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Evaluation of Histopathological Changes in *Cantang* Groupers' Brain and Gill Infected with *Streptococcus Iniae*

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ABSTRACT

Cantang hybrid grouper is the result of hybridization between female *Macan* grouper (*Epinephelus fuscoguttatus*) and male *Kertang* grouper (*Epinephelus lanceolatus*). Hybrid fish have better performance in terms of growth, resistance to diseases, and tolerance to environmental factors, compared to the parents. One of the diseases that can attack fish is an infection induced by *Streptococcus iniae* (*S. iniae*), which is quite a dangerous bacterium since it can cause mass death of fish. The present study aimed to investigate the pathological changes of the brain and gill of *Cantang* hybrid grouper *(Epinephelus fuscoguttatus* and *Epinephelus lanceolatus)* infected with *S. iniae*. A total of 180 groupers from the Management Unit of Brackish Water Aquaculture, Situbondo, Indonesia, were included in the current study with a length of 7 cm. The experimental study was conducted using a complete randomized design, including three treatments and three repetitions for each one (n = 20). The main parameters to observe the histopathological changes in the fish internal organs, namely the gill and the brain at the beginning and the end of the study. Moreover, the supporting parameter was the water quality which included the temperature, oxygen content, pH, and salinity. Several observations including the pathogenicity test, Lethal Dosage 50 test, observation of clinical symptoms, and observation of histopathology were done during the treatment. The obtained results were indicative of the histopathology damages in the brain and gill tissues of the *Cantag* hybrid groupers infected with *S. iniae*. Different scores of lesions, infiltration, congestion, and degeneration were indicated in the brain tissue. Furthermore, the gill damages consisted of hyperemia, congestion, and infiltration. In conclusion, *Cantang* hybrid groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with *S. iniae* indicated the clinical symptoms, anatomical pathology, and histopathological changes.

Keywords: Bacteria, Epinephelus fuscoguttatus, Epinephelus lanceolatus, Histopatology, *Streptococcus iniae*

INTRODUCTION

Southeast Asia is the leading global producer of groupers, and Indonesia is in the second rank for aquaculture of grouper production (Rimmer et al., 2004). Indonesia has abundant potential for the development of the aquaculture industry (Rimmer et al., 2004). These potentials include national waters covering 3.1 million km^2, the exclusive economic zone area of about 2.8 million km^2, the coastline length reaching 81,000 km, and a total of 17,499 islands which can be used to strengthen the capacity of sea fish production. Based on the obtained statistical data of Kementerian Kelautan dan Perikanan Marine and Fisheries Ministry (MFM, 2009), the utilization of marine aquaculture potential is around 0.3% with 12,502,369 ha of aquaculture potential land that can be developed.

The grouper fish is one of the important fishery commodities with a high selling price and demand (Szuster and Albasri, 2010). *Cantang* hybrid grouper is the result of hybridization between female *Macan* grouper (*Epinephelus fuscoguttatus*) and male *Kertang* grouper (*Epinephelus lanceolatus*). The name *Cantang* is an abbreviation for Macan and Kertang. The fish crossbreeding was first studied by researchers at Balai Budidaya Air Payau (BBAP, Brackish Water Aquaculture Center), Situbondo (Nurhayati et al., 2014). According to the data reported from the official site of the BBAP Situbondo, this hybrid fish has a better performance than the two parents in terms of growth, resistance to diseases, and tolerance to environmental factors. The hybridization process is one of the solutions to the aquaculture process, because the *Cantang* grouper can grow fast, and survive in a new environment (Rimmer and Glamuzina, 2019).

The fish disease is a direct or indirect interference to fish health. It is important to gain sufficient knowledge about fish diseases to minimize the related causes of failure in the aquaculture business. One of the diseases that can attack fish is Streptococcosis which is induced by *Streptococcus spp*. bacteria (Chang and Plumb, 1996). This *Streptococcus* infection develops due to low resistance of the fish to bacterial diseases, poor environmental situation, and poor feed management leading to an imbalance (Watts et al., 2017).

