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(RESEARCH ARTICLE)



## Histo-pathology of the *Vibrio alginolyticus* bacteria on tiger grouper (*Epinephelus fuscoguttatus* Forskal)

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### Abstract

The purpose of this study was to determine histo-pathology of bacterium *V. alginolyticus* against several tiger grouper fish organs. The pathogenicity test was carried out by injecting intramuscularly (IM) 0.1 ml of *V. alginolyticus* bacterial suspension with bacterial density of  $10^7$ ,  $10^8$ ,  $10^9$  cells/ml/fish and injected with 0.1 ml of PBS solution (phosphate buffer solution) as a control. Results showed that bacterium *V. alginolyticus* causes degeneration, damage and structural changes in the tissues of gill organs, kidneys, liver, and brain of tiger grouper fish. Pathogenicity challenge tests also prove that this bacterium is a true pathogen for test animals. The degree of density of the suspension of pathogenic cells affects mortality and incubation period. The bacterial density of  $10^7$  cells/ml/fish has caused death of 33.33%, density of  $10^8$  cells/ml/fish causes' death of 66.67%, and density of  $10^9$  cells/ml/fish causes death of 73.33%, while without bacteria (control) there is no death.

**Keywords:** Histo-Pathology; Pathogenicity; *Vibrio alginolyticus*; Tiger grouper

### 1 Introduction

Tiger grouper (*Epinephelus fuscoguttatus* Forskal) is one of the fishery commodities that has high economic value. The demand and opening of market opportunities for tiger grouper fish can have an intensive exploitation impact (Antoro *et al.*, 2001), so that it can disrupt the balance in nature considering that the habitat of this fish is in clear shelled waters, at this time the coral reef ecosystem continues to degrade and decrease both in quality and quantity. This fish has advantages, such as it can be farmed in floating net cages (FNC), ponds or controlled tubs, is quickly large and in demand both domestic and international markets.

One of the basic needs in cultivation is the procurement of seeds. Good fish fry will affect the success of cultivation such as the level of health, fish morphology, namely the uniformity of the size of the fry. This fish is a type of carnivorous fish and is cannibalistic and nocturnal. Today tiger grouper fry come from hatchery and from nature. Fry of natural origin can obviously become career fish disease.

One of the obstacles faced by tiger grouper farmers is the presence of pathogenic bacterial infections, which in peak conditions can result in mortality reaching 100%. Pathogenic bacterial infection is suspected to be the cause of the low hatching rate of 1.2-2.9% (Yuasa and Song, 1990). Bacteria *Vibrio* sp. has been reported to be pathogenic (Austin and Austin, 1988) against grouper fish, namely *V. alginolyticus* type bacteria (Aryani *et al.*, 2004). Efforts to deal with the consequences of infection are carried out using antibiotics, unfortunately there is resistance and the presence of residues in the fish product. The International Market rejects all products containing antibiotics in it, so to reduce mortality it takes information on the histo-pathology caused by the bacterium *V. alginolyticus* against tiger grouper.

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Bacterial diseases at this time are often quite a serious problem due to the rapid rate of spread and lead to high mortality. The presence of pathogenic bacteria on the body of fish will lead to damage to organ tissues and disruption of the working system of organs, so that the physiological processes of fish are disturbed. Fish that are attacked by pathogenic bacteria will be seen to experience clinical symptoms before mortality occurs. Information about histo-pathology studies, especially in farmed fish, has not been widely reported, even though it has a considerable meaning in handling cases of diseases that occur.

## 2 Methodology

This research method used a complete randomized design (RAL) of one factor, four levels and three tests. Pathogenicity tests were carried out by injecting intramuscularly (IM) 0.1 ml of *V. alginolyticus* bacterial suspension with a density of  $10^7$ ,  $10^8$ ,  $10^9$  cells/ml/fish and injecting fish with 0.1 cell/ml/fish PBS solution as a control.

