Histopathology of the gill of Vaname Shrimp (*Litopenaeus vannamei*) infested by protozoan ectoparasite

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Abstract. The aim of this study were to determine the changes of vaname gill which infested by ectoparasites and the correlation between the degree of ectoparasite infestation. The results showed that ectoparasites were found in the gill of vaname shrimp were *Zoothamnium* sp. and *Epistylis* sp.. The degree infestation of the infested gills showed moderate infestations for *Zoothamnium* sp.; moderate and mild infestations for *Epistylis* sp. Histopathology analysis found haemorrhagi and hyperplasia. The correlation between the degree of ectoparasite infestation and the changes of vaname gill histopathology infested by ectoparasite showed a positive correlation (r = 0.926), which means that the high degree of the infestation, the higher histopathological changes.

1. Introduction

The disease that is often faced by farmers is the infestation of parasites. Parasites are harmful organisms because of the parasitic nature of life that takes nutrients from their hosts. According to [1], *Zoothamnium* sp., *Vorticella* sp., and *Epistylis* sp. is a type of protozoa or parasite attached to the body of the shrimp. These parasites are usually found in stressed shrimp and are affected by changes in water quality conditions, especially temperature, as well as shrimp culture ponds that contain a lot of feed remnants that will increase ammonia levels and decrease the dissolved oxygen content in the waters of the pond.

Histopathology can determine the condition of cells, tissues, and organs that are attacked by ectoparasites. Histopathological analysis was carried out by comparing the condition of healthy tissue to shrimp tissue samples infected with ectoparasites [2]. The first step that needs to be considered in diagnosing the disease is to see clinical signs that include behavior, external and internal characteristics and pathological changes [3]. Therefore, it is necessary to do a further examination to determine the histopathology of vaname shrimp that is infested with ectoparasites.

The purpose of this study was to determine histopathological changes and the correlation between changes in the histopathology of the gill of the white shrimp which were infected by ectoparasites in the village of Tanjangawan. The benefits obtained are that it can provide information and knowledge about the analysis of histopathological changes in the gills of the vaname shrimp (*Litopenaeus vannamei*) infested by ectoparasites.
vannamei) which were infected by ectoparasites in the village of Tanjangawan, Ujungpangkah sub-district, Gresik district, East Java.

2. Materials and methods
   2.1 Sample collection
   100 samples of Vaname shrimp were taken from Tanjungawan Village, Gresik, East Java, Indonesia. Shrimp examination by taking target organs from Zoothamnium, Vorticella, and Epistylis, that was the gill. After the identification process is carried out counting and grouping according to the degree of parasitic infestation.

   2.2 Histopathological examination
   Histopathological sample preparation begins with the collection of gills Vaname shrimp (Litopenaeus vannamei) infested with ectoparasites. Part of The vaname shrimp gills is preserved in Davidson's fixative solution during 1-2 days. Observations were made using a microscope to simplify the process of observing histopathological changes in tissue vaname shrimp gills (Litopenaeus vannamei) with a magnification of 400x.

3. Results
   3.1 Ectoparasites observation
   The results of ectoparasites observation in vaname shrimp found ectoparasite infestations from Protozoa phylum namely Epistylis sp. and Zoothamnium sp. The observation of Zoothamnium sp. and Epistylis sp. can be seen in Figure 1.

   ![Figure 1](image_url)

   Figure 1. A. Zoothamnium sp. and B. Epistylis sp. on the gills of vaname shrimp (400x)

   From the observations of Zoothamnium which was found to have a rounded body shape, transparent color, has a food vacuole and has a stem (stalk) which serves to attach itself to the host, the body shape that is owned in accordance with the statement [4]. Zoothamnium sp. has a transparent body that has an average zooid size of 30-45 μm and a stem of 10 μm in diameter. Whereas, Epistylis which has been observed in this study has a body shape such as elongated bells, transparent in color, has a non-contractile stalk. Epistylis sp. has a branching stalk and 10 μm in diameter, there is anterior zooid cilia. From the results of the observation, Epistylis was found to have characteristics and body shape following the statements of [4]. We found 29 shrimps were in a moderate level of infestation of Zoothamnium, 5 shrimps were lightly infested by Epistylis, 49 shrimps were moderate infested by Epistylis and 18 shrimps were infested by Zoothamnium and Epistylis.

   Based on the data obtained, the ectoparasites that are commonly found are Epistylis. this is due to the condition of the waters that support the growth of Epistylis parasites. Epistylis parasites are found in substrate waters with organic and low dissolved oxygen [5].

