Fisheries Journal, 15 (1), 446-453 (2025)
http://doi.org/10.29303/jp.v15i1.1434

HISTOPATHOLOGIC FEATURES OF HEPATOPANCREAS INFECTED WITH *Vibrio parahaemolyticus* IN VANNAMEI SHRIMP (*Litopenaeus vannamei*)

Gambaran Histopatologi Hepatopankreas yang Terinfeksi *Vibrio parahaemolyticus* Pada Udang Vannamei (*Litopenaeus vannamei*)

Avira Salsabila Wijaya*, Era Insivitawati, Budi Sugianti

Fisheries Pathology Handling Engineering Study Program, Sidoarjo Marine and Fisheries Polytechnic

Buncitan Kotak Pos 1 Kecamatan Sedati, Kabupaten Sidoarjo, Jawa Timur 61253

*Coresponding author: avirasalsabilla@gmail.com

(Received February 4th 2025; Accepted February 28th 2025)

ABSTRACT

Shrimp farming faces a major problem, which is the disease caused by Vibrio parahaemolyticus infection, leading to vibriosis and damage to the shrimp's hepatopancreas. This study aims to analyze the pathological changes that occur in the tissues and organs of vannamei shrimp due to Vibrio parahaemolyticus infection using histopathological testing methods. The research findings show that this bacterial infection causes damage to the hepatopancreas, including hemocyte infiltration, melanization, sloughing, necrosis, and bacterial colonization. This damage is caused by toxins produced by Vibrio parahaemolyticus, leading to the degeneration of cells and tissues. This study provides important information that can be used to develop prevention and treatment strategies for diseases in vannamei shrimp farming, as well as enhance understanding of the impact of bacterial infection on the hepatopancreas organ of shrimp.

Keywords: Histopathology, Vannamei Shrimp, Vibrio parahaemolyticus

ABSTRAK

Budidaya udang dihadapkan pada masalah utama, yaitu penyakit yang disebabkan oleh infeksi *Vibrio parahaemolyticus*, yang dapat menyebabkan vibriosis dan kerusakan pada hepatopankreas udang. Penelitian ini bertujuan untuk menganalisis perubahan patologis yang terjadi pada jaringan dan organ udang vannamei akibat infeksi *Vibrio parahaemolyticus* menggunakan metode uji histopatologi. Hasil penelitian menunjukkan bahwa infeksi bakteri ini menyebabkan kerusakan pada hepatopankreas, termasuk infiltrasi hemosit, melanisasi, sloughing, nekrosis, dan kolonisasi bakteri. Kerusakan ini disebabkan oleh toksin yang diproduksi oleh bakteri *Vibrio parahaemolyticus,* yang mengarah pada peluruhan sel dan jaringan. Penelitian ini memberikan informasi penting yang dapat digunakan untuk mengembangkan strategi pencegahan dan pengobatan penyakit pada budidaya udang vannamei, serta meningkatkan pemahaman tentang dampak infeksi bakteri pada organ hepatopankreas udang.

Kata Kunci: Histopatologi, Udang Vannamei, *Vibrio parahaemolyticus*

INTRODUCTION

Vannamei shrimp is one of the fishery commodities that is widely cultivated by the Indonesian people because the demand is quite high. Vannamei shrimp has several advantages such as being more resistant to disease attacks and having a high level of productivity (Ariadi et al., 2021). In addition, other advantages of vannamei shrimp are rapid growth, tolerance to water temperature, high productivity levels, the presence of disease-free seeds, and low protein feed requirements (Putri et al., 2020). In 2021, Indonesia was the 2nd largest fishery product exporter in Southeast Asia. Shrimp contributed 21.29 percent of Indonesia's fishery product exports in terms of tonnage and 40.37 percent in terms of value (FAO, 2023). The largest production of vannamei shrimp in Indonesia is in the province of West Nusa Tenggara (NTB), which is 160,000 tons. Meanwhile, in East Java, the value of vannamei shrimp production is 120,000 tons (Edwin, 2022). The increasing volume of shrimp exports is also in line with the increasing shrimp production every year.