Test bacteria were isolated from the kidneys of tiger grouper infected with *V. alginolyticus* and then carried out biochemical and inoculum tests on test bacteria (Murdjani, 2002; Taslihan *et al.*, 2001; Kurniasih, 1999). Test fish before infection is carried out acclimatization for 7 days. The suspension of test bacteria is injected intramuscularly. Pathogenicity test is carried out for 7 days, observations are carried out visually physiology and morphology. During the challenge test, measurements of water quality, feeding and calculation of the density of *V. alginolyticus* bacteria in water and in the kidney organs were carried out. The calculation of bacterial density is carried out by making a dilution series, then inoculated on a selective agar medium (TCBSA). Then, the calculation of the density of test bacteria on the water medium is carried out on the first day, the fourth and seventh days. The target organs in this examination are carried out on the tissues of the organs of the kidneys, gills, liver, spleen and brain of tiger grouper fish. The data obtained were analyzed descriptively.

## 3 Results and discussion

The results of the identification of test bacteria by conducting colony morphological observations, Gram painting and biochemical tests showed that bacteria isolated from the kidneys of tiger grouper were infected with *V. alginolyticus* bacteria, the same as the injected bacteria. The morphology of the bacteria found is comatose, red or Gram-negative. The results of biochemical tests showed that there was a bacterial overhaul of the substrates, motile, positive indole, positive oxidase, positive H<sub>2</sub>S and positive nitrate reduction. The same thing as stated by Syafriadiman (2006) generally the morphology of bacteria looks comma-shaped, and red in color.

Visual observations, physiology and morphology of tiger grouper fish infected with the bacterium *V. alginolyticus* showed clinical symptoms. Clinical symptoms were morphologically seen starting in the test fish on day 3, such as pale body color, reddish mouth color and base of the dorsal fin, swimming activity slightly and moving sluggishly, impaired body balance and appetite and response to feeding and response to feed decreased and on day 5 had no response to feed. the whole body is slimy. On the 4th day generally the fish experience abscesses and ulcers (ulcers) at the site of the injection mark. This is caused by injection wounds and rupture of blood vessels so that the fish respond to the incoming bakteri by producing excessive mucus or mucus so that it looks like a milky white ulcer (Figure 1).



**Figure 1** Ulcers (→)

Other visible symptoms are bulging eyes (exophthalmia), and flatulence filled with light yellow liquid. If surgery is performed on the abdominal cavity, there will be bleeding (haemorage) on the gills, mouth, intestines, and internal organs. If in this phase the fish has not died then the symptoms of the disease occur and develop looks like peeling skin, scabs or necrosis on some parts of the body. The test of the challenge of *V. alginolyticus* bacteria on tiger grouper with

different levels had an effect on mortality rates and incubation periods. Pathogenicity test results against tiger grouper assays in Table 1.

**Table 1** Mortality of tiger grouper fish (%) infected with the *V. alginolyticus* bacteria

Density of Vibrio Bacteria (cell/ml/fish)	Mortality of Tiger Grouper Fish			Average (%)
	Replicate 1	Replicate 2	Replicate 3	
0 (control)	0	0	0	0
A ( $10^7$ )	20	40	40	33.33
B ( $10^8$ )	60	60	80	66.67
C ( $10^9$ )	60	80	80	73.33

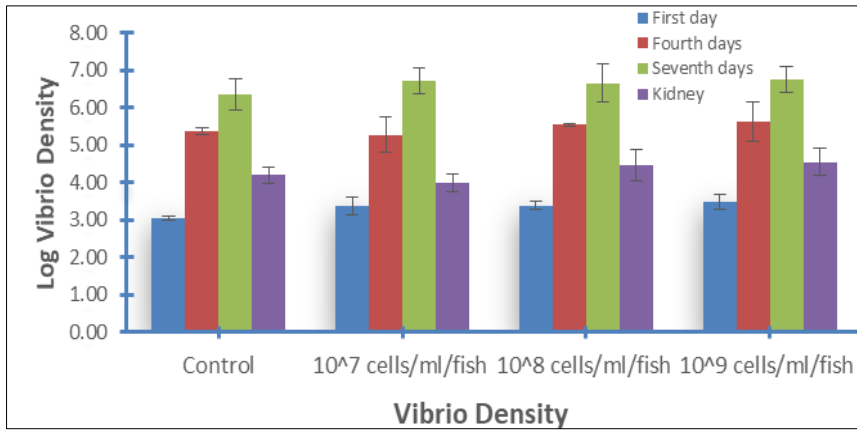
Table 1 shows that an increase in the density of the injected bacteria *V. alginolyticus* will be able to lead to an increase in the mortality number of tiger grouper fish. Fish mortality in treatment A (suspension of population density of  $10^7$  cells/ml/fish) was different from treatment B (suspension of density of  $10^8$  cells/ml/fish), and treatment A (suspension of population density of  $10^7$  cells/ml/fish) was different from treatment C (suspension of population density of  $10^9$  cells/ml/fish). Meanwhile, treatment B (suspension density of  $10^8$  cells/ml/fish) did not differ from treatment C (suspension of population density of  $10^9$  cells/ml/fish). The results showed that the *V. alginolyticus* bacteria infects tiger grouper fish has different pathogenesis. The order of the number of mortality decreased was C (suspension of population density of  $10^9$  cells/ml/fish) > B (suspension density of  $10^8$  cells/ml/fish) > A (suspension of population density of  $10^7$  cells/ml/fish).