Streptococcus is a spherical cell, single-rod, or ovoid-shaped coccus, and structured similar to a chain, Coccus cleaves in the perpendicular area to the long axis of the chain. The chain's length may vary due to environmental factors.

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Streptococcus is a gram-positive bacterium, but in the old culture, *Streptococcus* looks like a Gram-negative after overnight incubation (Jawetz, 2007).

Streptococcus iniae (*S. iniae*) is a bacterium which greatly influences the success of marine aquaculture activities (Watts et al., 2017). The disease is believed to be dangerous since it can cause mass death to the fish. Therefore, the present study was conducted to explore the clinical symptoms of bacterially infected groupers, and observe the changes in histopathology of the brain and gill of *Cantang* hybrid groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with *S. iniae*.

MATERIALS AND METHODS

Ethical approval

All of the processes were monitored and approved ethically with the animal ethical committee of Universitas Airlangga, Indonesia.

Study design

The current study was conducted at Brackish Water Aquaculture Center (BBAP) Situbondo, Indonesia. The production process of the histopathology samples of the brain and gill of *Cantang* groupers was conducted at the Microbiology Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia. The pure isolate of *S. iniae* was obtained from the First Class Fish Quarantine Center, Juanda, Surabaya, Indonesia.

A total of 180 groupers from the Management Unit of Brackish Water Aquaculture, Situbondo, Indonesia, were included in the current study. The investigated experimental animals were *Cantang Epinephelus sp* groupers with a length of 7 cm. Brain Heart Infusion Agar (BHIA) and Brain Heart Infusion Broth (BHIB) media were used as the bacterial culture obtained from Balai Besar Laboratorium Kesehatan Surabaya (Health Laboratory Center of Surabaya), Indonesia. The implemented maintenance media included salinity seawater 32-35 ppm from the sterile reservoir, brackish water with the salinity of 30-35 ppt with the acidity pH of 7-9, oxygen > 5 ppm, and temperature range of 24-32°C. The experimental diet was commercial grouper feed given *ad libitum*. For histopathological evaluations of the groupers' gill and brain, the samples were fixed in formalin 10% and all process of preparing the microscopic slides were done according to the Bernet et al. (1999) and Sultana et al. (2016). Finally, the slides were stained by Hematoxylin-Eosin dyes and were investigated under a light microscope (Olympus, Japan).

This experimental study followed a complete randomized design method using three treatments and three repetitions (n = 20) resulting in nine-unit tests. The observation toward histological preparations was performed using the semi-quantitative scoring method (Gibson-Corley et al., 2013), then analyzed statistically using the Kruskal-Wallis (Riffenburgh, 2006). Treatment A in the present study included *Cantang* groupers *(Epinephelus fuscoguttatus* and *Epinephelus lanceolatus)* uinfected with *S. Iniae,* and injected by phosphate-buffered saline (PBS). Treatment B entailed *Cantang* groupers *(Epinephelus fuscoguttatus* and *Epinephelus lanceolatus)* infected with 10^6 at volume of 0.1 ml of *S. iniae*, and treatment C consisted of *Cantang* grouper *(Epinephelus fuscoguttatus* and *Epinephelus lanceolatus)* infected with 10^8 at volume of 0.1 ml of *S. iniae*. The observation of pathologial changes in body`s organ was done during 8, 16 and 24 hours after tearment. The feeding was given twice a day using the *ad libitum* method by providing feed until the fish was no longer hungry. The observation of clinical symptoms was included the observation of behavior, such as the movement, activity, appetite, morphology and physical condition (eyeballs, operculum, gills, and scales). The observation of appetite was carried out by looking at the response and feeding activity to the given food, compared to the response and feeding activity of the control fish. It was stated to have a normal response in case the fish responded spontaneously and engaged in feeding activities when they were fed.

The investigated variables in this study were the dilution of *S. iniae* to infect *Cantang* grouper as an independent variable, histopathology of the brain and gill of the groupers as a dependent variable, and the type and size of the groupers as the control variables. The main parameter was anatomic pathology, addressing the macroscopic changes of the fish body organ. The changes in histopathology of internal organs, including the gill and brain, were conducted at the beginning and the end of the study. Meanwhile, the supporting parameter was water quality, especially the temperature, oxygen content, pH, and salinity. The temperature measurement was carried out using a thermometer, the dissolved oxygen content was evaluated by a dissolved oxygen (DO) meter, and pH of the water was gauged using a universal paper indicator of pH (pH paper). The measurement of water quality was carried out after bacterial infection on the *Cantang* groupers. The current study was conducted through several observations by conducting the Pathogenicity Test and LD50 Test, observation of clinical symptoms, and observation of histopathology.

