

Diseases of Crustaceans — **Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP)**

Signs of Disease

Disease signs at pond level

- There are no specifically distinctive gross signs of infection by EHP;
- Infection may be suspected with the occurrence of unusually retarded growth in the absence of other gross signs of disease;
- Infection must be confirmed by microscopic or molecular methods.

Disease signs at animal level by histopathology

- In hepatopancreatic (HP) tissue sections stained with hematoxylin and eosin (H&E), HP tubule epithelial cells show the presence of cytoplasmic, basophilic inclusions containing clusters of elliptical to somewhat ovoid spores of 1.1 ± 0.2 by $0.6-0.7 \pm 0.1$ μm (Fig. 1);
- Sometimes free spores released from lysed cells may be seen in the tubule lumens;
- Because of their small size, use of an oil immersion lens is recommended in searching for spores, although with experience, tissue sections and smears may be first scanned using a 40x objective;
- HP tissue smears to screen for spores may also be prepared by stunning shrimp in ice water followed by aseptic removal of the carapace, followed by holding the outer region the HP with a pair of forceps before cutting off a portion of tissue, gently placing the cut surface in a drop of 2.8% sodium chloride solution containing 10% formalin near the frosted end of a microscope slide and smearing the length of the slide in one

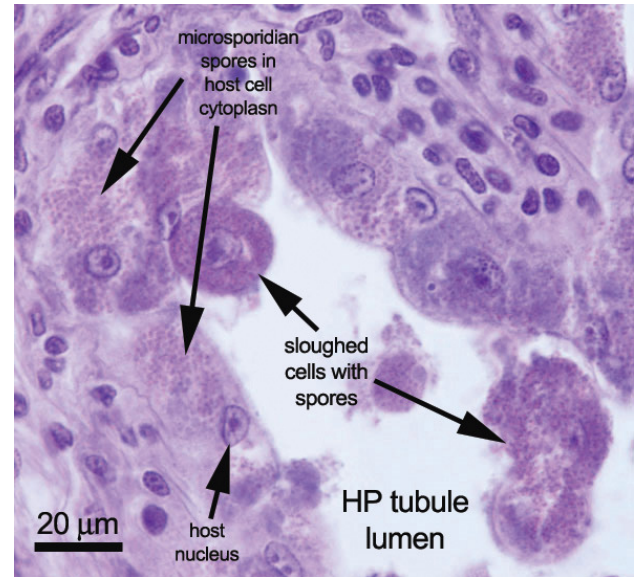


Figure 1. Photomicrograph of H&E stained HP tissue showing tubule epithelial cells infected with EHP.

Source: T Flegel

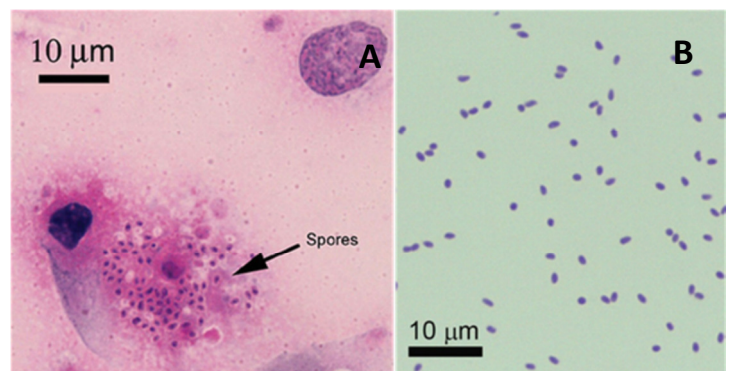
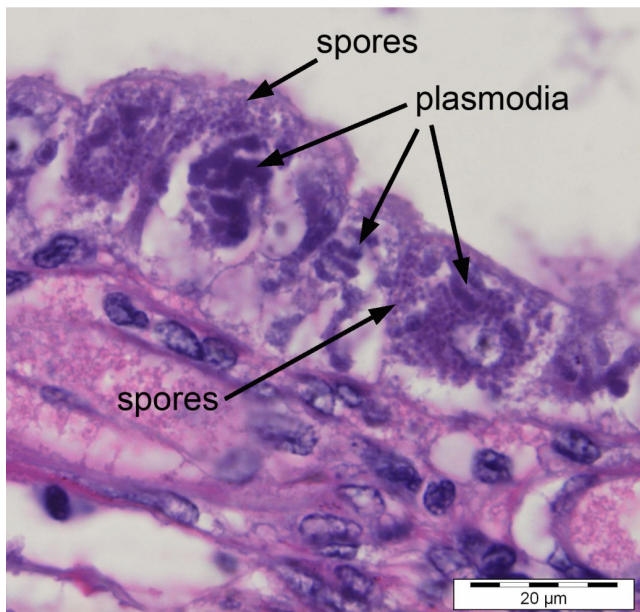