Along with the increase in shrimp production, vannamei shrimp farming faces various challenges. Lack of understanding of farmers regarding good farming practices, such as excessive stocking density, high salinity levels, poor water quality, and inadequate pond preparation, can trigger the emergence of diseases in shrimp (Sarah et al., 2018). Shrimp disease is a crucial problem for farmers and hampers the development of shrimp farming (Selvin et al., 2015). Poor water quality creates favorable conditions for Vibrio spp. to infect vannamei shrimp. One of the common diseases that attacks farmed shrimp is vibriosis. Bacteria from the genus Vibrio are pathogens that are often found in marine and brackish water environments. Sarjito et al. (2016) explained that Vibrio bacteria are the main cause of vibriosis and can cause death in farmed shrimp and marine fish. Vibrio can be found in various marine organisms, both vertebrates and invertebrates, aquatic plants, sediments, or living freely in waters (Chase et al., 2015).

Vibrio spp. have high adaptability, strong resistance, and are able to survive in aquatic environments even in extreme conditions (Vezzulli et al., 2015). One type of deadly disease caused by a specific strain of *Vibrio* spp. is infection by *Vibrio parahaemolyticus*. This bacteria has a pVA1 plasmid that carries the genes encoding the Pir-like toxins ToxA (PirA) and ToxB (PirB). The presence of this toxin is characterized by damage to the hepatopancreas (Sirikharin et al., 2015). In detecting disease in vannamei shrimp, histopathology test methods can be used. Histopathology aims to identify cell or tissue damage due to bacterial infection. According to Sarah et al. (2018), changes in shape or structure in the body parts of infected shrimp are often difficult to observe with the naked eye. These changes can only be seen clearly if the shrimp body tissue is observed using a microscope. Therefore, histopathology tests are very sensitive and important parameters in determining changes in cell or tissue structure that occur in the internal organs of shrimp.

The purpose of this study was to analyze and identify pathological changes that occur in shrimp tissues and organs due to *Vibrio parahaemolyticus* infection. This study is important because vibriosis is one of the main problems in vannamei shrimp cultivation. Vibriosis can cause damage to the shrimp hepatopancreas, which is important for digestion and metabolism, and can cause death in shrimp. Understanding the impact of this bacterial infection at the tissue and cell levels will greatly assist in the development of effective prevention and treatment strategies.

METHODS

Time and Place

This research was conducted on September 21 - December 13, 2024 at PT. Central Proteina Prima, Sidoarjo Regency, East Java Province.

Tools and Materials

The tools and materials used in histopathology testing are vannamei shrimp, microscope, davidson, xylene, 96% reintz ethanol, absolute ethanol, 37% HCL, 100% acetic acid, 25% ammonia solution, distilled water, 96% technical alcohol, formalin, eosin, hematoxyline, APS dodecahydrate, entellan, coverglass, mercury oxide, cutting board, tweezers, blade, petri dish, scissors, microtome, waterbath, slide warmer, tissue processor, tissue embedding, tissue staining, erlenmeyer, magnetic stirrer, measuring cup, beaker glass, hot plate, cassettes.

Research Methods

This research was conducted using a case study method, namely observing the hepatopancreas organ of whiteleg shrimp (*Litopenaeus vannamei*) which showed histopathology or changes in cell and tissue structure.

Research Procedure

The diagnostic procedure for histopathology testing includes several stages of the process, namely, fixation, processing, embedding, sectioning, staining, mounting, and reading histopathology slides. According to Prasetyani (2017), the preparation of histology preparations can be carried out based on the following procedures: fixation, dehydration, clarification, impregnation, blocking, block cutting, floating and coloring. Whiteleg shrimp samples were fixed using Davidson's fixative solution. The sample was injected with 0.1 to 10 ml of Davidson's fixative solution depending on the size of the whiteleg shrimp in the lateral part of the hepatopancreas, the anterior area of the hepatopancreas and the lateral abdomen in segments 1,3 and 6. After the fixation process, store at room temperature for 3 x 24 hours. Next, the sample is processed and embedded using paraffin. The next stage is sectioning with a thickness of 4 - 5 µm. The sample is then stained using Hematoxyline and Eosin and observed using a microscope.

