Role of Macrofauna in Farm-Level Prevalence of *Enterocytozoon hepatopenaei* (EHP) in Shrimp Aquaculture Systems in North Western Province of Sri Lanka

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Enterocytozoon hepatopenaei (EHP) is the most significant microsporidian pathogen infecting *Litopenaeus vannamei*, causing hepatopancreatic microsporidiosis (HPM) disease. EHP causes significant economic losses in shrimp aquaculture worldwide. This pathogen was first reported in 2022 in the Puttalam District, North Western Province of Sri Lanka. Farm-level macrofaunal communities are considered to play a major role in disease transmission. Various macrofaunal groups, including crabs, molluscs, fish, and insects, are commonly associated with shrimp farming environments and natural aquatic ecosystems. This study aimed to investigate the association between macrofaunal communities and EHP prevalence across shrimp farms in the North Western Province of Sri Lanka. A total of 55 shrimp farms in the North Western Province were surveyed, and 158 macrofaunal samples were collected based on accessibility, comprising fish, molluscs, crabs, and aquatic insects. Species identification was conducted based on morphological characteristics. Organs from potential macrofaunal carriers were collected according to sample type, as follows: crabs (hepatopancreas, stomach, and gills); fish (gills); molluscs (gills and stomach); and insects (whole body). Organs were collected and pooled from five samples per farm. Detection of EHP was performed using nested polymerase chain reaction (PCR) targeting the spore wall protein (SWP) gene. The dominant macrofaunal species identified included *Glossogobius giuris* (n=32), *Perisesarma bidens* (n=54), *Oreochromis niloticus* (n=6), *Cerithidea cingulata* (n=18), *Gerris spp.* (n=12), *Euphausiacea spp.* (n=31), and *Turritella spp.* (n=5). Three species each of Arthropoda, Chordata, and Mollusca were identified based on morphological characters. All samples tested positive by nested PCR using the SWP primers. EHP was detected across multiple macrofaunal groups, with the highest prevalence observed in *Glossogobius giuris* (68.75%; 22/32), followed by *Perisesarma bidens* (59.26%; 32/54), *Gerris* spp. (58.33%; 7/12), and *Cerithidea cingulata* (50.00%; 9/18). EHP was not detected in *Euphausiacea* spp., *Oreochromis niloticus*, or *Turritella* spp. These findings provide strong evidence that macrofauna contribute to the environmental persistence and transmission of EHP in shrimp aquaculture systems and to the spread of disease into natural aquatic ecosystems. The results emphasize the need for enhanced biosecurity measures, including the management of macrofaunal populations within farms, to reduce the risk of disease spread at both the farm level and in natural aquatic ecosystems. This study advances the current understanding of EHP epidemiology and offers practical insights for improving disease control strategies in Sri Lanka's shrimp farming industry.

Keywords: EHP, shrimp aquaculture, macrofauna, *Glossogobius giuris, Perisesarma bidens*

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