Iraqi Journal of Veterinary Sciences, Vol. 39, Supplement III, 2025 (207-215)
Proceedings of the 4th International Conference on Veterinary Medicine and Health Sciences (ICVMHS), Airlangga University

Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Macroscopic and microscopic analysis of Aeromonas hydrophila infection in Nile tilapia (*Oreochromis niloticus*) in traditional fish farms in Surabaya

K. Desiandura, R. Solfaine, I. Rahmawati and A. Arifin

Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, East Java, 60225, Indonesia

Article information

Article history:
Received 10 October 2024
Accepted 08 December 2024
Published 05 October 2025

Keywords:
Aeromonas hydrophila sp
Nile tilapia
Histopathology
Microbiology
Hematology

Correspondence:
K. Desiandura
kurniadesiandura@uwks.ac.id

Abstract

Through a comprehensive pathological analysis, this study investigates bacterial diseases in Nile tilapia (*Oreochromis niloticus*). *Nile tilapia* is a freshwater fish commonly cultivated and has great potential to be developed for fisheries businesses in Indonesia because it has high economic value. However, this business is open to various problems, like pests and diseases. These problems have negative impacts, such as a decrease in production, a decrease in water quality, the death of many fish or total death. The cause of the disease comes from pathogenic agents, such as viruses, parasites and bacteria. The fish, owned by a traditional farmer in Surabaya, exhibited clinical symptoms such as lethargy, surface swimming, hemorrhage in fins and abdomen, exophthalmia, and jaundice. Laboratory examinations included macroscopic (anatomic pathology) and microscopic (histopathology) assessments, microbiology and clinical pathology (hematology) analyses. The result of microbiology analysis was that the fish were infected by the bacteria *Aeromonas hydrophila sp*. The macroscopic and histopathology results are pale gills and rupture in the secondary lamella with telangiectasis and erosion of the primary lamella, hemorrhage, necrosis and infiltration of inflammatory cells. The liver organ is pale yellowish, necrotic and hemorrhagic hepatitis and hemosiderosis, and the pectoral fin is hemorrhage and inflammatory cell infiltration. The clinical pathological results are macrocytic hypochromic anemia, hyperfibrinogenemia, neutropenia, lymphocytopenia, monocytopenia, and basophilia.

DOI: 10.33899/ijvs.2024.154294.3948,

Introduction

Tilapia was first cultivated in Kenya in 1924. It is one of the most popular fish to be widely cultivated, and it contributes to improving local livelihoods, especially in developing countries (1). More than 140 countries use Tilapia for commercial trade and fish farming (2). Tilapia cultivation reaches 6 million tons (MT), placing Tilapia in second place as the world's most widely cultivated freshwater fish after carp (3). There are around 70 species of Tilapia that have been identified globally, and Nile tilapia (*Oreochromis niloticus L.*) is the most widely cultivated species, so various efforts are needed to ensure the quality and quantity of Tilapia needed by the community (4). In line with the development of cultivation businesses, several disturbing problems, such as pests and diseases, hinder the development of cultivation businesses. Various diseases are the main problem in the cultivation process because of various negative impacts, such as decreased production, decreased water quality and even total death. This disease can be caused by several pathogenic agents such as viruses, parasites or bacteria (5). One of the bacterial diseases that often becomes an obstacle in tilapia cultivation is *Aeromonas hydrophila (A. hydrophila)*. Aeromonas is a gram-negative bacterium that belongs to the *Aeromonadaceae* family of *Gammaproteobacteria*, motile bacilli or coccobacilli rods,

non-spore-forming with rounded ends that size 1–3.5 µm (6). Haemolysin, aerolysin, cytosine, gelatinase, enterotoxin and antimicrobial peptides have been identified as virulence factors in *A. hydrophila*. The habitat of these bacteria is often found in freshwater, aquatic plants and fish bodies, and it is known as Motile Aeromonas Septicemia (7) or Hemorrhagic Septicemia. This disease is common in tropical fish such as Nile tilapia, gourami, and goldfish. Infected fish usually have bruises on their bodies but can also show other signs, such as protrusion of the eyeballs (exophthalmia) and bleeding in various parts of the body. Another condition is a red mouth, bloated abdomen, blood on the exterior surface and around the anal scale sloughing, surface lesions and septicemia (8). Infected fish with open wounds can spread this disease to other fish, and fish that appear healthy but carry this disease (sub clinical carriers) may be present and will release the bacteria in their feces into the aquatic environment. Aeromonas bacteria are opportunistic human pathogens that can cause septicemia, wound infections, and gastroenteritis, especially in children (9). Like other enteropathogenic bacteria, the pathogenicity of Aeromonas bacteria is always associated with mechanisms for producing toxins, such as cytotoxins and enterotoxins (including those with hemolytic activity) and attachment to host tissue. Apart from toxins, Aeromonas bacteria also produce other extracellular fluids such as protease, amylase, chitinase, lipase and nuclease (10).