Test Parameters

The main parameters in this study include histology and histopathology of the hepatopancreas organ of vannamei shrimp (*Litopenaeus vannamei*).

Data Analysis

Data analysis is the process of systematically searching for and compiling the data obtained. This study includes observation, interviews, documentation, literature studies, and conducting direct research on histopathological testing of vannamei shrimp (*Litopenaeus vannamei*) at PT. Central Proteina Prima, Sidoarjo Regency, East Java Province.

RESULTS

Based on the results of the study, it shows a normal histological picture for the hepatopancreas in vannamei shrimp (Figure 1) and the histology of the hepatopancreas that has tissue damage (Figure 2, 3, 4 and 5). The hepatopancreas consists of many tubules, with the condition of the tubules still filled with lipid droplets (fat granules), has a uniform shape, there is a lumen or hollow part in the tubule, and the epithelial cells are perfectly attached. Microscopically with a magnification of 400x, the histological picture of normal shrimp hepatopancreas shows that the epithelial cells of the hepatopancreas tubules consisting of E cells, F cells, R cells and B cells (Dwiono, 2018) are normal and the hepatopancreas lumen is normal. Tissue damage observed in the research sample showed hemocyte infiltration, melanization, sloughing, necrosis and bacterial colonization.

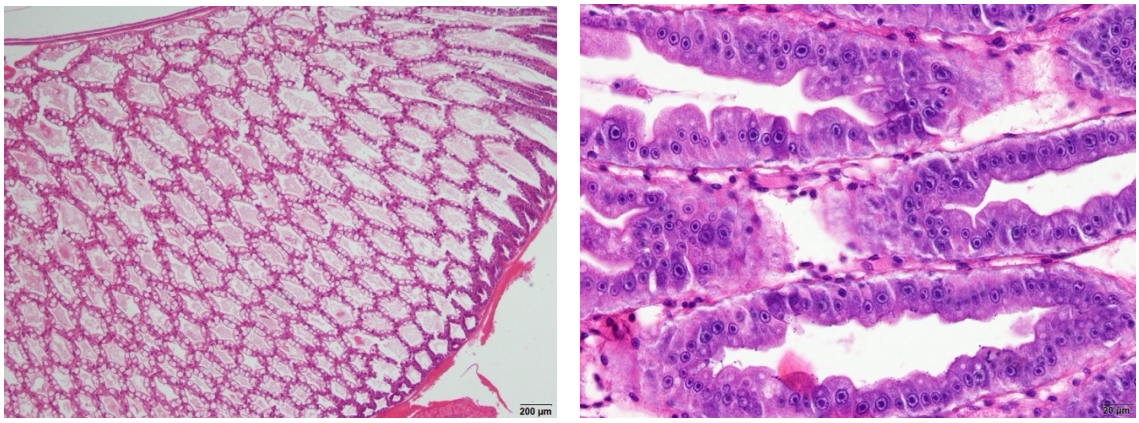

Figure 1. Histology of Hepatopancreas

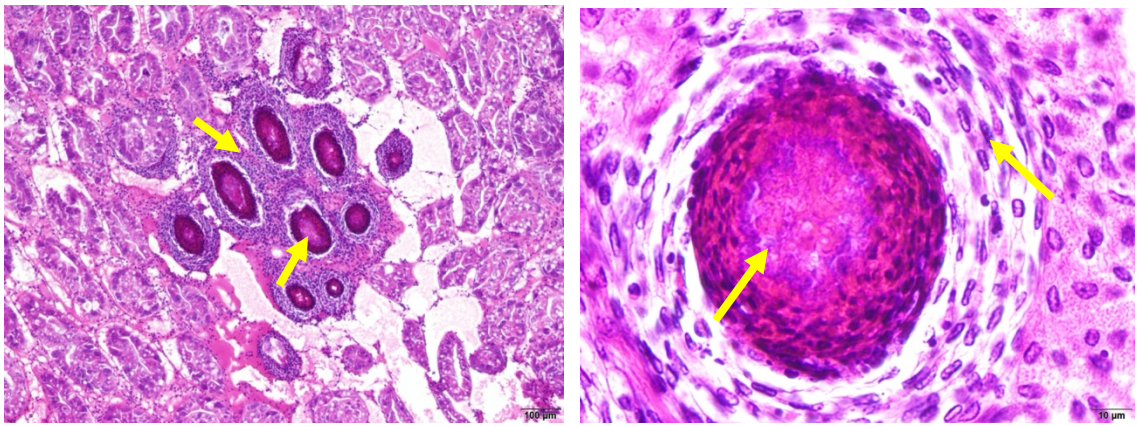

Figure 2. Hemocyte Infiltration and Melanization

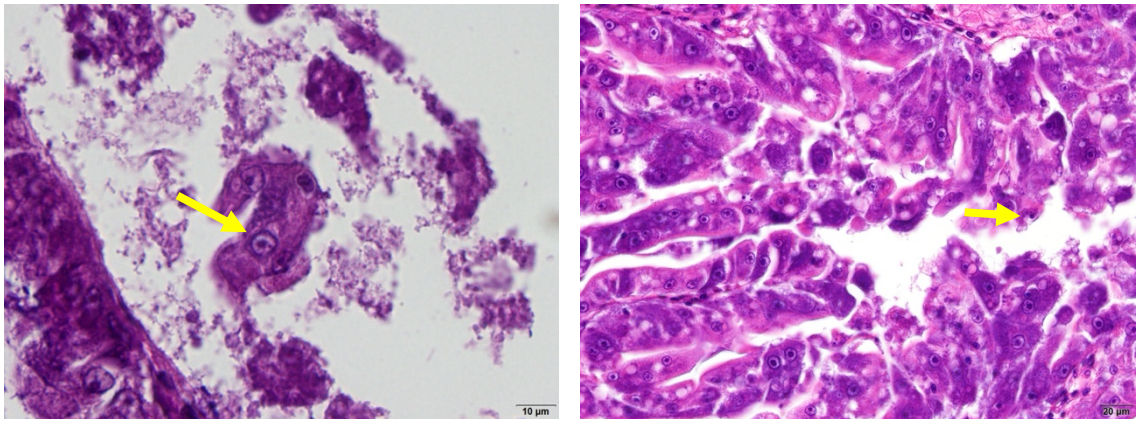

Figure 1. Sloughing

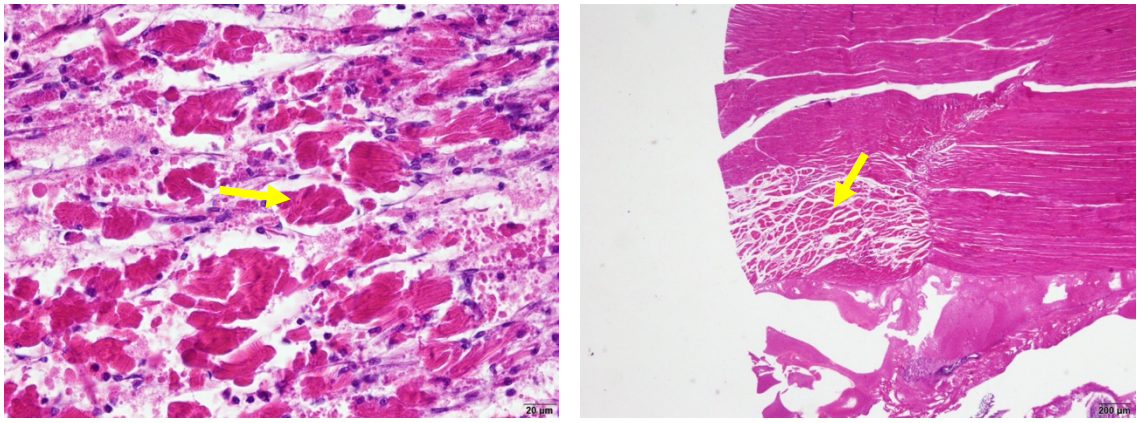

Figure 4. Necrosis

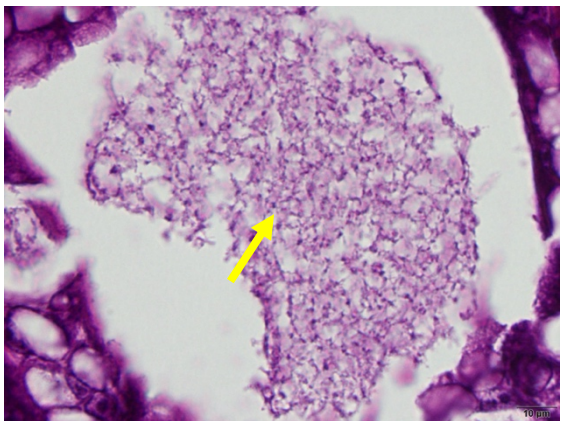

Figure 5. Bacterial Colonization

DISCUSSION