The disease arises always preceded by the occurrence of an imbalance of three main factors. These factors include the presence of diseases seedlings or pathogens, poor environmental carrying capacity and weak host body resistance. Changes in the environment or not supportive for life will affect physiological processes and in the end the fish become weak and stressed. Stressed fish are more easily affected by diseases, both primary pathogens and secondary pathogens.

Vibriosis bacteria are classified as Gram-negative septicemia. Many attacks occur in the blood causing a high temperature that is spread throughout the host's body. This bacterium can be transmitted and spread quickly through contact in fish that are kept with high stocking density or through media that already contain bacterial pathogens. Furthermore, these pathogenic bacteria do not have a specific host and can live outside the host's body as in the air. An environment that is not good low carrying capacity will increase the reproduction speed of attackability and malignancy of bacteria. This vibriosis bacteria always appears periodically due to seasonal changes, such as fluctuations in temperature, volume and water discharge as well as an increase in the content of organic matter in the mouth of the river and the texture of the shelled bottom which is the habitat of the tiger grouper.

Pathogenicity depends not only on the bakteri, but is influenced by the host's ability to resist the entry of pathogens. Some of the factors affecting the pathogenicity of bacteria are the ability to produce toxins, enzymes, host resistance and multiplication speed. The Gram-negative bacterial group is known to produce toxins or toxins is one of its ability to cause disease. The toxin secreted by this bacterium is an exotoxin, that is, the poison is excreted into the surrounding medium. One of the enzymes produced by this bacterium is leocusidine, which is an enzyme that is secreted by bacteria and can kill red blood cells (leukocytes), which is one of the defenses in fish against incoming antigens such as microbes. While hemolysine is an enzyme that analyzes red blood cells and frees hemoglobin.

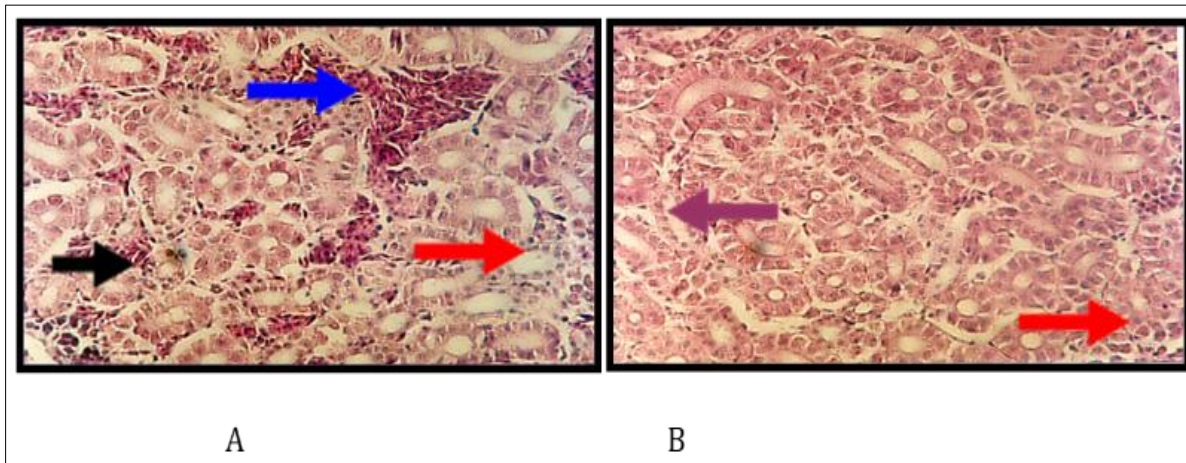
The environment is influenced by several factors, physical, chemical, and biological factors greatly affect the balance between fish as hosts and bacteria as the cause of disease. The *V. alginolyticus* bacteria is opportunistic, an attack will arise when there is a growth of a high bacterial population followed by a weakened host's immune system. Figure 2 shows a graph of the results of the log calculation of the number of *V. alginolyticus* bacteria on the maintenance water medium and on the kidney organs.