Figure 2. (A) Photomicrograph of a smear of HP tissue stained with H&E and showing a cluster of EHP spores next to the nucleus of the lysed cell that contained them. (B) Photomicrograph of spores purified by density gradient separation.

Source: T Flegel

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- swipe. The slide should then be thoroughly dried before staining with H&E, mounting with permount, and searching for spore clusters and free spores (Fig. 2);
- Plasmodia of the microsporidian may also be seen in tissue sections but cannot be used for diagnosis in the absence of spores (Fig. 3);
 - In some situations, spore production may be low or not yet initiated, making confirmatory diagnosis difficult or impossible. In such cases, PCR detection is recommended.

Figure 3. Photomicrograph showing plasmodia and spores of EHP.
Source: T Flegel

Disease Agent

- *Enterocytozoon hepatopenaei* is a microsporidian first discovered in *Penaeus (Penaeus) monodon* in Thailand in 2004 (Chayaburakul, et al., 2004) and later described in detail and named (Tourtip, 2005; Tourtip, et al., 2009). It infects only the tubule epithelial cells of the hepatopancreatic (HP) tissue of shrimp;
- It was later found to infect also *Penaeus (Litopenaeus) vannamei* cultivated in Thailand and is suspected to have been reported from *Penaeus (Marsupenaeus) japonicus* in Australia in 2001 (Hudson, et al., 2001; Tourtip, et al., 2009);
- EHP has been reported from Vietnam as associated with white feces syndrome (WFS) (Ha, et al., 2010 ; Ha, et al., 2010), and from China (Liu et al., in press)
- The spores are very small (1.1 ± 0.2 by $0.6-0.7 \pm 0.1$ µm) and show the presence of a polar filament of 4-5 coils (Fig. 4);
- The association was later challenged (Tangprasittipap, et al., 2013) when it was shown in laboratory infections did not result in white feces syndrome. However, EHP may be present in shrimp exhibiting WFS or other diseases such as WSSV;
- EHP should not be confused with *Agmasoma penaei*, another microsporidian that infects muscle tissue and connective tissue in *P. monodon*, *P. merguensis* and *P. vannamei* in Asia leading the gross signs of “cotton shrimp disease” or “white back” disease (Laisutisan, et al., 2009; Pasharawipas Flegel, 1994; Pasharawipas, et al., 1994). In rare cases, lesions of *A. penaei* may extend into the connective tissue of the shrimp hepatopancreas, but infections never extend into the tubule epithelial cells of the HP;

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- Moreover, unlike the microsporidian *A. penaei*, EHP can be transmitted horizontally among shrimp in a rearing ponds (Tangprasittipap, et al., 2013) meaning that infections can spread progressively as cultivation continues.