Epithelial cells consist of four types of cells, namely E (embryonic) cells, F (fibrillar) cells, B (blister) cells and R (reabsorption) cells (Figure 1). The top of the tubule contains undifferentiated embryonic (Embryonalzellen) or E cells. Moving away from the top, the cells begin to differentiate into absorbing, storage (Restzellen) or R cells. The F cells furthest from the tubular top are more basophilic and larger than those closest to the top. The nuclei of F cells are larger than those of R cells and usually contain a single promotive nucleolus. The cytoplasm of R cells typically contains numerous vacuoles. The luminal surface of all cells has a brush border of microvilli. B cells usually have a very large convex luminal surface. B cells are sometimes seen to be binucleate, with the nucleus or nuclei shifted peripherally to the basal region of the cell.

Based on the research results, the damage that occurs to the hepatopancreas organ is characterized by the presence of hemocyte infiltration, melanization, sloughing, necrosis and bacterial colonization. Khusnah, et al., (2023) stated that shrimp infected with *Vibrio parahaemolyticus* showed tissue damage that occurred in the hepatopancreas. The damage that occurs in the hepatopancreas is cell decay, cell infiltration, tissue necrosis and the presence of bacterial masses. The hepatopancreas also experiences melanization and hemocyte infiltration (Figure 2). Melanization is characterized by the presence of dark pigment due to pathogens. Hemocyte infiltration can occur as a response caused by pathogens. According to Oktaviana and Febriani (2019) hemocytes are one of the defenses in shrimp that are responsible for phagocytosis, nodulation, and encapsulation. The high number of hemocytes indicates a good level of shrimp health.

