risk factor in the development of AHPND.

Importantly, EHP infection is characterized by low growth (growth retardation) and by size disparity and can be observed at the early stage of postlarvae farming in hatcheries. This condition suggests that vertical transmission is also possible. EHP can occur in a wide range of pond conditions, and has been prevalent at salinities between 2 ppt to 30 ppt. Yet, severity of EHP infection increases at higher salinity levels (30 ppt). EHP becomes especially problematic when the salinity is high (30 ppt) and drops with mid salinity environments (15 ppt) or low salinity waters (2 ppt). Nevertheless, EHP infection and PCR detection of the microsporidian can be observed in a wide salinity range from close to 0 ppt to more than 30 ppt.

Causative agents of EHP. Infectious *Enterocytozoon hepatopenaei* (EHP) is a microsporidian parasite classified within the fungal family Enterocytozoonidae. The disease was first discovered in *Panaeus monodon* in Thailand in 2004. It has been shown that EHP infection may be present in shrimp that concurrently have white faeces disease. Nevertheless, this condition has unknown causative agents thus it is unclear if it has a role in EHP-affected shrimp health deterioration.

Clinical signs of EHP. The appearance of clinical signs can occur as early as in postlarvae stages and then during the juvenile stage in growout ponds. Clinical signs of EHP infection include low growth rates and wide size distribution, with more than 5-fold difference in sizes in affected populations. Weight variation coefficient in these ponds usually becomes >30%. Differential diagnosis includes IHHNV and postlarvae stocking with populations affected by high size distribution. Association of EHP with EMS was acute during a large cohort study identifying microsporidian infection prevalence as high as 60% by PCR.

Dual infection of EHP and Vibriosis has been associated with the presence of white faeces syndrome. Nevertheless, not all EHP positive ponds present shrimp affected with white faeces or vice versa, which indicates an indirect relationship between EHP and white faeces syndrome. Histopathological lesions observed in EHP diseased shrimp include basophilic inclusion bodies within the hepatopancreatic cytoplasm cells, either with or without EHP spores.

Early detection using Shrimp MultiPath (SMP). Early testing and detection with SMP can give farmers two to three weeks notice before clinical signs appear and prior to low growth onset. In commercial shrimp ponds, EHP infection can be detected early and farmers advised as soon as postlarvae are received, either in raceways, nursery ponds or growout ponds. This information is an **early warning system** preparing farmers for a critical period where slowing the spread of the disease and maximizing production outputs is still possible.

Early detection empowers the implementation of prompt mitigation strategies. These can include:

- Suspending pond stocking with PLs from infected hatcheries
- Avoiding live and fresh feeds (especially for broodstock) with the option to heat them at 70°C for 15 minutes to kill infective EHP structures
- Not feeding female broodstock 6 hours before moving to spawning tanks and so reducing egg contamination with faeces, and reinforcing egg and nauplii washing and disinfection before transferring to hatchery tanks to reduce possible EHP contamination from broodstock faeces
- Soil treatment with quick lime (CaO) before stocking in ponds previously affected by EHP, pond stocking only with EHP PCR negative tested PLs and, frequent pond surveillance for EHP using molecular tools, are procedures that will help control EHP.

Changing pond management when EHP is detected reduces horizontal transmission by:

- Increasing water exchange
- Removing sick or dead shrimp (to prevent transmission through cannibalism)
- Reducing pond density (partial harvest) and faeces removal (syphoning and/or bacterial bioremediation when possible), must be considered as priority tasks.

Proper technical assistance for periodic monitoring with appropriate diagnostic tools will allow for discrimination between EHP and other disease causing microsporidia-forming microorganisms. Biosecurity around infected ponds must be increased, for example making management of affected ponds in daily routines, separating nets and equipment, physical barriers put in place, inform adjacent farmers of the infection, and first to harvest when commercial size is reached. Disease mitigation plans should include pathogen exclusion programs.

The Shrimp **Multi**path PCR is used to confirm when broodstock or PL are infected with EHP infective structures. This data can be used to eliminate infected broodstock and/or PL batches from production systems before stocking ponds.

Susceptible species for EHP infection include *P. monodon, P. vannamei, P. stylirostris, Artemia spp.*, polychaetes and clams. *P. japonicus* is a suspected EHP susceptible species.

Target life-history stages for accurate early detection include broodstock, late PL and juveniles stages (both at hatchery and/or at farm raceways and nursery ponds). Clinical signs with wide size distribution have been observed in juveniles just few days after pond stocking.

Target organ for sensitive SMP detection is the hepatopancreas (HP). Adequate HP sampling is essential for accuracy of EHP molecular detection and quantitation. Uneven distribution of EHP in the HP has been observed; thus, it´s recommend to make a whole HP homogenate in order to take an aliquot for DNA extraction and a subsequent PCR analysis for EHP detection. Faeces can be used to detect the presence of EHP as well. Faecal samples are useful if a farmer is testing valuable broodstock.

Sampling and preservation of tissues for PCR tests should be done in labelled vials and/or tubes that seal. The fixative should be 70-95% laboratory grade ethanol or RNALater. With Shrimp **Multi**Path EHP testing, typically customers take a 2 mm^2 piece of hepatopancreas to pool with other target organs for sensitive pathogen detection. However, in instances where shrimp farmers want to increase representation of the potential sites where microsporidians are present, it is recommended to make a whole HP homogenate and then take a small aliquot equivalent to the 2 mm^2 size piece of HP.

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. There is also the option to pool samples for Shrimp **Multi**Path testing to maximize value for money with PCR testing.

Longer term solutions to disease caused by EHP include breeding for tolerance and biosecurity measurements implementation as a preventative strategy. Good sanitary and good management farming practices may help to control the disease. These include, among other things, improvement of maturation and hatchery sanitary conditions, frequent broodstock faeces and PL PCR-screening, adequate broodstock management (especially females prophylactic measurements), use of EHP-negative 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 EHP infections.

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.

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
- + Much more...

Questions?

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EHP

Did you know...

Shrimp rarely harbour only one pathogen and farmers often don't know which ones they are. This is a huge economic risk for farmers. **Genics has solved this problem with** Shrimp **Multi**Path. It stacks up as the ultimate early warning system for farmers, detecting 13 pathogens in a single automated test that is unparalleled in today's industry for its sensitivity and accuracy.