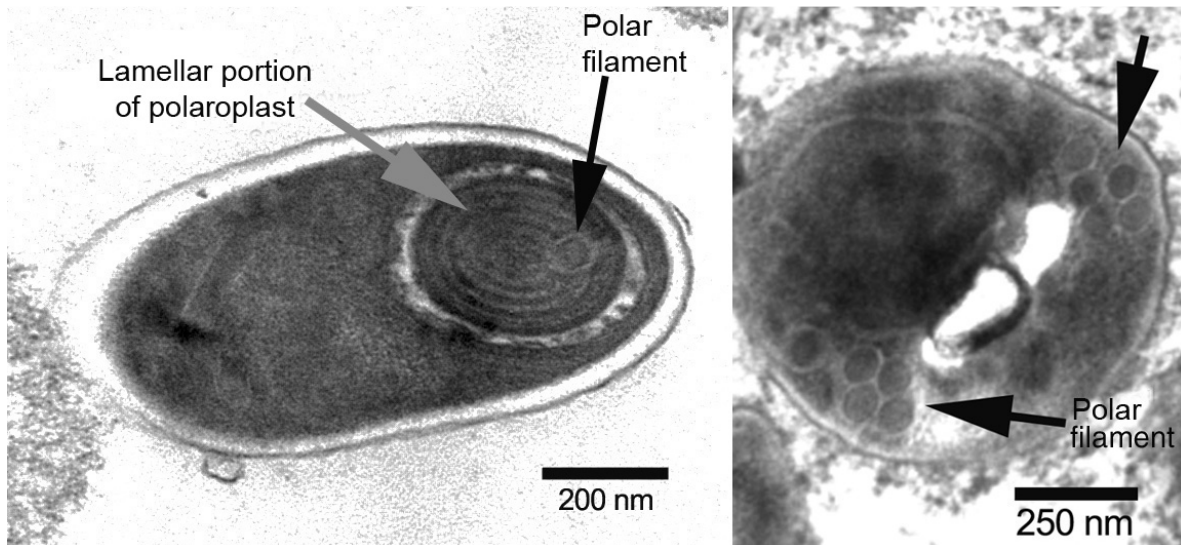


Figure 4. Electron micrograph of spores of EHP showing a polar filament of 5-6 coils..

Source: T Flegel

Molecular Diagnostics

- PCR and *in situ* hybridization methods for EHP were initially described in 2009 (Tourtip, et al., 2009). The PCR detection method was later improved to a more sensitive PCR method (Tangprasittipap, et al., 2013). More recently, alternative *in situ* and PCR detection (Tang, et al., 2015), real time PCR (Liu et al., 2014) and LAMP-nanogold method (Suebsing, et al., 2013) have also been described.
- Because of the difficulty in resolving spores of EHP, and because the microscopic method is destructive and unsuitable for non-destructive screening of shrimp feces, more sensitive molecular methods such as nested PCR, LAMP or real-time PCR should be the choice for EHP detection.



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Host Range

EHP affects both *P. monodon* and *P. vannamei* and is suspected to also infect *P. japonicus* (Tangprasittipap, et al., 2013) (Hudson, et al., 2001).

Presence in Asia-Pacific

- EHP was first detected in *P. monodon* in Thailand in 2004, later reported from Vietnam (Ha, et al., 2010 ; Ha, et al., 2010; Tang, et al., 2015);
- It resembles an unnamed microsporidian reported in the HP of *P. monodon* in Malaysia in 1989 (Anderson, et al., 1989) and in *P. japonicus* in Australia in 2001 (Hudson, et al., 2001).
- PCR positive results were also obtained from *P. vannamei* cultivated in Indonesia and India (unpublished). Thus, it is probable that EHP is endemic in the Australasian region.
- It is also possible that it may be able to infect other species of penaeid shrimp in the region;
- Since some microsporidian species are known to have alternative hosts with different spore stages in different animal species (sometimes in completely different phylogenetic groups), it is possible that different spore stages also exist for EHP but have not yet been discovered



Further information

An additional published report from NACA, which includes control measures at hatchery and farm levels, as well as prevention of international/trans-boundary spread can be obtained at the following link: http://www.enaca.org/modules/news/article.php?article_id=2039

Additional Notes:

It is still common practice for many hatcheries to routinely feed live polychaete worms and mollusks to broodstock to increase nauplii production, even though this presents a significant biosecurity risk. We have obtained PCR positive results for EHP from living polychaetes and clams (unpublished) but have not confirmed that they are mechanical or infected carriers of EHP. Thus, in order to reduce the risk of EHP transmission, we recommend that live or fresh feeds not be used and that they be at least frozen before being used to feed clean broodstock.

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List of Experts:

Dr. Kallaya Sritunyalucksana

Shrimp-virus Interaction Laboratory
(ASVI),
National Center for Genetic Engineering
and Biotechnology (BIOTEC),
National Science and Technology
Development Agency (NSTDA)
Yothi Office, Rama VI Rd., Bangkok,
10400, Thailand.
kallaya@biotec.or.th

Dr. Siripong Thitamadee

Centex Shrimp, 4th Floor
Chalermprakit Building
Faculty of Science, Mahidol
University
Rama 6 Road, Bangkok 10400
Thailand
siripong.thi@mahidol.ac.th

Prof. Timothy W. Flegel

Centex Shrimp, 4th Floor
Chalermprakit Building
Faculty of Science, Mahidol
University
Rama 6 Road, Bangkok 10400
Thailand
tim.flegel@gmail.com

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Note: All information included in this disease card were provided by Prof. Timothy W. Flegel, Centex Shrimp, Bangkok, Thailand.