Pathogenicity test and lethal dose test

This test was conducted by treating 5 bacterial suspension dosages started from the dilution of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} up to 10^{-8} CFU/ml. Each treatment consisted of five fish. The treatment was distinguished from the density of *S. iniae*

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(cell/ml) injected by 0.1 ml per head intramuscularly. Lethal Dosage 50 (LD50) calculation was carried out based on the Dragstedt Behrens method (DBM, Hubert, 1980).

Observation of clinical symptoms

Observation of clinical symptoms was conducted by observing the changes in the gill and brain of *Cantang* groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with *S. iniae*, and also observing the changes in behavior, and the outer body organs of *Cantang* groupers during maintenance.

Pathological observations

Histopathology observation was conducted by making histopathological preparations of the brain and gill tissues of the *Cantang* groupers (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*). Sample preparations were performed by taking an experimental fish from each treatment where clinical symptoms indicated that the fish was infected with *S. iniae*. After that the brain and gill organs were soaked using 10% of Pro Analyst (PA) formalin solution, followed by soaking gill tissue in Nitrates acid leading to soft and easy-cutting tissue. The assessment of the brain and gill tissue damages was performed using the Histological Activity Index (HAI; Setyowati et al., 2010).

Statistical analysis

Kruskal-Wallis statistical test was applied for the detection of differences between traits. SPSS version 22 was used as statistical software. The obtained results of brain organs and gill organs were then further analyzed using the Kruskal-Wallis statistical test. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

After the bacterial infection, a lot of groupers showed severe clinical symptoms. Clinical symptoms were in the form of slow movement and poor appetite. In the 8th hour of the observation, it was found that the fish died after getting a treatment dose of 10^8 cell /ml at each repetition. In the 16th hour, more clinical symptoms were found, including faded patterns of the body surface, slower swimming movement, sometimes swimming to the surface and spinning (whirling), and lesions on the body surface after getting a dose of 10^6 cell /ml and 10^8 cell /ml. The fish looked dying, and in fact, some of them were dead. Other clinical symptoms during the observation were convex stomach, dark body color, lesions and hemorrhagia on the dorsal, ventral and the surface of the body as well as on the internal organs. The infection treatment at the dose of 108 cell/ml led to a welling in the kidney and liver organs of some fish. Other internal organs, such as the spleen, heart, intestines, and gills were pale. Swimming bubbles in some treatment samples were infected, broken, and damaged. The body condition of a grouper with some clinical symptoms can be observed in Figure 1.

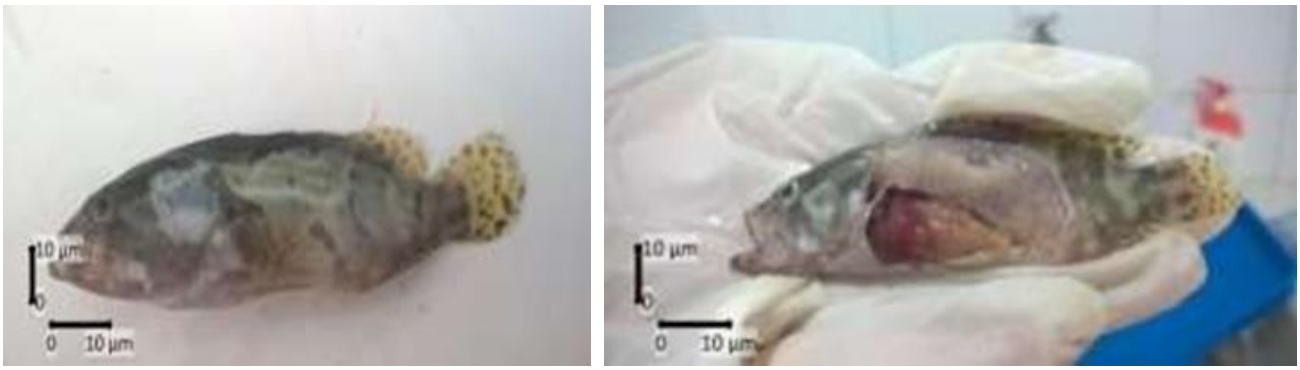

Figure 1. The body condition of *Cantang* groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with *Streptococcus iniae*

