

First report on *Enterocytozoon hepatopenaei* (EHP) infection in Pacific whiteleg shrimp (*Litopenaeus vannamei*) cultured in Sri Lanka

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Microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) that causes Hepatopancreatic Microsporidiosis (HPM) has emerged to be one of the most important pathogens in Asia for the exotic, cultivated Pacific Whiteleg shrimp (*Litopenaeus vannamei*). This parasite replicates in the hepatopancreas and the midgut. Infected shrimp exhibit reduced feeding and growth retardation. Since February 2022, some farms in the Puttalam district that have stocked *L. vannamei* post larvae (PL) originated from imported SPF broodstock reported retarded shrimp growth and white faeces. Therefore, live shrimp were sampled from fifteen affected farms (8-10 shrimp per farm) during the period from February 2022 to June 2022 to identify the etiology. A polyphasic diagnostic approach that included direct microscopy of faecal samples (wet mounts and smears stained with modified Trichrome stain), histopathological examination of internal organs (hepatopancreas and midgut), and a nested PCR targeting SSU rRNA gene of EHP was employed. History (slow growth apparent by 35-40 days post stocking, white floating fecal strings) and external examination findings (high size variation among shrimp) were suggestive of HPM. Wet mounts and stained smears of faeces showed spores suggestive of EHP. Histopathological examination revealed degeneration and necrosis in the hepatopancreas and midgut, and microsporidian spores in the tubular epithelial cells. PCR confirmed the presence of EHP in all fifteen farms tested, severe infection in six farms and a low-level infection in the rest. Bacterial co-infection with *Vibrio* species was also detected. This is the first report that confirmed the presence of EHP infection in *L. vannamei* shrimp cultured in Sri Lanka using a polyphasic diagnostic approach. Timely control and prevention measures including screening of PLs by PCR to confirm the absence of EHP prior to stocking and appropriate pond preparation between culture cycles should therefore be implemented to limit the impacts of this disease on shrimp production in the country.

Keywords: *Enterocytozoon hepatopenaei*, *Litopenaeus vannamei*, slow growth, white feces

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