**Figure 2** Graph Log calculation of the number of populations of *V. alginolyticus* bacteria on the media and kidneys

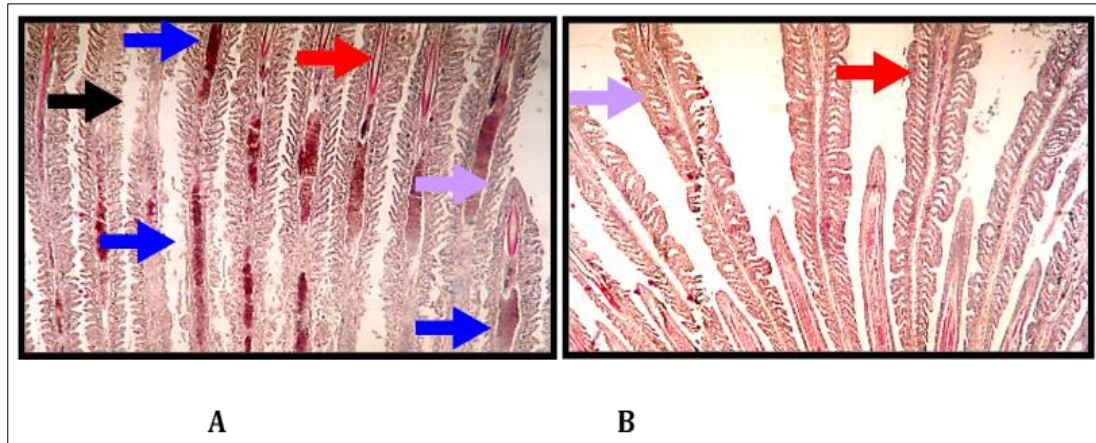
Figure 2 shows an increase in the number of *V. alginolyticus* bacteria on the maintenance water medium. The density of the bacterial population in the media is influenced by the density of injections to test fish, as well as in the kidney organs directly proportional to the density of injections. In the media and kidneys of the control test fish injected with PBS, it was found that the *V. alginolyticus* bacteria. This is suspected to be the bacteria derived from fresh trash fish feed and wild shrimp which are used as feed that has contained *Vibrio*. Wild shrimp originating from pond or marine waters can be career-specific, in addition to non-sterile maintenance equipment can contaminate test animals. The increase in bacterial density is more in the ulcerated test fish media, because there is direct contact between the ulcers which are a collection of bacterial populations and the media water.

Histo-pathology results show that the *V. alginolyticus* bacteria causes degeneration, damage and structural changes in the tissues of the kidneys, gills, liver, spleen and brain of the test fish. Transverse micrographs of tissues resulted from histo-pathology studies of the organs of tiger grouper fish in Figure 3-7.