The changes in anatomic pathology of the *Cantang* grouper infected with *S. iniae* can be described as lesions in the injection area, swollen abdomen, swollen and pale liver, swollen spleen, out of scales, and enlarged swimming bubbles. The descriptive features of the changes in anatomic pathology of the samples can be seen in Table 1. The fish given infection treatment with a dose of 10^6 cell /ml, fell out fish scales and experienced lesions on the injection marks in the eighth hour of the observation. In hour 16, the abdomen began to enlarge, hemorrhagia and lesions spread out on the

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injection site. In hour 24, beside the hemorrhagia in abdomen, the liver was swollen, heart, spleen, gill, and kidney were pale, however, swimming bubbles were still intact.

Table 1. Pathological changes in *Cantang* groupers (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) infected with *Streptococcus iniae*

Treatment	Body's organ	8 hours after treament	16 hours after treament	24 hours after treament
Control	Body (Outer body)	Normal Color	Normal Color	Normal Color
	Fin	Normal	Normal	Normal
	Liver	Normal	Normal	Normal
	Heart	Normal	Normal	Normal
	Spleen	Normal	Normal	Normal
	Kidney	Normal	Normal	Normal
	Intestine	Normal	Normal	Normal
	Eye	Normal	Normal	Normal
	Gill	Normal	Normal	Normal
	Swimming Bubbles	Intact	Intact	Intact
10^6 cell/mL	**Body's organ**	**Lesion**	**Lesion**	**Lesion**
	Fin	Normal	Normal	Hemorrhagic
	Liver	Normal	Pale	Pale Swollen
	Heart	Normal	Normal	Pale
	Spleen	Normal	Pale	Pale
	Kidney	Pale	Pale	Pale Swollen
	Intestine	Normal	Normal	Pale
	Eye	Normal	Normal	Normal
	Gill	Normal	Pale	Pale
	Swimming Bubbles	Intact	Intact	Intact
10^8 sel/mL	**Body's organ**	**Lesion**	**Lesion**	**Lesion**
	Fin	Normal	Hemorrhagic	Hemorrhagic
	Liver	Pale	Pale Swollen	Pale Swollen
	Heart	Pale	Pale	Pale
	Spleen	Pale	Pale	Pale
	Kidney	Pale	Pale Swollen	Pale Swollen
	Intestine	Normal	Pale	Pale
	Eye	Normal	Normal	Normal
	Gill	Normal	Pale	Pale
	Swimming Bubbles	Intact	Intact	Broken

Regarding the dose of 108 cell/ml of the treatment, the color of the fish faded, and some scales began to fall out after 8 hours of the observation. In 16 hours of the observation, the fish body was pale and hemorrhagic, the experimental samples were abnormal in shape and did not have an average size with the convex stomach. After 24 hours of observation, fish body was pale and hemorrhagic, there were significant changes in internal organ damages, namely the broken swimming bubbles, as well as pale gill, heart, heart, and swelling indicating degeneration (Table 1). These observations were similar to the findings of Chang and Plumb (1996).

The body condition of the *Cantang* grouper undergone anatomic pathology changes can be seen in Figure 2. The behavior of the *Cantang* groupers during the study can be seen in Table 2. Pathological changes occurred most in the brain organ. The form of histopathological changes in the organs of the brain and gill included hyperemia, congestion, cell infiltration, lesions, and cell degeneration. The assessment result of the changes in brain and gill tissues' damage of the groupers can be seen in Tables 3 and 4. Scoring in Tables 3 and 4 was according to Sah Putra et al. (2020).

