Histopathological analysis of Pangasius sp. infected by Edwardsiella tarda causes Edwardsiellosis disease

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Abstract. Pangasius sp are included in the group of catfish that have high economic value. Catfish are susceptible to infection with Edwardsiellosis caused by Edwardsiella sp. Edwardsiellosis is one of disease which causes declining of catfish production in Indonesia. Histopathological test can be one way to diagnose a disease infection by observing cell or tissue changes. The method used in this study is descriptive and sampling method by ex situ, by taking samples from the field and observing in the laboratory. The results showed tissue changes in the organs of Pangasius sp. due to bacterial infection which leads to damage hyperplasia, lamella fusion, and necrosis.

1. Introduction
Catfish (Pangasius sp.) is a type of freshwater fish that has a silver long body [1]. Based on statistical data in 2012 catfish production in Indonesia is 347,000 tons [2]. Catfish cultivation in Indonesia has developed in recent years and many have applied intensive cultivation systems [3]. Intensification of cultivation can cause air pollution in ponds and cause disease outbreaks with economic losses [4]. Intensive cultivation can cause outbreaks of disease such as Edwardsiella tarda [5].

Edwardsiellosis, caused by Edwardsiella tarda, has been reported worldwide in economically important fish species. This infection also leads to serious economic losses in the aquaculture. Edwardsiellosis in fish usually occurs under imbalanced environmental conditions, such as high water temperature, poor water quality, and high organic content. Fish infected with E. tarda show depigmentation, swelling of the abdominal surface, hemorrhage in fin and skin [6].

Histopathology is performed to provide an overview of changes in tissue infected with the disease. Diagnosis of disease infection is the first step that needs to be applied in determining the disease. Histopathology is the gold standard and only accurate way for assessing the severity of organs damage [7]. How to find out pathological changes in tissues infected with the disease, need to histopathological examination. The aim of this research is the effect of E. tarda bacterial infection on the histopathology of catfish (Pangasius sp.).

2. Materials and methods
The research method used is the experimental method. Using Complete Random Design (CRD).
Infection is carried out 7 days after being transferred from the maintenance aquarium to the research aquarium, this serves to adapt the fish. The infection of *E. tarda* bacteria was carried out by immersion method with a bacterial density of 10^7. Histopathology analysis using a microscope in the form of preparations. The first step for organ (gill, kidney, and spleen) histopathology observation in catfish is making preparations i.e. tissue preparation, tissue fixation, tissue paper, tissue embedding, sectioning, and staining.

3. Results and discussion

3.1. Clinical symptoms

The clinical symptoms of catfish infected with *E. tarda* show that the body's color depigmentation becomes more faded or pale. The dorsal and caudal fins were flaky, and inflammation occurs in the anus to the base of the tail. The caudal fin was severely bleeding. The movement of the fish slowly, more silent and passive (Figure 1B). Infected fish commonly showed loss of appetite, along with obvious symptoms including a swollen abdomen, and many streaks of blood on the body surface, a swollen liver and kidney [8]. Clinical symptoms due to *E. tarda* infection were lesions on the skin and develop into necrosis, pale color, swollen kidneys, enlarged abdomen and swollen anus [9]. Fish infected with *E. tarda* cause hemorrhage in the stomach and anus, mucus that appears excessively and necrosis in the fins. Severely infected fish will become lethargic, swim irregularly, pale gills, and enlarged liver and kidneys [10].

![Figure 1. Clinical Symptoms of Catfish. (A) Healthy catfish without any clinical symptoms of *E. tarda* infection (1B) Dorsal fin rot, (2B) Body's color depigmentation, (3B) Inflammation of the anus to base of the tail, (4B) Bleeding and necrosis on the tail.](image)

3.2. Gill histopathology

Histopathology of gill in catfish infected by *E. tarda* showed kidney tissue damages were edema, necrosis, and congestion. Edema is the gill tissue damage on the part of the secondary lamella. Secondary lamella swells filled with fluid which is a gill response due to environmental changes and disease infections (Figure. 2). Swelling of the secondary lamella containing mucus which is a gill response because of the presence of dangerous substances such as chemicals or bacterial infections of the gills. Gill organs were semipermeable so the liquid in the form of mucus can enter the gill tissue and swelling occurs in the secondary lamella [11].

Necrosis is damage to the *Pangasius* sp. gills which are characterized by blurry, unclear tissue, widening tissue so that it does not appear to be tight (Figure. 2). Necrosis is cell death which affects the function of the gill organs. Necrosis in cell death caused by disease infections and exposure to chemicals. Tissue cells that experience necrosis will lose their function. Characteristics of necrosis can be characterized by cell membranes and cell nuclei that fade [12].

The infection of *E. tarda* bacteria results in inflammation and damage to the gill tissue. Gills of fish can also experience blood damages in the gill lamella and if it continues it will cause congestion...
damage. Congestion is characterized by the damaging of blood in the gill lamella, a tightly packed blood clot and visible cell nucleus in a blood clot (Figure. 2). The presence of blood damages in the gill tissue is caused by the presence of increased blood volume in the blood vessels in the blood gill lamella depending on the level of oxygenation in the vessels [13]. Gill tissue damage in the form of congestion can be caused due to bacterial infections and the presence of chemicals in the waters. Gills are sensitive organs because they come into contact with the environment (water) and become the main target organ for disease infections. Gills are important organs that function as respiration, acid-base balance, and osmoregulation [14]. Stress is an adaptation response to changes in environmental conditions. The presence of stress and physical injury during handling can cause infectious diseases that are lethal and increase tissue damage, the level of damage can be affected by the presence of chemicals in the form.