This study aims to be a comprehensive analysis whose results can provide insight into the effects of *Aeromonas hydrophila* bacterial infection on Tilapia, which affects aspects of blood results and macroscopic and microscopic tilapia organ tissue. These findings also contribute to a better understanding of the etiology and pathogenesis of the observed clinical manifestations, guiding future research and intervention strategies in fish health management.

Materials and methods

Ethical approval

All treatments on animals have been approved by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, University of Wijaya Kusuma (No. 157 – KKE).

Study period and location

This research was carried out from April to May 2024. The research used the biosurvey method with fish sampling locations at Kayoon Fish Market, Gunungsari and Bratang, Surabaya, East Java. Necropsy and preparation for sampling organs, microbiology and hematology analysis at the Pathology, Microbiology and Clinical Pathology Laboratory of the University of Wijaya Kusuma Surabaya. Histopathology preparations were made at the Integrated Research Laboratory, Faculty of Dentistry, Gajah Mada University.

Taking of sample

A sampling of Tilapia fish in freshwater weighing 950-1000 g, and physically, there are abnormal lesions around its body. The method used to examine Tilapia with protocol number F-19 consists of necropsy and blood sampling. Macroscopic and microscopic organ examinations (histopathology) were carried out. Microbiological examination consists of Tryptone Soya Agar (TSA) media or Mac Conkey Agar (MCA) media, gram staining, catalase test, then continued with biochemical tests including Triple Sugar Iron Agar (TSIA), Simmon's Citrate Agar (SCA), Urease, Sulfide Indole Motility (SIM) (11), and clinical pathology examination with hematology analysis which includes observing the number of erythrocytes/red blood cells (RBC), leukocytes/white blood cells (WBC), hemoglobin, hematocrit/packed cell volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), initial and final Total Plasma Protein (TPP), and differential calculations.

Making histopathology preparations

Organ samples were fixed with 10% BNF (Buffered Neutral Formalin) for 24-48 hours and then processed for histology preparations by rehydrating using graded alcohol, embedding in paraffin, and cutting to a thickness of 5 microns. Then, the samples were painted using Haematoxylin and Eosin (HE). The parameters observed were inflammatory cell infiltration, hemorrhage, necrosis, and abnormalities in the organ (12).

Results

Pathology examination

Tilapia fish with protocol number F-19 (because of the category of fish) have a body weight of 1 kg with anamnesis; the fish aquarium is cleaned once a week. Nine fish are in one large aquarium, but they must be more specific about the material and size. They are given pellet food. The clinical symptoms are that the fish looks weak, swims to the surface has reddish spots (hemorrhage) on the surface of the body, and the fish's eyes become bulging (exophthalmia) and yellowish (icterus). The results of a macroscopic examination of the organs (anatomical pathology) show that there is an ecchymosis hemorrhage on the abdomen and pectoral fin of the fish, and the gills look pale and ruptured. The liver organ is pale yellowish (icterus), there is linear hemorrhage, a rubbery consistency, and the incision area is wet. Meanwhile, his tail experienced linear hemorrhage (Figure 1).

