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The presence of disease in growing spiny lobster *Panulirus* spp. in floating net cages at Tanjung Putus Island, Lampung

Keberadaan penyakit pada pembesaran lobster *Panulirus* spp. di keramba jaring apung di Pulau Tanjung Putus, Lampung, Indonesia

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ABSTRACT

Spiny lobster is a member of crustaceans that has high economic value. The demand for export markets in Asian, European and American countries as well as locally, is quite high with an expensive selling price. However, there are obstacles that often occur in spiny lobster rearing cultivation, namely infectious diseases that will cause high mortality. The purpose of this study was to investigate infectious diseases in rearing spiny lobster *Panulirus* spp. that were kept in floating net cages at Tanjung Putus, Lampung. The research method involved sampling five spiny lobsters in floating net cages, observation of clinical symptoms, bacterial isolation, identification of bacteria, detection of Milky Hemolymph Disease of Spiny Lobster (MHD-SL) through polymerase chain reaction (PCR) analysis and histopathology. The sampling location was at PT. Saibatin Perikanan Indonesia. The bacteria *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were detected. Five lobsters were infected by MHD-SL, characterized by milky white hemolymph. All five lobster samples were positively infected with Milky Hemolymph Disease of Spiny Lobster (MHD-SL) through PCR analysis. Histopathological observations showed pathological microanatomical changes in the lobster hepatopancreas tissue, indicating that the tissue changes, in the form of encapsulation and infiltration were due to Rickettsia-like bacteria (RLB) infection. The management of spiny lobster stocking density, maintenance biosecurity, and increased lobster immunity must be implemented in order to prevent MHD-SL disease in floating net cages.

Keywords: bacteria, histopathology, MHD-SL, *Panulirus* spp.

ABSTRAK

Lobster merupakan anggota *crustacea* yang memiliki nilai ekonomis yang tinggi. Permintaan pasar ekspor pada negara Asia, Eropa, dan Amerika maupun lokal cukup tinggi dengan nilai jual yang mahal. Akan tetapi, terdapat kendala yang sering terjadi pada budidaya pembesaran lobster yaitu infeksi penyakit yang akan menyebabkan tingginya mortalitas Tujuan penelitian ini adalah untuk menginvestigasi penyakit infeksi pada pembesaran lobster *Panulirus* spp. yang dipelihara di keramba jaring apung Tanjung Putus, Lampung. Metode penelitian ini meliputi pengambilan sampel lobster sebanyak lima ekor dalam karamba jarring apung (KJA), pengamatan gejala klinis, isolasi bakteri, identifikasi bakteri, deteksi *Milky Hemolymph Disease of Spiny Lobster* (MHD-SL) melalui analisis *Polymerase Chain Reaction* (PCR), dan histopatologi. Lokasi pengambilan sampel di PT. Saibatin Perikanan Indonesia, Lampung. Terdapat bakteri *Vibrio parahaemolyticus* dan *V. alginolyticus*. Lima ekor lobster terinfeksi oleh MHD-SL yang ditandai dengan hemolim berwarna putih susu. Lima sampel lobster positif terinfeksi penyakit *Milky Hemolymph Disease of Spiny Lobster* (MHD-SL) melalui analisis PCR. Hasil pengamatan histopatologi terdapat perubahan mikroanatomi patologi pada jaringan hepatopankreas lobster menunjukkan bahwa adanya perubahan jaringan berupa enkapsulasi dan infiltrasi karena adanya infeksi *Rickettsia-like bacteria* (RLB). Pengelolaan kepadatan penebaran lobster, biosekuritas pemeliharaan, dan peningkatan imunitas lobster harus ditingkatkan untuk mencegah penyakit MHD-SL di KJA.

Kata kunci: bakteri, histopatologi, MHD-SL, *Panulirus* spp.

INTRODUCTION

Spiny lobster is a member of crustaceans that has high economic value. Export market demand in Asian, European, and American countries as well as locally is quite high with a high selling price. In 2020 there are 12 provinces in Indonesia that are lobster producers with a total production of 206.7 tons. The largest volume was produced by West Nusa Tenggara Province of 68.01 tons while the lobster production volume of Lampung Province was 13.71 tons (KKP, 2020). Spiny lobster farming in Indonesia is mostly by utilizing resources derived from fishing activities.

Sustainable fishing activities can cause lobster availability in the wild to decrease. The availability of spiny lobster stocks in nature affects fishing activities to be cultivated in floating net cages (KJA) (Pranata *et al*., 2017). In addition, there are seed export activities that continue to increase so that seed stocks in nature decrease (Anggraini *et al*., 2021). Six types of spiny lobsters found in Indonesia are *P. homarus*, *P. longipes*, *P. penicillatus*, *P. versicolor*, *P. polyphagus*, and *P. ornatus*. Intensive lobster fishing activities and poor fisheries management can threaten the sustainability of lobster in nature (Damora *et al*., 2018; Suman *et al*., 2019).