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At the end of the study, the result of water quality parameters' measurement consisted of the condition of temperature, pH, DO, salinity, and ammonia can be seen in Table 5.

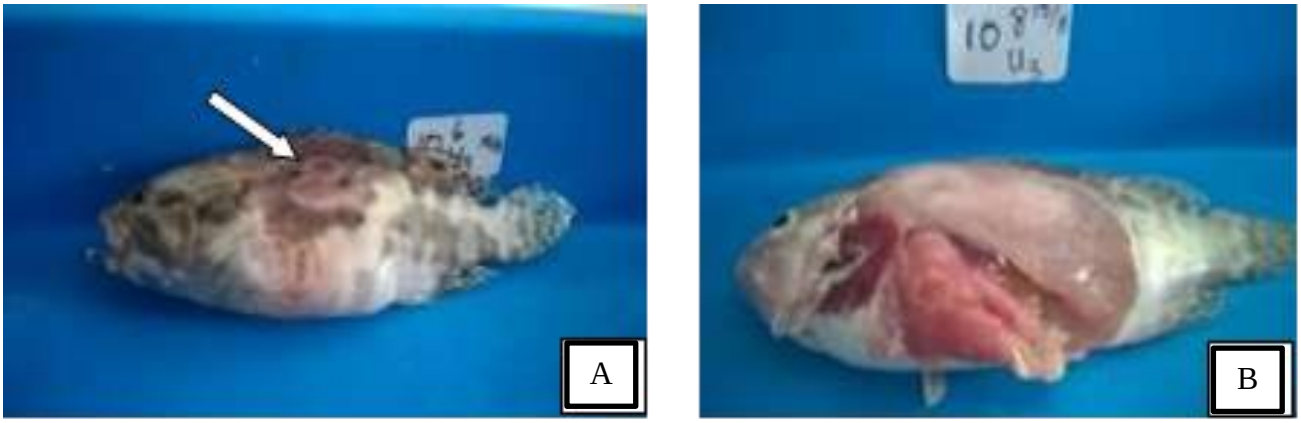

Figure 2. The anatomical pathology (hemorrhagic lesions) in Groupers infected with *Streptococcus iniae*. **A:** Swollen abdomen. **B:** The internal organs of the fish were severely damaged in the treatment at the dose of 10^8 cell/ml.

Table 2. The behavior of *Cantang* groupers during the study

Treatment	Behaviorial status
A	Fish swimming in the bottom, there is not any movement, open-close operculum is normal
B	Fish swimming in the bottom, there is not any movement, open-close operculum is slow
C	Fish swimming in the bottom, there is not any movement, open-close operculum is slow

Note: A: *Cantang* groupers as the control were injected with phosphate-buffered saline, B: *Cantang* grouper was injected with 10^6 dilution of *Streptococcus iniae* culture, C: *Cantang* grouper was injected with 10^8 dilution of *Streptococcus iniae* culture.

Table 3. Scoring of the changes in brain and gill tissues' damage of the *Cantang* Groupers *(Epinephelus fuscoguttatus* and *Epinephelus lanceolatus)* infected with *Streptococcus iniae*

Treatment	Brain tissue				Gill tissue		
	Lesions	Infiltration	Congestion	Degeneration	Hyperemia	Congestion	Infiltration
P0	0*	0	0	0	0	0	0
P1	1.6	1.6	2	1.1	1.6	2	2.1
P2	2.3	2.3	2.3	2.1	2.6	2.8	2.3

Scoring is calculated according to the method of Sah Putra et al. (2020). ***** Score of '0' was considered as normal for all descriptors.
P0: *Cantang* groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) uninfected with S. Iniae, and injected by phosphate-buffered saline (PBS). P1: *Cantang* groupers (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with 1060.1 ml of *S. iniae*, P2: *Cantang* grouper (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with 1080.1 ml of *S. iniae*.

