



LINKING *VIBRIO* AND *ENTEROCYTOZOOM HEPATOPENAEI* (EHP) IN SHRIMP: DOES THE EXISTENCE BETWEEN THIS TWO PATHOGENIC MICROORGANISM INTERTWINED WITH EACH OTHER?

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KeyWords

Shrimp, *Enterocytozoon Hepatopenaei* (EHP), *Vibrio*

ABSTRACT

Shrimp is one of the leading national commodities and has high economic value. Indonesian cultivated shrimp production in 2012-2017 has increased significantly. Even though it has increased, there are still problems that must be overcome, one of which is the disease caused by the parasite *Enterocytozoon Hepatopenaei* (EHP). EHP infection disturbs the hepatopancreatic tubular cells and existing *Vibrio* cells form colonies on these cells. While *Vibrio* is a well known pathogenic microorganism in shrimp creating a multiple disease and health problems. This article will discuss about EHP and also its relationship with Vibriosis in Shrimp.

INTRODUCTION

Shrimp is one of the leading national commodities and has high economic value. Based on the Statistical Data Center, [1], the production of cultured shrimp in 2012 reached 415,517 tons, 2013 reached 645,955 tons, 2014 reached 639,369 tons, 2015 reached 607,152 tons, 2016 reached 692,568 tons, 2017 reached 919,987 tons and years 2018 semester 1 reached 398,007 tons. Overall shrimp culture production in Indonesia has increased. Although the production of shrimp farming has increased, there are still problems in shrimp culture that must be overcome, one of which is infection. Shrimp disease is the main obstacle for the development of shrimp culture [2]. One of the diseases that appears in shrimp is caused by the parasite *Enterocytozoon Hepatopenaei* (EHP).

Enterocytozoon hepatopenaei (EHP) is a microsporidian parasite in tiger shrimp and vannamei. EHP parasites were first identified and characterized in tiger prawns (*P. monodon*) in Thailand in 2009. EHP infects the cytoplasm of epithelial tubular cells of the shrimp hepatopancreas [3] In laboratory tests, white shrimp infected with EHP increased susceptibility to *Vibrio* infection associated with Acute Hepatopancreatic Necrosis Diseases (AHPND). AHPND is one of the most important diseases because it causes shrimp mortality and a significant decrease in production in countries where EHP is reported such as China, Vietnam, Malaysia, Thailand, Mexico [4-6] and then in the Philippines (Peña, et al., 2015). EHP infection disrupts the hepatopancreatic tubular cells and existing *Vibrio* cells form colonies on these cells [7].

DISEASES IN SHRIMP AQUACULTURE

Tiger shrimp (*Penaeus monodon*) and vannamei shrimp (*Litopenaeus vannamei*) are classified in the Arthropoda phylum, Crustacean subphylum, Decapoda order [8]. The carapace and abdomen of the tiger prawns have black bands,

giving them a shapetiger-striped. Vaname shrimp has a white body [9]. Vannamei shrimp are widely cultivated because of their fast growth, tolerance for high density, and high resistance to disease [10].

Decapod crustaceans, including shrimp, experience gradual growth resulting in the release of the exoskeleton. The removal of the exoskeleton is also known as (moltingmolting), which aims to increase body size [11]. Apart from molting, shrimp have cannibalistic properties (Romano, 2017). Weak and sick shrimp tend to be eaten by other shrimp [12]. Shrimp cannibalism is also one of the causes of disease spread in shrimp.

There are several types of diseases that attack shrimp, namely (1) Infectious Myonecrosis Virus (IMNV) shows symptoms of muscle necrosis [13], (2) White Spot Syndrome Virus is characterized by the appearance of white spots on the shrimp cuticles [14] and (3) *Enterocytozoon hepatopenaei* (EHP) is characterized by inhibited shrimp growth [7].

***Enterocytozoon Hepatopenaei* (EHP)**

Enterocytozoon hepatopenaei (EHP) is a microsporidian parasite, first recognized as an unidentified microsporidian, causing slow growth in tiger prawns in Thailand [15]. In 2009, EHP was first identified and characterized in tiger prawns (*Penaeus monodon*) in Thailand [3].

EHP is mostly found in Asian regions, such as in China, Malaysia, Thailand, Indonesia and Vietnam, possibly in India and Mexico [16]. In Indonesia, EHP has been detected in vaname shrimp populations in East Java, West Java, North Sumatra, South Lampung, Bali, Lombok and Sulawesi [17].

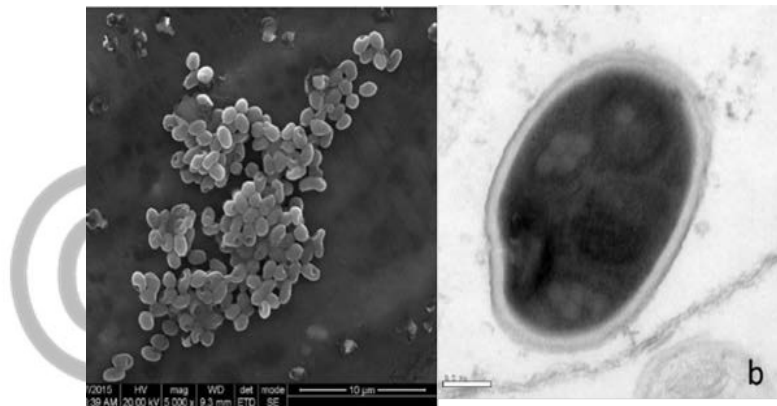


Fig. 1. *Enterocytozoon hepatopenaei* under Microscope. (a) Scanning Electron Microscope [18] and (b) Transmission Electron Microscope [19]

EHP infects the cytoplasm of epithelial tubular cells of the shrimp hepatopancreas [3]. EHP only infects the hepatopancreatic tubules in shrimp, thus damaging the ability of the hepatopancreas to absorb nutrients from food and causing stunted shrimp growth [16].