Among several species of lobster, sand lobster *P. Homarus,* batik lobster *P. longipes*, and pearl lobster *P. ornatus* are species that are more cultivated than other species (Setyanto *et al*., 2018). One of the provinces that has potential in the development of lobster cultivation is Lampung. The lobster potential is in the waters of South Lampung, Pesawaran, Tanggamus, and West Coast which are included in the State fisheries management area of the republic of Indonesia (WPPNRI) 572 (Diah *et al*., 2018). However, there are still many challenges in lobster rearing cultivation that are often found in the field, including disease, feed availability, and a relatively long maintenance period (Susanti *et al*., 2017). Diseases in spiny lobster farming in general are divided into two groups, namely non-infectious and infectious diseases (Trisnayanti *et al*., 2019).

Non-infectious diseases are diseases that are not caused by infectious processes and are not contagious. In general, it is caused by physical, chemical and biological environmental conditions, genetics, poor management of cultivation activities so that it will cause toxic compounds due to contaminants. Infectious diseases can be caused by pathogenic organisms that are able to spread through the movement of infected hosts (Sianturi & Lestari, 2022). Diseases that generally occur in lobster farming can be caused by bacteria, parasites, fungi, and viruses (Andrykusuma *et al*., 2022). Spiny lobster farming with high mortality is caused by cannibalism, disease infection, transportation stress, water quality, and maintenance management that is not yet standardized. Some types of diseases in spiny lobsters include milky hemolymph disease, red body disease, black gill disease, red tail disease, and WSSV (white spot syndrome virus) (Clark *et al*., 2013).

Losses due to disease affect environmental conditions decrease, resistance to disease and mass mortality (Haryanti *et al*., 2017). Bacterial diseases in spiny lobster farming can cause high mortality and large-scale losses are bacterial infections of milky hemolymph disease of spiny lobster (MHD-SL) (OIE, 2008). The productivity of spiny lobster farming will decrease if there is mass deaths due to disease attacks, so efforts are needed to improve spiny lobster disease control in the field (Prasetya & Hasidu 2021). Diseases caused by bacterial infections can appear and be detrimental in lobster farming (Radhakrishnan & Kizhakudan, 2019). Knowledge of diseases that arise in spiny lobster culture is essential so that disease management can be carried out effectively and efficiently and increase opportunities for good cultivation of lobster commodities. This study aims to investigate infectious diseases in growing spiny lobsters *Panulirus* spp. reared in floating net cages in Tanjung Putus Island, Lampung.

MATERIALS AND METHODS

Time and place

The research was conducted from October to December 2021 at PT. Saibatin Perikanan Indonesia, Lampung RW 06, Tanjung Putus, Sukarame Village, Punduh Pedada District, Pesawaran Regency. The test were carried out at the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

Floating net cages and spiny lobster rearing

Spiny lobster (*Panulirus* spp.) rearing is carried out in cultivation containers in the form of floating net cages (KJA) measuring 3×3×4 m. The KJA used is made of wood and nets. The stocking density of lobsters in KJA was 5 ind/

m^2 with a weight measurement of 171.32 ± 12.63 g. Feeding is done once a day at 15.00. The feed given is in the form of trash fish that comes from fishermen's catches. Measurement of the quality parameters of lobster culture water ranged from 28–31°C, salinity 29–35 mg/L, pH 7.7–8.2 and dissolved oxygen 3.4–9.1 mg/L.

Sampling and sample collection

A total of five spiny lobsters were taken from the KJA. Sampling was carried out by observing spiny lobsters that showed clinical symptoms of contracting the disease. Initial observations were made through the color of the gills and body of the spiny lobster as an indicator of disease infection. Furthermore, the spiny lobsters were dissected and observed the condition and color of the hepatopancreas and muscles. Then the target organ hepatopancreas was collected for bacterial analysis and the presence of Rickettsia-like bacteria (RLB) in the spiny lobsters using PCR.

Clinical symptoms

Observations were made on five spiny lobsters with clinical symptoms observed in the behavior of spiny lobsters tending to be inactive, decreased appetite, and there were several lobsters that experienced death. Observation of the morphological condition of the lobster was carried out by observing the external organs (physical condition) and the condition of the lobster's internal organs to determine any signs of disease infection. Observation of the physical condition of the spiny lobsters was carried out on gills, hepatopancreas, meat, and hemolymph by looking at the condition and color of the organs, while laboratory tests were carried out on the condition of the internal organs (Sudewi *et al*., 2018).