   The value of the degree of an infestation can be caused by environmental conditions or water quality. Vaname shrimp are animals that live on the bottom of the water. Traditional vaname shrimp maintenance ponds with soil base easily occur in the sedimentation process. The sedimentation process causes a high content of organic matter which is a good place for ectoparasite growth [6]. Vaname shrimp that live on the bottom of the water will be in direct contact with sediments, thus making it easy for this species to be infected with ectoparasites. According to [7], the parasite...
population will significantly increase in the stress condition caused by water quality. The optimum salinity that supports is 17 ppm, the optimum pH for ectoparasites to attack the host is 7.63 - 8.80 while for dissolved oxygen which supports the development of ectoparasites is 3-4 ppm.

Another cause of ectoparasite infestation is that the traditional maintenance system applied does not use water changes, so that if there is a decrease in water quality, it can make ectoparasites grow rapidly. The source of water obtained by the farmers of vaname shrimp comes from the river which is directly inserted into the maintenance pond. The absence of treatment or filtering systems that are applied make ectoparasites and other pathogenic agents easily enter maintenance ponds [6].

3.2 Histopathological observation

Histopathological changes of vaname gill were infested by ectoparasites is hyperplasia and hemorrhage. hyperplasia is characterized by increased cell nucleus in the gill tissue and hemorrhage characterized by bleeding.

Scoring results were obtained from observing histopathological of normal vaname gill shrimp and infected by ectoparasites. Scoring results of 0 or the absence of ectoparasite infestations in the vaname shrimp gills; the results of scoring 1 or the light category were obtained with a percentage change of less than 30% and for the score 2 that is being obtained by the percentage of histopathological changes in the gill of shrimp vaname by 30-70%. Scoring 0, scoring 1 and scoring 2 can be seen in Figure 2

Figure 2. Histopathological changes of vaname shrimp gills (Litopenaeus vannamei) which were infested by ectoparasites (1000x) (a) scoring 0, (b) scoring 1, (c) scoring 2. A: Normal gill, B: Hyperplasia, C: Haemorrhage

Correlation analysis between the degree of ectoparasite infestation and changes in the vaname gill histopathology that were infected by ectoparasites was carried out using the Pearson Correlation, which showed a positive correlation with an R-value of 0.926. A positive correlation means that an increase in one variable will be followed by an increase in another variable. Any increase in the degree of infestation of ectoparasites in vaname shrimp will be followed by an increase in the score of histopathological changes in vaname shrimp.

From the results of this study obtained mild and moderate scoring on the changes in vaname gill histopathology that were infected by ectoparasites, this is caused by the number of parasites that infest each shrimp gill is different according to the degree of infestation of the ectoparasites. From the results of the vaname gill histopathology scoring that was infected with ectoparasites showed that the gills of the vaname shrimp had hemorrhage and hyperplasia. At this observation, the presence of red blood cells in the tissue caused by the release of blood cells from blood vessels. Hemorrhagic indicates the release of blood from blood vessels, both out of the body and into body tissues [8]. Hemorrhagic can occur due to ectoparasite infestation when Zoothamnium attaches to vaname shrimp, the ectoparasites will release specific substances that can cause hemorrhage [2]. Hyperplasia is the increase of the cell nucleus in the gill. Hyperplasia is caused by Epistylis which infest the surface of the gills. Epistylists use host cells that are released as the source of their nutrition. Optimal water quality in the growth of
Epistylis can cause hyperplasia of the gills and skin of the host as well as an increase in loose of epithelial cells [9].

The correlation between the degree of infestation and histopathological changes showed a positive correlation of 0.926. Then it can be concluded that in each increase in the degree of infestation of ectoparasites in vaname shrimp followed by an increase in the histopathological change scoring in vaname shrimp. According to [10], a perfect positive correlation is obtained if the value of r reaches +1. A positive correlation means that an increase in one variable will be followed by an increase in another variable.

Other parameters that support the growth of ectoparasites are water quality from vaname shrimp pond waters. Water quality has an important role in the life and growth of shrimp [11]. From the results of measurement of water quality in Table 1 shows that the temperature in vaname shrimp ponds has poor water quality because the method of shrimp maintenance is still using traditional methods.

High temperatures in shrimp farms reached 31.3-31.70°C. The high temperature in maintenance ponds causes increased growth of ectoparasites. [12] said that the optimal temperature for ectoparasite growth ranged from 28-310°C. One of the water quality parameters that support the growth of ectoparasites is dissolved oxygen (DO) [5]. [13] said that the optimum dissolved oxygen level for vaname shrimp 3 - 7,5 ppm, and pH with a value of 8 [14], nitrite <1 ppm and ammonia <0.1 ppm.

### Table 1. Water quality measurement

| Water Quality Parameters | Temperature (°C) | pH | DO (ppm) | Salinity (ppt) |
|--------------------------|------------------|----|----------|----------------|
|                          | 32               | 8  | 3,4      | 18             |

4. Conclusion

This research found haemorrhagi and hyperplasia on vaname gill histopathology which was infected by ectoparasites and there was a positive correlation (r = 0.926) between infestation of vaname shrimp ectoparasites (*Litopenaeus vannamei*)

5. References

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