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INFECTION WITH ENTEROCYTOZOON HEPATOPANAEI (EHP)

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Fungus.

1.2. Disease name and synonyms

Infection with *Enterocytozoon hepatopenaei* (EHP).

1.3. Pathogen common names and synonyms

Hepatopancreatic microsporidiosis.

1.4. Taxonomic affiliation

EHP is a microsporidian, spore forming unicellular parasite belonging to the Family Enterocytozoonidae and Phylum Microsporidia. The pathogenic agent has four intracellular life stages in the infected cells.

1.5. Authority (first scientific description, reference)

EHP was first discovered in *Penaeus monodon* in Thailand in 2004 (Chayaburakul *et al.*, 2004) and later described in detail and named (Tourtip, 2005; Tourtip *et al.*, 2009).

Based on morphological and molecular analyses, a new genus, *Ecytonucleospora*, was suggested which includes EHP (Wang *et al.*, 2023).

1.6. Pathogen environment (fresh, brackish, marine waters)

Brackish (> 2 ppt) and marine waters. An EHP infection can occur at a salinity as low as 2 ppt; however, the prevalence and the severity of the EHP infection is higher at a salinity of 30 ppt (Aranguren *et al.*, 2021).

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)

EHP can be transmitted horizontally among shrimp through cannibalism and co-habitation in rearing ponds (Tangprasittipap *et al.*, 2013) meaning that infections can spread progressively as cultivation continues. Dragonflies can be natural EHP hosts and have the potential to horizontally transmit the pathogen to shrimp (Kumar Dewangan *et al.*, 2023).

EHP has a relatively simple (direct) life cycle compared to other microsporidia with a single spore type facilitating horizontal transmission among a limited number of penaeid shrimp species.

2.2. Reservoir

Infected populations of shrimps, both farmed and wild.

Marine crabs are a potential vector for EHP (Mani *et al.*, 2022).

2.3. Risk factors (temperature, salinity, etc.)

Polychaetes, artemia, molluscs, squid and other animals used as live or fresh shrimp feeds have been reported to be PCR-positive for EHP and capable of causing the infection when fed to shrimp (Chaijarasphong *et al.*, 2021).

The infectivity of EHP is higher at a salinity of 30 ppt than at lower salinities (Aranguren *et al.*, 2021).

Multiple co-infections with white spot syndrome virus and EHP has been reported (Thamizhvanan *et al.*, 2019).

3. HOST RANGE

3.1. Susceptible species

Giant tiger prawn *(Penaeus monodon)* (Chayaburakul *et al.*, 2004), whiteleg shrimp *(Penaeus vannamei)* (Tangprasittipap *et al.*, 2013) and blue shrimp *(Penaeus stylirostris)* (Tang *et al.*, 2015) have been reported to be susceptible to infection with EHP.

An uncharacterized microsporidian with ultrastructure that resembles EHP has been reported from kuruma prawn (*Penaeus japonicus*) (Hudson *et al.*, 2001).

12, rue de Prony
75017 Paris, France

T. +33 (0)1 44 15 18 88
F. +33 (0)1 42 67 09 87
woah@woah.org
www.woah.org

A new distinct strain of EHP has been reported in giant river prawn (*Macrobrachium rosenbergii*), which differs from the common strain isolated from *Penaeus vannamei* (Wang *et al.*, 2022).

3.2. Affected life stage

All life stages are affected. Clinical signs caused by infection with EHP in the early life stage are not so obvious, while the infection will cause very severe economic losses during the grow-out stage.

3.3. Additional comments

EHP increases susceptibility of shrimp to *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) (Aranguren *et al.*, 2017).

EHP should not be confused with *Agmasoma penaei*, another microsporidian that infects muscle tissue and connective tissue in *P. monodon*, *P. merguiensis* and *P. vannamei* in Asia leading the gross signs of 'cotton shrimp disease' or 'white back' disease (Laisutisan *et al.*, 2009; Pasharawipas *et al.*, 1994).

4. GEOGRAPHICAL DISTRIBUTION

Infection with EHP has been reported in China (People's Rep. of) (Liu *et al.*, 2016), Thailand (Chayaburakul *et al.*, 2004; Tourtip, 2005; Tourtip *et al.*, 2009, Network of Aquaculture Centres in Asia-Pacific (NACA), 2021-2024), Vietnam (Ha *et al.*, 2010a; Ha *et al.*, 2010b; Tang *et al.*, 2015), and in Chinese Taipei, India and Philippines (NACA, 2021-2024).

Uncharacterised microsporidians resembling EHP have been reported from Malaysia (Anderson *et al.*, 1989) and Australia (Hudson *et al.*, 2001).

Unpublished findings of PCR positive results for infection with EHP have been reported from Indonesia (Tang *et al.*, 2016).

5. CLINICAL SIGNS AND CASE DESCRIPTION

5.1. Host tissues and infected organs

The main organ where pathology is observed is the hepatopancreatic tissue.