Bacterial isolation

Isolate diseased spiny lobsters by transferring them to different floating net cages. The bacterial isolation method used is the scratch method on a Petri dish. Diseased spiny lobsters were dissected aseptically using sterile surgical instruments. The organ used for identification is the hepatopancreas. The organ is then streaked on the bacterial growth medium using aseptic techniques with an ose needle.

The media used were sea water complete (SWC) and thiosulfate citrate bilesalt sucrose agar (TCBS) in petri dishes. The scratched media was then incubated in an incubator at 27–37°C for 24 hours. Seawater bacteria were grown on SWC media while for *Vibrio* spp. grown on TCBS specific media. Purification of bacterial isolates was carried out using the streak plate method on bacterial colonies showing different morphology and color. Purification was carried out repeatedly by separating the colonies until a pure culture was obtained using the 3-stroke technique. This is meant to separate the colonies. After the pure culture is complete, the bacteria are incubated at 27–37°C for 24 hours (Fitriasari *et al*., 2020).

Bacterial characterization

The method used to identify bacteria in spiny lobsters is a conventional method involving biochemical tests which is a way to identify bacteria through their physiological and biochemical characteristics. The results of biochemical tests and gram staining were written down in test blanks and then identified manually with the help of reference book Cowan and Steel (2003). Subsequently, testing in the form of an API KIT (analytical profile index) determines the species of five pure cultured bacterial isolates. Identification of bacteria was carried out by isolation from the target organ, namely the hepatopancreas, then incubated for 24 hours at 37°C. Then it was purified using TCBS media and incubated for 24 hours at 37°C (Widyastana *et al*. 2015).

Detection of milky hemolymph disease of spiny lobster by PCR

The organ used to identify Milky Hemolymph Disease of Spiny Lobster is the hepatopancreas with the target pathogen being Rickettsail-like bacteria (RLB) and natural feed in the form of mangrove shellfish (*Telescopium Telescopium*). Milky hemolymph disease of spiny lobster was detected using PCR amplification using primer (254 F/R) with a target of 254 bp based on OIE (Office International des Epizooties) (OIE, 2008). The primary sequence of 254F is 5'-CGA-GGA-CCA-GAG-ATG-GAC-CTT-3' and 254R is 5'-GCT-CAT-TGT-CAC-CGC-CAT-TGT-3'. DNA extraction from lobster hepatopancreas samples was carried out by adding 200 µL GT buffer in 1.5 ml microtubes and then grinding. Then 20 µL of proteinase K was added and homogenized.

The first incubation was at 60°C for 30 min. Then add 200 µL of GBT Buffer, homogenize and incubate the second at 60°C for 20 min. Then centrifuged at 14–16,000 ×g for 2 min. Add 200

µL of absolute ethanol, homogenize and transfer to a GS column placed in a 2 mL collection tube. Then centrifuge at 14–16,000 ×g for 2 min and transfer the GS column to a new 2 mL collection tube. Add 400 µL W1 Buffer to the GS column and centrifuge at 14–16,000 ×g for 30 s.

Then add 600 µL Wash Buffer to the GS column and centrifuge at 14–16,000 ×g for 30 s. Discard the flow through then centrifuge at 14–16,000 ×g for 3 min to dry the GS column. The final stage of the GS column was transferred to a new 1.5 mL microcentrifugation tube, adding 100 µL of Elution Buffer which had been heated on a hot plate. Then let stand for five min and centrifuge at 14–16,000 ×g for 30 s. The supernatant was taken and stored at 20°C until used. The PCR reaction for detection of milky hemolymph disease was red mix 5 µL, primer 254F (1 µL), primer 254R (1 µL), NFW 2 µL and DNA sample 1 µL with a total reaction of 10 µL.

Amplification was carried out under initial denaturation conditions at 96°C = 3 min, 40 doubling cycles consisting of denaturation: 96°C = 15 s, annealing: 65°C = 30 s, and extension: 72°C = 15 s, followed by final extension: 72°C= 1 min (Koesharyani *et al*., 2016). The obtained amplicon was separated with 0.2% Agarose in 20 mL TBE. In the agarose gel there are wells that are used to enter the sample to be electrophoresed. The first well was filled with 3 µL of DNA marker, the second well was a negative control adalah no-template control (NTC), samples one to six were put into the next well and positive control (+) is a sample that has been confirmed positive for infection (RLB). The electrophoresis process lasted for 40 min at 100 V to determine which lobsters were infected with milky hemolymph disease of spiny lobster.

Histopathology

Preparation of histological preparations was carried out by fixation using a fixative solution (Davidson) for 24 h. Next, the processes of dehydration, clearing, impregnation, embedding, blocking, tissue cutting, coloring and tissue gluing were carried out, referring to the method of Ihsan *et al*. (2017). The stain used was Hematoxylin-Eosin. Then the finished preparations were observed with an Olympus CX21 microscope with a magnification of 400×.