Observations in Figure 3 show sloughing, which is a condition where epithelial cells are released from the hepatopancreas tubules. Manan et al. (2015) explained that healthy hepatopancreas has E, B, and R cell structures and fixed and unchanged tubular lumen. In addition to sloughing, necrosis is also observed in the hepatopancreas (Figure 4). Necrosis, which is cell and tissue death due to irreversible degeneration (Berata et al., 2015), often accompanies diseases caused by Vibrio species. Cells that experience necrosis will be damaged and disappear. The results of this study indicate that necrosis is characterized by abnormalities due to cell death that cause shrinkage and changes in muscle shape. This is in line with the opinion of Putri et al. (2015) who stated that necrosis is acute cell damage that can occur locally or widely, causing tissue to lose its original shape due to shrinkage or shrinkage of the nucleus. Necrosis can be triggered by various biological agents such as viruses, bacteria, fungi, and parasites. In the case of *Vibrio parahaemolyticus* infection, the bacteria that cause AHPND damage the hepatopancreas and release the toxic proteins Pir-like ToxA (PirA) and ToxB (PirB) which cause severe damage and necrosis of the tubular epithelial cells (Lai et al., 2015).

Bacterial colonization is widespread in the hepatopancreas filling the lumen of the tubules (Figure 5). This is possible because in addition to the hepatopancreas being the target organ of *Vibrio parahaemolyticus*, the bacteria enter through wounds in the hepatopancreas or from a contaminated environment. *Vibrio parahaemolyticus* has the ability to attach to hepatopancreas cells using phili. After attaching, *Vibrio parahaemolyticus* will begin to multiply and form colonies in the hepatopancreas tissue. Bacterial infections, especially in the hepatopancreas, occur due to poor water quality and bacterial activity (Sukarno, 2016).