Table 4. The results of Kruskal-Wallis statistical analysis among treatments in the histopathology of brain and gill tissues of the *Cantang* groupers *(Epinephelus fuscoguttatus* × *Epinephelus lanceolatus)* infected with *Streptococcus iniae*

Analysis result	Brain					Gill	
	Lesions	Infiltration	Congestion	Degeneration	Hyperemia	Congestion	Infiltration
Kruskal-Wallis	0.041	0.023	0.045	0.028	0.031	0.03	0.045

Table 5. Range of the water quality parameters during the study

Parameter	Range
Temperature (°C)	27-28
pH	7.3-7.6
DO (mg/l)	5.5-7.8
Salinity (‰)	31-34
Ammonia/NH3 (mg/l)	0.054-0.226

DO: Dissolved oxygen

As can be seen, the obtained results of brain tissue analysis were statistically significant for the damage to lesion, infiltration, congestion, and degeneration among the treatments ($p < 0.05$). Whereas, the result of gill tissue analysis

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showed statistically significant changes for the damage to hyperemia, congestion, and infiltration ($p < 0.05$) meaning that there were significant differences among the treatments.

The response to the stimuli, swimming movement, and operculum movement were the parameters of the health level of the fish. The poor appetite in the experimental fish could result from the stress they experienced in the course of treatment and entry of extraneous materials or objects into the body. The decreased response to the reaction of stimuli and appetite, irregular swimming, and skin discoloration were some of the clinical symptoms of the samples infected with the pathogenic bacterium (Miyazaki et al., 1984; Declercq et al., 2013). The difference in the level of bacterial density was also expected to affect the stress level of each treatment group.

After the fish fell prey to bacterial infection, the clinical symptoms of slow movement and poor appetite appeared due to the weakened defense mechanisms of the groupers. To protect the body from bacterial infection, the fish secreted mucus continuously, thus the body's metabolism increased leading to higher energy consumption. Consequently, the groupers became weak, had no appetite, and were easily stressed. This made it easier for bacteria to infect the body by removing toxins through open areas, such as the gills (Supriyadi, 1990). The working power of the toxin found in bacteria was related to the specific receptor cells. Interaction between receptor cells in the body with the hemolysin had a wound effect on the body. The extracellular toxin had two virulence determining regions, namely the attachment area (i.e., the area where the toxin attached to the specific receptor cells) and the active region as the main cause of cell infection (Virella, 1997).

In the treatment with a dose of 10^8 cell /ml, the wound in the injection area developed into an ulcer. The occurrence of the ulcer was caused by the high density of bacteria accumulated in the injection area, thus the intensity of the toxin released in the infection process was higher in the area (Smith, 1997; Overstreet and Hawkins, 2017). *Streptococcus iniae* infection was often associated with the infection of the lining of the brain and parenchyma cells (meningoencephalitis) which was characterized by exophthalmia or protruding eyes and corneal opacity. In the present study, both the dose of 10^6 cell /ml and 10^8 cell /ml did not cause histopathological changes (Tables 2 and 3), whereas histopathological changes occurred most in the brain organ (Table 3). The form of histopathological changes in the organs of the brain and gill included hyperemia, congestion, cell infiltration, lesions, and cell degeneration. *Streptococcus iniae* is a group of Gram-positive bacteria that causes septicemia where it is able to survive and replicate in the circulatory system, and to several specific target organs, and is suitable to their standard requirements, so they would stick and replicate to the target organ.

Streptococcus iniae produces α hemolysin toxin, and its infection routes through the blood circulatory system (Maryadi, 2009). When entering the blood vessels, *S. iniae* would produce hemolysin toxin which causes the rupture or lysis of the red blood cells (Locke et al., 2007). The bacteria move very fast in the blood vessel, it could easily reach the important organs of fish such as the gill which has many blood vessels where the location is utilized by bacteria as a medium for living and breeding, and uses the nutrients in the vicinity for metabolic processes (Overstreet and Hawkins, 2017). One of the clinical symptoms of fish infected with *S. iniae* is swimming spinning (Supriyadi et al., 2016). Fish with abnormal behavior (swim in sideways and spins) due to the effect of pathogenic bacteria indicating that infection is accumulated in brain liquid (Supriyadi et al., 2016). The pathogenicity of each pathogenic agent is also closely related to its ability to produce enzymes, toxins, and in overcoming its host immune system (Russo et al., 2006). *Streptococcus iniae* is also zoonotic, where they could also infect humans, and causes cellulitis (Bowser et al., 1998).