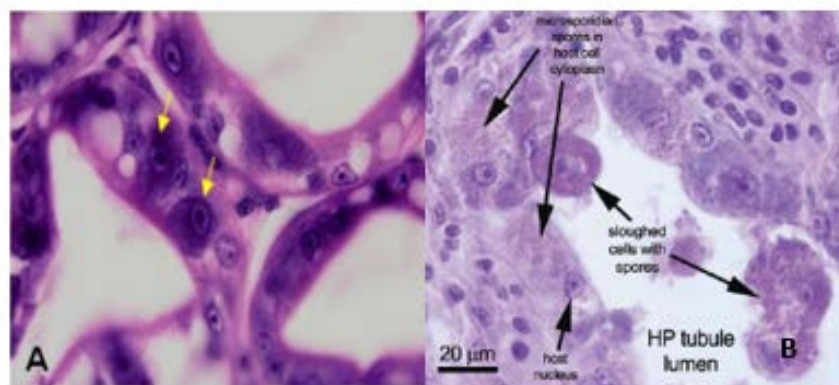


Fig. 2. Hepatopancreatic Tissue with Hematoxylin Eosin Staining. (A) In normal cells [20] and (B) infected with EHP [21]

In addition to tiger shrimp, EHP can also infect vaname shrimp (*Litopenaeus vannamei*). It is thought that EHP parasites can be transmitted from one shrimp to another via oral (cannibalism) [22]. EHP infection does not cause death, but causes stunted shrimp growth [21].



Fig. 3. Stunted Growth of Vaname Shrimp, observed after 90 days of culture [18]

VIBRIO

Bacteria *Vibrio* are straight or curved rods, moving (motile) with flagella at one end (monotric), gram-negative bacteria, aerobic and anaerobic facultative, no spores, no capsules. The biochemical test showed positive results in the oxidase and indole test. The name "Vibrio" comes from the word *vibrate*, indicating the motility characteristics of the vibrator. *Vibrio* bacteria can be found in aquatic habitats with different salinity levels. *Vibrio* bacteria are commonly found in marine, brackish waters, on the surface and in the intestines of marine animals [23,24].

Vibrio bacteria can carry out respiration and fermentation. The optimum temperature for growth varies, it can grow at 20°C, most of them grow at 30°C. Most *vibrio* species require Na⁺ or sea water to grow and need 0.5-3% NaCl for optimum growth. Some species can glow (*luminescent*) (Brenner, *et al.*, 2004). *Vibrio* bacteria can use D-glucose and other carbohydrates such as maltose, d-manose and trehalose by fermentation and oxidation, producing acid but not producing gas except for *V. furnissii*, *V. gazogenes*, some strains of *V. (Listonella damsela)* [24].

Vibrio is bacteria that is in the digestive tract of shrimp. Number of species *Vibrio Normal alginolyticus* in shrimp gut can act as a probiotic [25]. *Vibrio* can also cause disease in shrimp if it is in large quantities [26]. The disease caused by *vibrio* bacteria is called vibriosis.

EHP and Vibrio

In laboratory tests, white shrimp infected with EHP increases the susceptibility to infection with diseases caused by *Vibrio*, such as *Acute Hepatopancreatic Necrosis Disease* (AHPND) and *White Feces Disease* (WFD). EHP infection disrupts cells in the hepatopancreatic tubules and existing *Vibrio* forms colonies on these cells [7].

Microbial diversity in healthy and sick shrimp has differences. class bacteria *Gammaproteobacteria* in healthy shrimp are more abundant than in sick shrimp (inactive, empty digestive tract and poor growth). But in the water from where the sick shrimp grew, it showed that bacteria from the *Gammaproteobacteria* and *Alphaproteobacteria* classes were higher than the water from where healthy shrimp grew [27].

Based on research by Fenny *et al.* 2020, The abundance of bacteria in water and feces of shrimp infected with EHP is higher than in healthy shrimp. The abundance of the suspected *Vibrio* bacteria in the hepatopancreas, water and feces of EHP infected shrimp was higher than that of healthy shrimp. *Vibrio* suspected green colonies of bacteria in water and shrimp feces had higher EHP. In EHP shrimp hepatopancreas, the number of yellow colonies of the suspected *Vibrio* bacteria was higher than in green colonies.

Conclusion and Future Research Prospect

Research on the association between the presence of EHP and *Vibrio* is still underreported. So for the next research conducted to examine *Vibrio* species that can survive on EHP infected shrimp. In addition, further research can be carried out to investigate the effect of viable *Vibrio* species on EHP shrimp on the susceptibility of shrimp to other diseases.

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