CONCLUSION

Based on the research conducted, it can be concluded that *Vibrio parahaemolyticus* infection in vannamei shrimp causes significant damage to the hepatopancreas. This damage includes hemocyte infiltration as an immune response, melanization due to dark pigments of pathogens, sloughing or detachment of tubular epithelial cells, necrosis or cell and tissue death, and extensive bacterial colonization in the tubular lumen. Necrosis is caused by *Vibrio parahaemolyticus* which produces toxic proteins Pir-like ToxA (PirA) and ToxB (PirB), causing shedding and necrosis of tubular epithelial cells. Extensive bacterial colonization in the hepatopancreas occurs because this organ is the main target of *Vibrio parahaemolyticus*, and bacteria can enter through wounds or contaminated environments. Poor water quality factors and bacterial activity also play an important role in this infection.

ACKNOWLEDGEMENT

The authors would like to thank the Sidoarjo Marine and Fisheries Polytechnic and PT. Central Proteina Prima, Sidoarjo Regency, East Java Province, who have helped facilitate the permits and use of the laboratory for this research.

REFERENCES

Ariadi, H., Wafi, A., Supriatna, S., & Musa, M. (2021). Tingkat difusi oksigen selama periode blind feeding budidaya intensif udang vaname (*Litopenaeus vannamei*). Rekayasa, 14(2), 152-158. https://doi.org/10.21107/rekayasa.v14i2.10737

Berata I Ketut, Winaya Ida Bagus Oka, Adi Anak Agung Ayu Mirah, Adnyana Ida Bagus Windia. (2015). Patologi Veteriner Umum Cetakan ketiga. Penerbit Swasta Nulus. Denpasar.

Chase, E., Young, S., and Harwood, V. J. (2015). Sediment and vegetation as reservoirs of *Vibrio vulnificus* in the Tampa Bay Estuary and Gulf of Mexico. Applied and Environmental Microbiology, 81(7): 2489-2494. https://doi.org/10.1128/AEM.03243-14

Dharmawan, M. R. M. B., Julyantoro, P. G. S., & Sari, A. H. W. (2020). Profil histologi hepatopankreas udang vanamei *(Litopenaeus vanamei)* dikultur dengan padat tebar berbeda. Current Trends in Aquatic Science, 3(1), 81-87.

Dwiono, A., Widigdo, B., & Soewardi, K. (2018). Pengaruh komposisi mineral air tanah terhadap fisiologi dan histologi udang vaname *Litopenaeus vannamei*. Jurnal Ilmu dan Teknologi Kelautan Tropis, 10(3), 535-546. https://doi.org/10.29244/jitkt.v10i3.21049

Edwin, Y. S. (2022). *Shrimp Farming Production in Indonesia*.

FAO. (2023). *Fishery and Aquaculture Statistics. Global Fisheries commodities production and trade* 1976-2021.

Khusnah, A., Satyantini, W. H., & Amin, M. (2023). Studi Perbandingan Histopatologi Udang Vaname yang Terinfeksi *Vibrio parahaemolyticus* dari Tiga Tambak Berbeda. Grouper: Jurnal Ilmiah Perikanan, 14(2), 144-151. https://doi.org/10.30736/grouper.v14i2.212

Lai, H. C., Ng, T. H., Ando, M., Lee, C. Te, Chen, I.T., Chuang, J. C., Mavichak, R., Chang, S. H.,Yeh, M. De, Chiang, Y. A., Takeyama, H. ,Hamaguchi, H. o., Lo, C. F., Aoki, T., & Wang,H. C. (2015). Pathogenesis of *acute hepatopancreatic necrosis disease* (AHPND) in shrimp. Fish and Shellfish Immunology, 47(2), 1006-1014. https://doi.org/10.1016/j.fsi.2015.11.008