Data analysis

The research data were analyzed descriptively. Analysis of bacteria using Gram stain characterization test and biochemical physiology. Analysis of the API Kit test data using the biorieux website. Histopathological observations are performed using images to describe tissue damage under a microscope.

RESULTS AND DISCUSSION

Results

Clinical symptoms

Clinical symptoms observed in the behavior of spiny lobsters in floating net cages were weak movements and decreased appetite. There are symptoms that appear on the morphology of the spiny lobster, namely a whitish color on the stomach muscles and the hemolymph infected with the disease becomes cloudy milky white. Based on observations of the internal organs of lobsters, the results are presented in Table 1. The following is a picture of the results of identifying clinical symptoms in the abdominal organs (Figure 1), gills (Figure 2) and lobster hepatopancreas (Figure 3), from lobster samples showing healthy and unhealthy conditions.

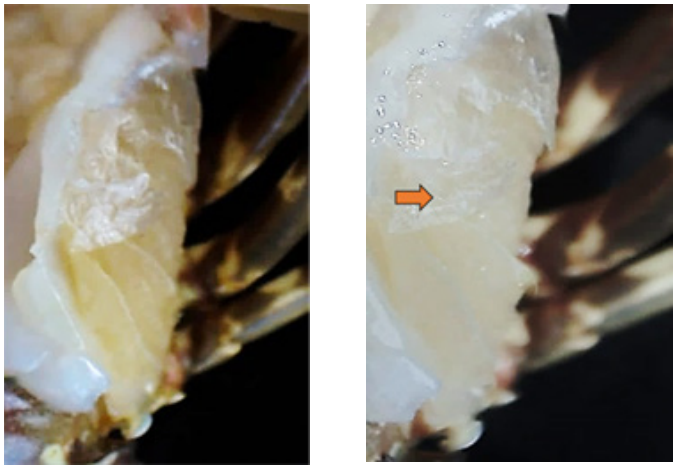
Based on the table above, it was found that the symptoms that arise in spiny lobsters infected with the disease differ depending on how severe the infection is. Spiny lobster samples 1 and 2 looked normal on the hepatopancreas, gills and muscle, while samples 3, 4 and 5 showed symptoms of severe disease. In the observation of the sample having symptoms of disease visible discoloration of the external organs (abdominal muscles) and internal organs (gills and hepatopancreas) was noted.

Table 1. Condition of the hepatopancreas, gills and lobster muscle *Panulirus* spp.

Sample	Hepatopancreas	Gill	Muscle
Lobster 1	Yellowish white	Normal	Yellowish white
Lobster 2	Yellowish white	Normal	Yellowish white
Lobster 3	Pale white	White	Pale
Lobster 4	Pale white	White	Pale
Lobster 5	Pale white	White	Pale

(a) (b)

Figure 1. Clinical symptoms in the abdominal organs of spiny lobsters (a) healthy: normal; (b) unhealthy: observed whitish color on the abdominal muscles and the hemolymph infected with the disease becomes cloudy milky white.



(a) (b)

Figure 2. Clinical symptoms in the gill organs of spiny lobsters (a) healthy: observed yellowish white on the gills; (b) unhealthy: observed pale white on the gills.

(a) (b)

Figure 3. Clinical symptoms in the spiny lobster hepatopancreas (a) healthy: observed bright yellowish color on the hepatopancreas; (b) unhealthy: observed pale white color on the hepatopancreas.

Characteristics of bacteria

Isolated bacteria from spiny lobster hepatopancreas are characterized. Testing the characteristics of bacteria with biochemical tests to determine the type of bacteria to the genus level. The results of the bacterial identification test on all samples using the biochemical method obtained one type of bacteria belonging to the genus *Vibrio* sp. Gram staining obtained negative results in the form of bacilli, fermentative, motile, catalase positive, and positive oxidase in all bacterial isolates. The following are the results of bacterial identification presented in Table 2. The following are the results of bacterial identification presented in Table 2.

Bacterial identification results using the API Kit test (Analytical Profile Index)

Bacteria that have been isolated are gram negative, so the API 20 NE (Biomerieux) kit is used to identify these bacteria. API 20 NE is used to identify bacteria by means of biochemical tests. The level of compatibility of the bacterial sample with the bacteria contained in the program database is considered valid if the match rate reaches 80% or more. The incompatibility of API 20 NE results can occur because each biochemical test has a percentage of possibilities to be positive or negative and does not have a 100% certainty of results.