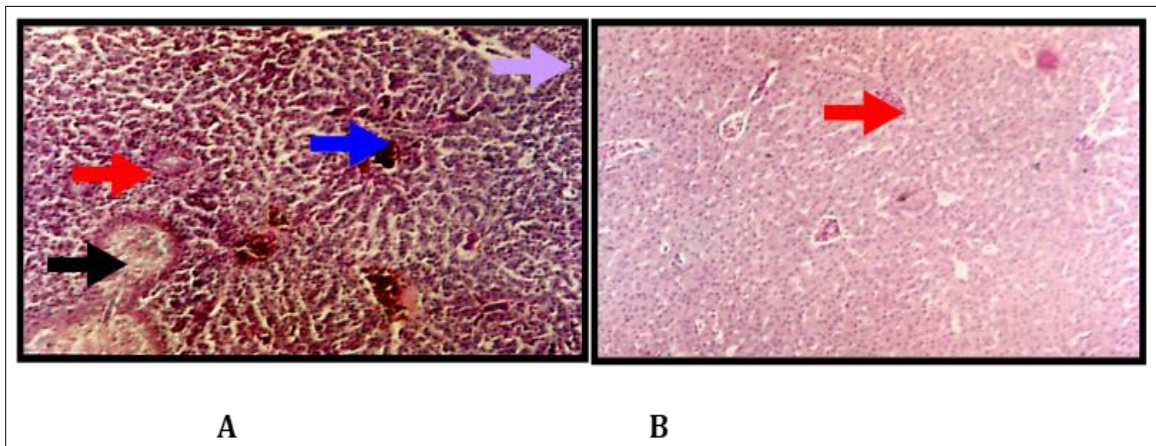
Description: there is hemorrhagatation (→), congestion (→), colonization (→), turbid swelling (→)

**Figure 3** Cross-sectional cross-section of the kidneys of tiger grouper infected with *V. alginolyticus* bacteria 10<sup>8</sup> cells/ml/fish, treatment with *Vibrio* (A), control (B), enlargement 200X



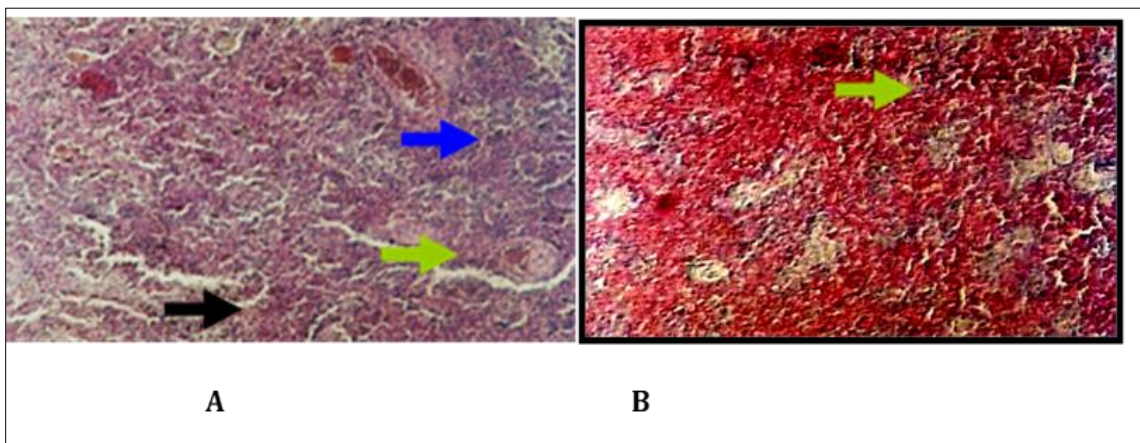
Description: occurs hemorrhagatation (→), Congesti (→), necrosis (→), proliferation (→).

**Figure 4** Cross-section of tiger grouper gills infected with *V. alginolyticus* bacteria  $10^8$  cells/ml/fish, hemorrhagic, congestion, prolimeration and necrosis occurred, magnification 200X. Vibrio treatment (A), control (B)