The obtained analysis results of histopathology damage in the groupers' brains were 0.041, 0.023, 0.045, and 0.028 for lesions infiltration, congestion, and degeneration, respectively, which were statistically significant ($p < 0.05$). The tissue damages in groupers' gill were estimated as 0.031, 0.03, and 0.045 for hyperemia, congestion, and infiltration, respectively ($p < 0.05$). This means that there was a significant difference among the experimental groupers in the control group and those involved in the treatment groups in terms of tissue appearance.

The data analysis continued using the Mann-Withney test which aimed to determine the response between P0 treatment with the P1, the P0 with the P2, and the P1 with the P2. The findings indicated a significant difference between P0 treatment and P1 ($p < 0.05$), a significant difference between P0 and P2 ($p < 0.05$), and an insignificant difference between P1 and P2 ($p > 0.05$). The control group of P0 differed significantly from P1 and P2, since in these two treatments (P1 and P2) the groupers were injected with bacteria, and the samples experienced tissue changes. However, the obtained results of the comparison of P1 and P2 treatment groups were not significantly different ($p > 0.05$), since samples in P1 and P2 were given an injection of bacterial treatment and experienced the same histopathic changes, but at the different levels of damage. The salinity at the end of the study was within the range of 31-34 percent.

In the brain organ of the *Cantang* groupers infected with *S. iniae*, encephalitis occurred which was characterized by cell degeneration, congestion, and cell infiltration. The changes of brain tissue infected with *S. iniae* caused the temporary cell metabolic disorders (degeneration) which was characterized by intracellular accumulation with the microscopic features, for instance, a lot of cells were jostled, cells swelled, paler color was observed, and cytoplasm was cloudy, scattered, and sometimes found vacuoles (Roberts, 2012). Meningoencephalitis is a special lesion commonly

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found in natural cases of *S. Iniae* infection. Systemic infection by *S. iniae* can cause damage to the blood vessels, thus oxygen supply is disrupted and induces ischemic lesions. In the process of reaction to lesions in the brain, microglia undergoes enlargement, hyperplasia, and neurophagia (Russo et al., 2006).

The result of histopathology examination (Tables 2-4) showed swollen gill lamella with an attachment (fusion) accompanied by branches. The branches were characterized by congestion, hemorrhagia, proliferation of chloride cells, and infiltration of inflammatory eosinophilic granule cell. Brunchitis was observed in the infection treatment of 10^6 cell /ml and 10^8 cell /ml, moreover, edema and congestion occurred in the primary lamella and secondary lamella. Infiltration of inflammatory cells showed the *S. iniae* bacteria were infected intramuscularly and could enter the gill blood vessels. The *S. iniae* bacteria caused the congestion and infiltration of inflammatory cells in the lamella tissue that looked like inflammation. Necrosa, such as excessive mucus excretion due to the chloride cell proliferation, the amalgamation of lamella (fusion) were indications of a natural immune response of the fish against toxic substances (Suhendrayatna et al., 2019).

At the end of the study, salinity was in the range of 31-34%, while the ammonia content ranged from 0.054 to 0.226 mg/l. The range of ecological parameters suitable for groupers' growth was 24-31ºC for the temperature, 30-33 ppt for salinity, > 4.9 ppm for dissolved oxygen, and 7.8-8.0 for pH (Thia-Eng and Seng-Keh, 1978). Ammonia tolerance limit for pond aquaculture was 0-0.25 ppm (Poernomo, 1992), while safe ammonia for grouper aquaculture needs to be less than 0.01 ppm (Supratno, 2006). The measurement facilities were a thermometer for temperature measurement, pH pen for pH, a refractometer for dissolved oxygen, and an ammonia test kit for the ammonia measurement. Water temperature at the end of the study was in the range of 27-28°C. Water pH at the end of the study was in the range of 7.3-7.6. The dissolved oxygen at the end of the study was in the range of 5.5-7.8 mg/l.

CONCLUSION

Hybrid *Cantang* grouper fish (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) infected with *S. iniae* showed clinical symptoms, anatomic pathology changes, and histopathological changes.

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