The results of API 20 NE that have been inoculated and incubated are recorded and entered into the Apiweb program. The results of the API 20 NE test kit are shown by the 20 carbohydrate components that have been analyzed in the Apiweb program. Bacterial species that have been identified in spiny lobster samples 1, 2 and 4 with a match rate of 99.9%; 99.9%; and 99.7% namely *Vibrio parahaemolyticus* bacteria, while in spiny lobster samples three and five with a match rate of 99.8% namely *Vibrio alginolyticus*. Identification results of bacterial isolates using API 20 NE are presented in Table 3.

PCR test results

The results of testing hepatopancreas and natural feed samples using PCR with specific primers formed DNA bands at 254 bp in size. The appearance of this DNA band indicated that all spiny lobster samples identified milky hemolymph disease caused by rickettsia-like bacteria. Lobster one and two, namely samples of lobster infected with mild milky hemolymph disease, Lobster 3-5 are samples of lobster infected with severe milky hemolymph disease. Sample P is a sample of lobster feed that does not contain rickettsia-like bacteria. The results of the PCR test are shown in Figure 4.

Histopathology

Histopathological examination of the hepatopancreas and gills of spiny lobsters. Changes in microanatomical pathology of the spiny lobster hepatopancreas tissue indicate changes in the tissue on the inside of the lumen, namely the tubule in the form of hemocyte infiltration and encapsulation (Figure 5). It was observed that the gill organs in the primary lamellae had hypertrophy of the connective tissue (Figure 6) infected with rickettsia-like bacteria. The observed damage to the hepatopancreas and gills was due to rickettsia-like bacteria infection.

Discussion

The productivity of spiny lobster cultivation has decreased due to the death of 2-5 lobsters per day. This is presumably due to disease attacks on spiny lobsters kept in floating net cages. Diseases in spiny lobster cultivation in general are divided into two groups, namely non-infectious and infectious diseases (Trisnayanti *et al*., 2019). Infectious diseases are health problems caused by organisms, such as bacteria, viruses, fungi or parasites. The cause of disease in spiny lobsters is caused by pathogenic bacteria which are commonly known as bacterial diseases, especially bacteria from the gram-negative group (Mindar *et al*., 2017).

Table 2. Results of identification of bacterial isolates from spiny lobster samples *Panulirus* spp.

Isolates	Gram	Shape	O/F	Motility	Catalase	Oxsidase test	Genus
Lobster 1	Negatif	Rod	F	Motile	Positive	Positive	*Vibrio* sp.
Lobster 2	Negatif	Rod	F	Motile	Positive	Positive	*Vibrio* sp.
Lobster 3	Negatif	Rod	F	Motile	Positive	Positive	*Vibrio* sp.
Lobster 4	Negatif	Rod	F	Motile	Positive	Positive	*Vibrio* sp.
Lobster 5	Negatif	Rod	F	Motile	Positive	Positive	*Vibrio* sp.

Table 3. Components of the test for carbohydrates in the API 20 NE KIT.

Test	Active Ingredients	Isolates				
		Lobster 1	Lobster 2	Lobster 3	Lobster 4	Lobster 5
NO_3	potassium nitrate	+	+	+	+	+
TRP	L-tryptophane	+	+	+	+	+
GLU	D-glucose	+	+	+	+	+
ADH	L-arginine	-	-	-	-	-
URE	urea	+	+	-	+	-
ESC	esculin ferric citrate	-	-	-	-	-
GEL	gelatin (bovine origin)	+	+	+	+	+
PNG	4-nitrophenyl-βDgalactopyranoside	+	-	-	-	-
GLU	D-glucose	+	+	-	+	+
ARA	L-arabinose	+	+	-	+	-
MNE	D-mannose	+	+	-	-	-
MAN	D-mannitol	+	+	-	+	+
NAG	N-acetyl-glucosamine	-	-	-	-	-
MAL	D-maltose	+	+	+	+	-
GNT	potassium gluconate	+	+	-	+	+
CAP	capric acid	-	-	+	-	-
ADI	adipic acid	-	-	-	-	-
MLT	malic acid	+	+	+	+	+
CIT	trisodium citrate	-	-	-	-	-
PAC	phenylacetic acid	-	-	-	-	-
OX	oxidase test	+	+	+	+	+
%ID		99,9%	99.9%	99.8%	99.7%	99.8%
Spesies		*Vibrio parahaemolyticus* (Excellent Identification)	*Vibrio parahaemolyticus* (Very Good Identification)	*Vibrio alginolyticus* (Good Identification)	*Vibrio parahaemolyticus* (Good Identification)	*Vibrio alginolyticus* (Very Good Identification)