![Figure 2. Histology of Gill Tissue in catfish (Pangasius sp.), (A) Normal Gill and (B) Gill Tissue Damage after E. tarda Infection.](image)

Infection caused damage to the gill tissue. Bacterial infection result in damage to the gills. Fish infected with gill bacteria appear swollen at autopsy, with hyperemic gill tissue protruding under the operculum. White to gray spots can be seen on the affected gill. Batches of bacteria organism can be firmly attached to the gill tissue. Infection can be unilateral or bilateral. The gill can be affected, giving a pale appearance initially confined mostly to the tips of the gill filament. This latter gradually progresses to the outer part. Bacteria can result to hyper-acute infection, if bacterial organisms invade the blood system through the gill or skin abrasion [16].

3.3. Kidney histopathology

The kidneys have the main function of fish. First, excreting most of the final product of the body's metabolism, and secondly, adjusting the concentration of body fluids. The kidney was composed normally of numerous renal corpuscles with glomerulus, Bowman's capsule and a system of tubules [17].
Histopathology of kidney in catfish infected by *E. tarda* showed kidney tissue damages were necrosis and edema. *E. tarda* was *edwardsiellosis* disease-causing bacteria on catfish. *E. tarda* secrete dermatotoxins and hemolysins which may confer pathogenicity on *E. tarda*. Certainly, the ability of *E. tarda* to produce toxin is an important part of the infection process. Both types of toxins produced are antigenic and are not found in the fish immune system. Toxins that have entered the body of the fish will cause necrosis and production of gas in the stomach. The liver and kidneys of the most examined fish showed congestion and edema [18]. Toxins produced by pathogenic bacterium and extracellular products such as hemolysin and elastase may cause severe necrosis in the kidney [19]. The pathology related to the *edwardsiellosis* in fish includes the presence of distended abdomen, cutaneous lesions inside the musculature, presence of gas pockets in the kidney and necrosis of renal tissue [20].

Necrotic renal tubules characterized by pyknotic or karyorrhectic nuclei (Figure 3B). Tubules were also replaced by granulomas containing pathogenic bacteria [21]. Common tissue damage found in the fish kidney infected pathogenic bacteria were degenerative and necrotic in the renal tubules and edema in Bowman’s capsules with atrophy in the glomeruli. The necrosis of the renal tubules affects metabolic activities and promotes metabolic abnormalities in fish [22].

Edema is damage to tissue filled with fluid so that the part enlarges and cannot function properly. Edematous be marked by infiltration of fluid between the tubules, hemorrhage, and diffusion the erythrocytes in the interstitial fluid [23]. Clinically edema in kidney cells is caused by erasification of proteins in the part of the renal tubules in the tissue. Kidney cells that have edema are usually in the tubules to glomerulus, and blood cells appear to bleed due to bacterial infection [24].

### 3.4. Spleen histopathology

Spleen is one of the organs that functions to fight bacterial infection through the immune system which is produced by the spleen. In histological structures, the spleen of catfish is macrophage cells. The spleen of fish is involved in the immune responses to pollutants and infection by bacteria [25]. In fish, spleen have important functions in clearing particulate and other antigens, microorganisms and aged red cells from the blood [26]. The structure of spleen is composed of white pulp and red pulp. The white pulp consists of T and B lymphocytes. The red pulp which filters the blood of foreign material and damaged erythrocytes [27].
Figure 4. Histology of Spleen tissue in catfish (Pangasius sp.), (A) Normal Spleen, (B) Spleen Tissue Damage after E.tarda infection. Olympus microscope with 400x magnification.

The results of histopathological analysis showed tissue damage including necrosis and vacuolization. The histopathological examination showed changes in the spleen because this organ infected by E. tarda. In the spleen, found vacuolization indicates injured on spleen tissue [28]. Vacuolation due to degeneration of cytoplasm that happened spleen tissue (Figure 4). Fat accumulation in the cytoplasm and causes the spleen organ to not work properly [29]. Vacuolization is also indicated by the disappearance of the boundary between red and white pulp [30].

There have been a few histopathological studies of bacteria in catfish, bacterial infection in fish causes changes in tissue structure [31]. For example in the lymphatic organs, found hemorrhage due to bacterial infection [32]. On this results of observations, it was found that necrosis damage in spleen by catfish. Secondary infection by bacteria causes damage to necrosis of the spleen organ [33]. Necrosis is cell death in a tissue. The research, parts of cells that necrosis damage and eventually become lysis cells (Figure 4) [34].

4. Conclusion
Infection of E.tarda causes tissue damage to the gills, kidneys, and spleen organs with this type of damage that is edema; Fusion lamella; necrosis; congestion, necrosis; edema; and necrosis; vacuolization respectively.

5. References
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