Manan, H., J. M. Zhong., F. Othman., and M. Ikhwanuddin. 2015. Histopathology of the Hepatopancreas of Pacific White Shrimp Penaeus vannamei from None *Early Mortality Syndrome* (EMS) Shrimp Ponds. Journal of Fisheries and Aquatic Science. Vol 10(6): 562-568 hlm. https://doi.org/10.3923/jfas.2015.562.568

Oktaviana, A. & Febriani, D. (2019). Jumlah Hemosit Total Pada Udang Vaname (*Litopenaeus vannamei*) yang Diberikan Tambahan Tepung Batang Pisang Pada Pakan. jurnal Perikanan, 9(2): 188-193. https://doi.org/10.29303/jp.v9i2.169

Prasetyani T. 2017. Gambaran Mikroskopis Histologi Bloksel Efusi Pleura dengan Menggunakan Fiksasi Alkohol 70% dan NBF 10% pada Pewarnaan HE. Karya Tulis Ilmiah. Universitas Muhammadiyah Semarang.

Putri, D. S., Affandi, M. I., & Sayekti, W. D. (2020). Analisis kinerja usaha dan risiko petambak udang vaname pada sistem tradisional dan sistem semi intensif di Kecamatan Labuhan Maringgai Kabupaten Lampung Timur. JIIA (Jurnal Ilmu-Ilmu Agribisnis), 8(4), 625-632. https://doi.org/10.23960/jiia.v8i4.4707

Putri, A. M., Prayitn, S. B., & Sarjito. (2015). Perendaman Berbagai Dosis Ekstrak Daun Bakau (*Rhizophora apiculata*) untuk Pengobatan Kepiting Bakau (*Scylla serrata*) yang Diinfeksi Bakteri *Vibrio Harveyi*. *Journal of Aquaculture Management and Technology,* 4(4), 141-149.

Sarah, H., S. B. Prayitno dan A. H. C. Haditomo. (2018). Studi kasus keberadaan penyakit IMNV (*Infection Myonecrosis Virus*) pada udang vaname (*Litopenaeus vannamei*) di pertambakan Pekalongan, Jawa Tengah. Jurnal Sains Akuakultur Tropis. 2 (1): 66-77.

Sarjito, S., Apriliani, M., dan Haditomo, A.H.C. 2016. Keanekaragaman agensia penyebab vibriosis pada udang vaname *(Litopenaeus vannamei)* dan sensi-tivitasnya terhadap antibiotik. Journal of Aquaculture Management and Technology. 5(1):98-107

Selvin, J., Ninawe, A., Ramu, M., & Kiran, S. (2015). *Control of Pathogenic Vibrios in Shrimp Aquaculture using Antiinfectives from Marine Natural Products. Conference Papper*. 102-141.

Sirikharin,R.,Sanguanrut,P.,Thitamadee, S.,Sritunyalucksana, K.,Taengchaiyaphum,S., & Flegel, T. W. (2015). AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. Aquaculture Reports, 2, 158-162. https://doi.org/10.1016/j.aqrep.2015.10.002

Sukarno, T. S. R. 2016. Studi Histopatologi Hepatopankreas Udang Vanname (*Litopenaeus vannamei*) yang Terinfeksi Penyakit WFS (*White Feses Syndrome*) di Desa Pandangan Kulon, Kecamatan Kragan, Kabupaten Rembang, Jawa Tengah. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Universitas Brawijaya. Malang

Vezzulli, L., Pezzati, E., Stauder, M., Stagnaro, L., Venier, P., and Pruzzo, C. (2015). Aquatic ecology of the oyster pathogens *Vibrio splendidus* and *Vibrio aestuarianus*. Environmental Microbiology, 17(4): 1065-1080. https://doi.org/10.1111/1462-2920.12484

Widanarni, W., Sukenda, S., & Septiani, G. R. (2016). Aplikasi Sinbiotik Untuk Pencegahan Infeksi Infectious Myonecrosis Virus pada Udang Vaname (Litopenaeus vannamei). Jurnal Kedokteran Hewan, 10(2): 121-127. https://doi.org/10.21157/j.ked.hewan.v10i2.5041