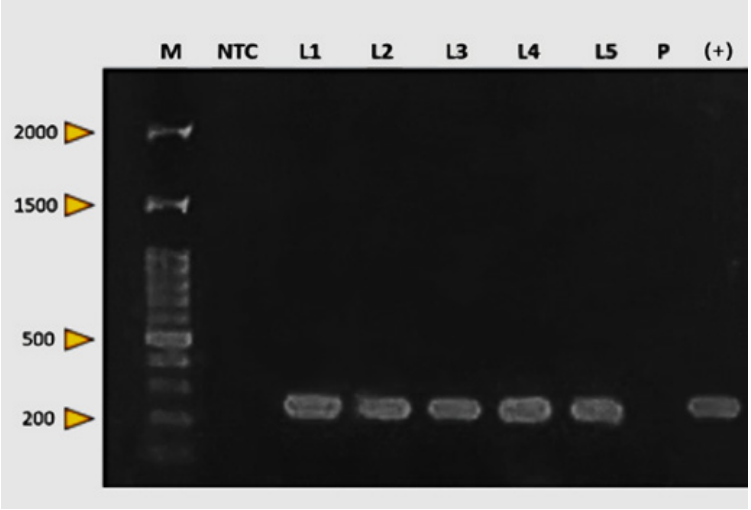
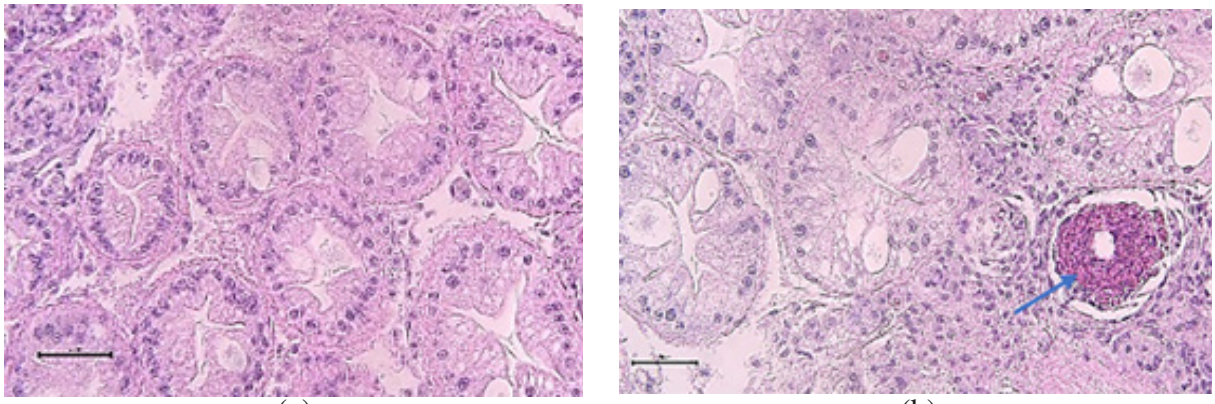


Figure 4. Detection results of milky hemolymph disease of spiny lobster (MHD-SL) M (DNA marker); NTC (no-template control); L1 (sample 1); L2 (sample 2); L3 (sample 3); L4 (sample 4); L5 (sample 5); and P (natural feed).

Clinical symptoms of spiny lobsters infected with infectious diseases are observed to be pale white in the hepatopancreas, gills, and muscle (Sudewi *et al*., 2018). Clinical symptoms caused in the study, namely there is a whitish color on the stomach muscles, decreased appetite and weak movement of spiny lobsters. Appetite observation was seen from the remaining lobster feed on anco. Unhealthy spiny lobsters show yellowish white, pale white or milky white hepatopancreas, gills and muscles. The bacterial colonies that were found in the yellow color of the study were caused by *Vibrio* spp. which can lower the pH and is able to ferment sucrose in TCBS media so that the bacterial colonies are yellow (Ihsan, 2021).