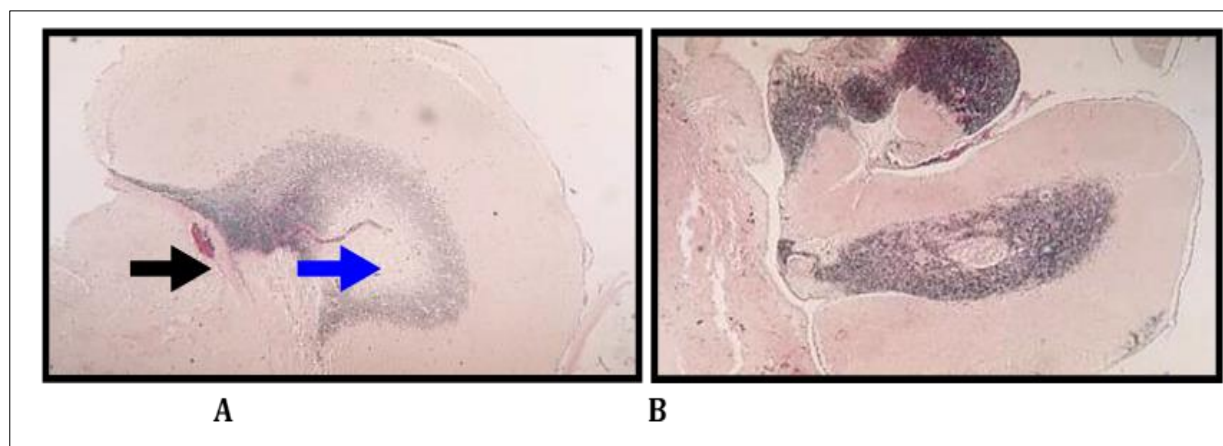
Description: hemorrhagatation occurs (→), congesti (→), turbid swelling (→) necrosis (→)

**Figure 5** Cross-sectional spleen of tiger grouper fish injected with *V. alginolyticus* bacteria density of  $10^8$  cells/fish (A) and with PBS on treatment (B), magnification 200X



Description: Hemorrhagatation occurs (→), congestance (→), fatty tissue (→)

**Figure 6** Cross section of tiger grouper liver infected with *V. alginolyticus* bacteria  $10^8$  cells/ml/fish (A) and control (B), magnification 200X



Description: hemorrhagation occurs on the molecular layer (→), congestion on the granular layer (→)

**Figure 7** Cross-section of the brain of a tiger grouper infected with *V. alginolyticus* bacteria (A) and control treatment with PBS (B), magnification of 40X

From the results of section in the target organ, it can be seen that there are damage and changes in tissue structure, both in the treatment with *Vibrio* injection and with PBS as a control treatment. The worst damage is seen in the tissues of the gill organs, this is due to the influence of the injections made and the presence of *Vibrio* populations on the water medium. The results of calculations at the beginning of the treatment of maintenance media turned out to have contained *vibrio* populations both on treatment and on controls. The presence of *Vibrio* in the media is suspected to be from feed. The feed used is fresh trash fish and wild shrimp obtained from the pond.

Damage and structural changes in organ tissues lead to disruptions to the functioning of organs, and organ systems so that in the end the physiological processes of fish cannot proceed as they should. Pathogenic bacteria in the fish's media and body attack the tissues of the incoming organs and attack first through the blood vessels, and spread throughout the body through blood circulation. The level of damage to organ tissues due to *Vibrio* is influenced by the level of population density of bacteria in the organ tissue, the denser the population of bacteria contained in the organ, the damage and structural changes of the tissues are faster and more severe, because the toxins produced by bacteria have dissolved the environment more and more. Basic knowledge and information regarding pathogenicity and histopathology studies of vibriosis fish disease are preliminary in the handling of bacterial diseases, such as knowledge of clinical symptoms both visually physiologically and morphologically. The best alternative prevention and the beginning of success in cultivation. Prevention can be done by carrying out good water and feed quality management, screening and monitoring of seeds or farmed animals, giving vitamins to increase fish immunity, indications of fish attacked by disease must be taken quickly and correctly by isolating and quarantining infected fish, for avoid transmission.

#### 4 Conclusion

The results of the study explained that the *V. alginolyticus* bacteria can cause mortality in tiger grouper. Fish infected with the *V. alginolyticus* bacteria may experience clinical symptoms and visually visible morphology and physiology. Clinical symptoms of fish infected with the *V. alginolyticus* bacteria look paler, loss of balance and appetite, swimming activity is slight. The density of *Vibrio* bacteria affects mortality and incubation period. The *V. alginolyticus* bacteria causes degeneration, damage and structural changes in the kidneys, gills, liver, spleen and brain organs so that it can interfere with the function and work system of these organs in the physiological processes of the fish body.

#### Suggestions

It is hoped that there will be further research on the histo-pathological study of tiger grouper fish in other target organs such as in digestive organs that are directly related to the food ingested, namely the intestines, and stomach.

#### Compliance with ethical standards

#### Acknowledgments

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