5.2. Gross observations and macroscopic lesions

Externally visible clinical signs are often absent, apart from retarded growth over time. White faecal strings existed in some cases but not in others indicating that the relationship between EHP and WFS appears to be conditional especially in the cases of animals infected with bacterial proliferation (Ha *et al.*, 2010a; Rajendran *et al.*, 2016). Food conversation ratio (FCR) is high (Geetha *et al.*, 2022).

The gross signs of white faecal syndrome such as floating whitish faecal strings is proposed to be used as an indicator of the presence of EHP in countries where EHP is endemic (Tang *et al.*, 2016).

5.3. Microscopic lesions and tissue abnormality

In hepatopancreatic (HP) tissue sections stained with haematoxylin 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 ± 0.1 µm (Tourtip *et al.*, 2009).

Sometimes free spores released from lysed cells may be seen in the tubule lumens.

5.4. WOAH status

Infection with EHP is considered to meet the WOAH definition of an 'emerging disease' and, as such, should be reported to WOAH in accordance with Article 1.1.4. of the *Aquatic Code*.

6. SOCIAL AND ECONOMIC SIGNIFICANCE

Although infection with EHP does not cause significant mortality in shrimp, it affects shrimp production due to growth retardation and its possible association with white faeces syndrome (Ha *et al.*, 2010a; Rajendran *et al.*, 2016).

The EHP loads in the hepatopancreas are negatively correlated with the shrimp growth rates. EHP loads above 103 copies/(ng HPDNA) indicate high risk (Liu *et al.*, 2016).

Infected populations show different growth rates, sizes of individual animals within the same group are uneven, and the food conversation ratio (FCR) is high (Geetha *et al.*, 2022). EHP infections have reached epidemic proportions in the Asian penaeid shrimp aquaculture industry.

7. ZOONOTIC IMPORTANCE

None.

8. DIAGNOSTIC METHODS

8.1. Definition of suspect cases

Infection may be suspected with the occurrence of unusually retarded growth in the absence of other gross signs of disease.

8.2. Presumptive test methods

A fluorescent stain, calcofluor white (CFW), can be used for detection of spores of the microsporidium EHP (Zhao *et al.*, 2020). An immunological influence assay (IFA) was also developed (Cho, *et al.*, 2024).

Sensitive molecular techniques such as one-step PCR, nested-PCR, LAMP, LAMP-based microfluidic chip, qPCR, ddPCR, ERA and RPA are available as presumptive test methods (Hu *et al.*, 2023; Jaroenlak *et al.*, 2016; Kanitchinda *et al.*, 2020; Koiwai *et al.*, 2017; Li *et al.*, 2023; Liu *et al.*, 2016; Liu *et al.*, 2018; Ma *et al.*, 2021; Sathish *et al.*, 2018; Suebsing *et al.*, 2013; Tang *et al.*, 2015; Tangprasittipap *et al.*, 2013; Tourtip *et al.*, 2009; Yang *et al.*, 2022; Zhang *et al.*, 2022; Zhou *et al.*, 2020).

8.3. Confirmatory test methods

Infection with EHP can be confirmed by one step-PCR and sequencing or nested-PCR and sequencing.

One-step PCR has detection limit ranges from 1,000-10,000 copies per reaction and may not be sensitive enough to detect carrier-state infections (Tourtip *et al.*, 2009; Tang *et al.*, 2015).

Nested-PCR has been developed with the detection limit of 10 copies per reaction (Tangprasittipap *et al.*, 2013; Jaroenlak *et al.*, 2016) and ddPCR can reach the detection limit of 2.3 copies per µL (Zhang *et al.*, 2022).

RPA can be carried out quickly (within 13 min) with a detection limit of 10 copies per µL and may be more practical than other assays in the field (Li *et al.*, 2023).

9. CONTROL METHODS

The use of EHP-free broodstock and post-larvae (PLs) is encouraged.

Appropriate biosecurity measures in aquaculture establishments before and after stocking are important to prevent introduction of EHP. This includes disinfection to inactivate EHP spores, particularly in ponds or hatcheries with a previous history of EHP infection.

Captured, live animals (e.g. live polychaetes, clams, oysters, etc.) from the wild should not be used as feed for broodstock. Fresh feed should be pre-treated at -20℃ for at least 48 h or 70 ℃ for 15 min before they are fed to broodstock.

Targeted surveillance for infection with EHP in early life stages of cultured susceptible species, especially before transferring to the ponds, is recommended.

10. TRANSMISSION RISK

Inactivation of purified EHP spores was achieved by exposure to freezing at -20°C for at least 2 hours (Aldama-Cano *et al.*, 2018).

11. ADDITIONAL USEFUL INFORMATION

The disease has been notifiable to NACA since 2015.

This disease card is based on the NACA disease card (Flegel, 2015).

For a recent review of EHP see Chaijarasphong *et al.*, 2021.

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