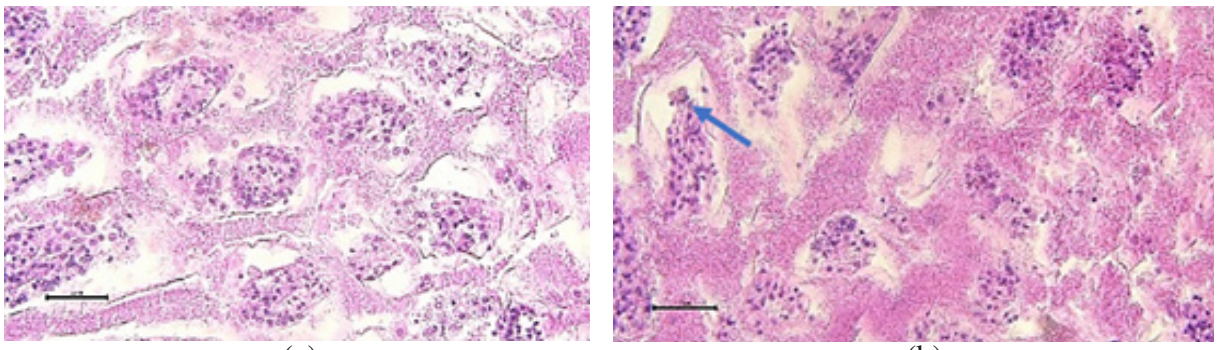
The isolated bacteria were identified using biochemical tests, including gram staining, oxidative or fermentative tests, motility tests, catalase tests, and oxidase tests (Sarjito *et al*., 2015). Gram staining aims to determine the nature of the bacteria (gram positive or gram negative) as well as the differences in cell wall structure which has a thick peptidoglycan content and shows a purple color are gram positive bacteria while gram negative bacteria contain thin peptidoglycan and show a red color (Ihsan *et al*., 2020). The bacterial isolates obtained were gram-negative in the form of short bacilli, fermentative, motile, catalase positive, and positive oxidase tests identified in the genus *Vibrio* spp as seen from Table Cowan and Steel (2003). The results of this identification are in accordance with the statement of Ihsan and Retnaningrum (2017), namely the results of identification at an early stage with biochemical tests showed that members of the genus *Vibrio* spp. including gram negative bacteria, bacilli forms, catalase positive, fermentative, and positive oxidase tests.

Vibrio bacteria is a very important pathogen causing cultivation failure. Several types of bacteria from the genus *Vibrio* cause vibriosis. *Vibrio* bacteria are pathogenic bacteria that live in waters and are opportunistic and can cause disease in humans (Ihsan, 2021). Identification using the API KIT method is one of the easiest ways to determine the species of a bacterium.



(a) (b)

Figure 5. Histopathology of the spiny lobster hepatopancreas (a) healthy; (b) unhealthy: hemocyte infiltration and encapsulation in the inside of the lumen.



(a) (b)

Figure 6. Histopathology of spiny lobster gill organs (a) healthy; (b) unhealthy: the primary lamellae had hypertrophy of the connective tissue.

The bacterial isolates obtained were *Vibrio* spp. Identification results of biochemical tests to determine the type of KIT API used.

Testing the bacterial sample to be tested is gram negative which is a non-Enterobacteriaceae species so that the KIT API 20 NE (Biomerieux) type is used (Sumini & Kusdarwati, 2020). The results obtained for the bacterial samples Lobster one, Lobster two, and Lobster four, namely *Vibrio parahaemolyticus* with %ID 99.9%, 99.9%, and 99.7%, respectively. Lobster three and Lobster five bacteria samples, namely *Vibrio alginolyticus* with % ID 99.8%. Therefore, the results obtained are in accordance with the literature according to Soedjatmiko and Ariesyady (2011) that the level of compatibility with the bacteria contained in the program database is considered valid if the match rate reaches 80% or more while less than 80% of the program database is considered invalid.

V. parahaemolyticus is a gram-negative rod-shaped bacterium with a diameter of 3–5 mm, which is oxidative and fermentative, facultative anaerobe, does not form spores, but has flagella, is motile (Chen *et al*., 2017). *V. parahaemolyticus* cannot ferment sucrose and the bacterial colonies are green in TCBS media. *V. alginolyticus* has the characteristics of yellow colonies, 3–5 mm in diameter. The physico-biochemical characteristics show that gram-negative staining is in the form of rods, has fermentative and motile properties (Rahmanto *et al*., 2014). *Vibrio parahaemolyticus* and *Vibrio alginolyticus* are some of the pathogenic bacteria that attack spiny lobsters (Dehkordi *et al*., 2014; Prastowo *et al*., 2022).

Spiny lobster mortality in PT. Saibatin Perikanan Indonesia is showing symptoms of being inactive and having a decreased appetite. Apart from that, the symptoms that arise are milky white abdominal muscles, swollen abdomen and milky white color. When the internal organs are dissected, the hemolymph is milky white and secretes a milky white liquid, then on the gills and most of it causes a white color. According to the literature Adiyana *et al*. (2014) stated that the symptoms showing pale white gills and hepatopancreas, as well as milky white hemolymph, these characteristics are symptoms of lobsters infected with MHD-SL caused by Rickettsia-like bacteria. Rickettsia-like bacteria is a genus consisting of intracellular parasites, in which these bacteria cannot reproduce or multiply outside of the host cell so they cannot be cultured in agar-based nutrient media. Rickettsia-like bacteria as pathogenic bacteria that attack the hemolymph are characterized by a swollen abdomen and a milky white color (Koesharyani *et al*., 2021).

MHD-SL disease caused by Rickettsia-like bacteria occurs with additional infections by bacteria from the genus *Vibrio* such as *V. parahaemolyticus* and *V. alginolyticus*. Both *Vibrio* bacteria are opportunistic pathogenic bacteria (Shields, 2011). The test was carried out using the polymerase chain reaction (PCR) method because Rickettsia-like bacteria cannot be cultured in agar-based nutrient media. The Polymerase Chain Reaction method is used to amplify certain (selective) DNA base sequences. A disease that attacks spiny lobsters that causes death in floating net cages at PT. Saibatin Perikanan Indonesia, Lampung was identified through PCR testing, namely milky hemolymph disease of spiny lobster.

PCR testing using primers 254F and 254R on five samples of the spiny lobster hepatopancreas showed a DNA band at 254 bp so that the samples tested positive for milky hemolymph disease of spiny lobster caused by Rickettsia-like bacteria (Koesharyani *et al*., 2016). The results of the PCR test for lobster natural feed were negative for Rickettsia-like bacteria. According to Sudewi *et al.* (2020) stated that the possibility of spreading Rickettsia-like bacteria from natural feed to cultivated spiny lobsters is very small. Identification of damage to the hepatopangcreas of spiny lobsters infected with milky hemolymph disease of spiny lobster was carried out by histopathological observation.

Hepatopancreas is a digestive gland (digestive gland) combined of the liver and pancreas. According to Ihsan *et al*. (2017) stated that the hepatopancreas has a very large role in the spiny lobster digestive system, namely being the center of nutrient metabolism in the spiny lobster body which includes absorption of nutrients, processing nutrients into ATP, and secretion of digestive enzymes. The high damage to the structure of the gills and hepatopancreas will affect the process of enzyme metabolism and osmoregulation. In addition, damage to cells caused by other factors, namely stress conditions, can increase sensitivity to bacterial infections (Coates & Söderhäll, 2021). Observation of histopathological changes in the gills observed in the primary lamellae showed connective tissue hypertrophy which was also reported by Ryazanova *et al*. (2023). Connective tissue hypertrophy in infected cells makes the

hemolymph turbid like milk in the most severely affected crustacean (Ryazanova *et al*., 2023).

Histopathology of the spiny lobster hepatopancreas suspected of being infected with bacteria due to granulocyte infiltration and encapsulation. Infiltration is an inflammatory cell which is shown in the results of histological preparations of the gill organs and the tubular part of the hepatopancreas. Granulocytes are cells that have segments or lobes in the cell nucleus and granules in the cytoplasm, consisting of neutrophils, basophils, and eosinophils. Encapsulation is a defense reaction against particles in large numbers and cannot be phagocytosed by hemocytes. The results of microscopic observations are shown in Figures 5 and 6 where there is a collection of inflammatory cells to form areas of necrosis.

According to de Souza Valente *et al*. (2021) stated that a collection of inflammatory cells will form an area of necrosis. In accordance with Sudewi *et al*. (2018), through histopathological observations of cross-sectioned hepatopancreatic tubules of a naturally infected spiny lobster, the necrotic tubular structure was revealed, and longitudinal sections through the gills with MHD-SL observed large amounts of RLB appeared in granular-like masses filling in the hemal sinus. Based on observations, the transmission of this disease is known through horizontal transmission. Transmission can occur through direct contact with infected individual spiny lobsters in the same floating net, or through contaminated water between floating nets that are located next to each other.

Spiny lobsters that show clinical symptoms of being infected by Rickettsia-like bacteria will die with an estimated 3-5 d, this is because the MHD-SL is considered the deadliest disease that causes up to 80% mortality and up to 100% morbidity (Sudewi *et al*., 2020). According to OIE (2008), states that MHD-SL in spiny lobster farming can cause high mortality and losses on a large scale. This greatly affects the cultivation of spiny lobsters kept in floating net cages, one of which is at PT. Saibatin Perikanan Indonesia. Therefore, as a suggestion for cultivating spiny lobsters in KJA, it is necessary to carry out lobster health management from the beginning to the lobster harvest, such as regulating stocking density, using immunostimulants and maintaining KJA through increasing biosecurity.

CONCLUSION

The bacteria found in the spiny lobsters *Panulirus* spp. are *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Five spiny lobsters from PCR results were infected with Milky Hemolymph Disease of Spiny Lobster (MHD-SL) ranging from mild to severe symptoms. Clinical symptoms that appear are characterized by a milky white lobster hemolymph. The histological data obtained contained granulocyte infiltration and encapsulation as symptoms of infection with Milky Hemolymph Disease of Spiny Lobster (MHD-SL). The management of spiny lobster stocking density, maintenance biosecurity, and increased lobster immunity must be enhanced in order to prevent an MHD-SL disease in floating net cages.

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