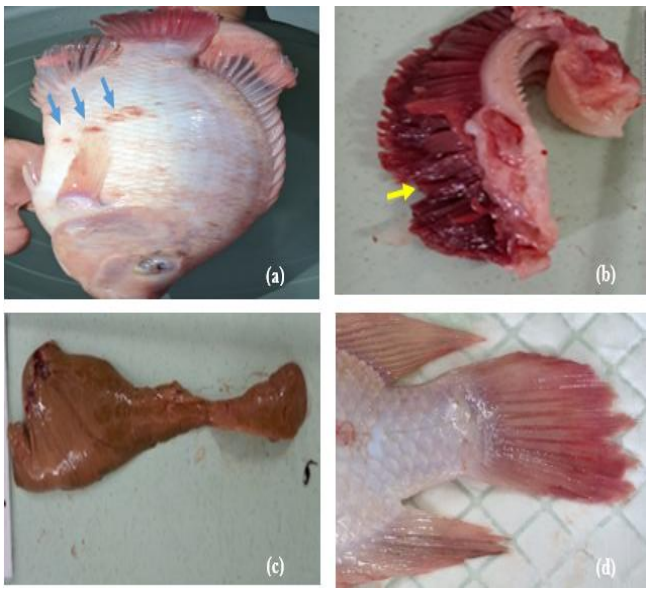

Figure 1: Results of Macroscopic Examination of Tilapia. (a) Abdominal ecchymoses hemorrhage (blue arrow). (b) Ruptured gills (yellow arrow). (c) Liver. (d) Tail.

Based on macroscopic observations, there were changes in the gills, liver and tail, so we continued with histopathological observations. The following is a histopathological description of the gills, liver and tail skin of the F-19 Tilapia fish. There is also a microscopic examination of gills experiencing rupture in the secondary lamella, telangiectasis, erosion of the primary lamella, hemorrhage, necrosis, and accompanied by fluid and inflammatory cell infiltration. The microscopic examination of the liver is congested in the hepatic portal vein and synocytic congestion, hemorrhage, inflammatory cell infiltration and hemosiderosis. Meanwhile, microscopically, the tail skin experienced inflammatory cell infiltration (Figure 2).

Microbiology examination

In diagnosing the cases of *Aeromonas hydrophila*, a swab is carried out on the eye, kidney and liver organs that are experiencing abnormalities. Then, the swab results are cultured on cultural media, such as for sample screening. The culture media was then incubated at 37°C for colonies to appear when examined on Tryptone Soya Agar (TSA) media. *Aeromonas hydrophila* is a gram-negative bacterium, has a short rod morphology with varying sizes (width: 0.8-1.0 microns; length: 1.0-3.5 microns), does not have spores, bacteria are motile because they have one flagellum (monotrichous flagella), which comes out from one of their poles. The surface morphology of the colony is slightly prominent, round, shiny, and creamy, with entire colony edges and a diameter of 1-3 mm (Table 1).

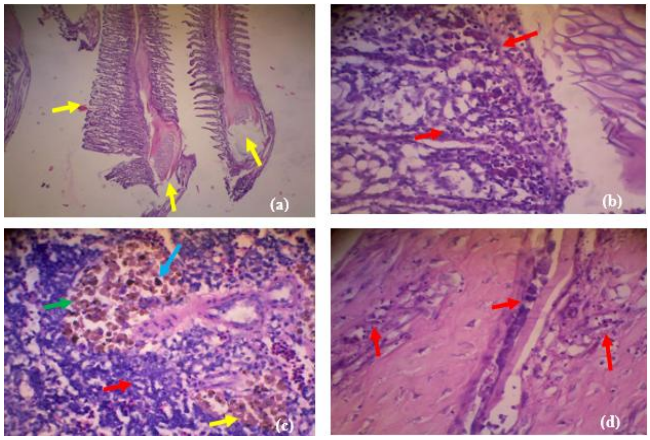

Figure 2: (a) & (b) Gills histopathology. (c) Liver histopathology. (d) Microscopic view of the tail of the skin. (HE stain 40-100×).

Table 1: Biochemical Test Results

No.	Biochemical Test	*A. Hydrophila*
1	Katalase	+
2	TSIA	+
3	SCA	+
4	Urease	-
5	SIM	+
6	MR	+
7	VP	-

In the subsequent examination, gram staining was carried out after bacterial colonies had formed for 24 hours; then, to see the morphology of the colonies formed, gram staining was carried out. This test aims to determine the nature of the bacteria being tested. The nature of gram-positive bacteria is characterized by purple-coloured bacterial cells, and the gram-negative bacteria are red/pink-coloured. The primary characterization *of A. hydrophila* is gram-negative, rod-shaped, motile, non-capsulated and non-sporulated. Can appear singly or in pairs with or without short chains. After gram staining identifies the sample, biochemical tests are continued. The media used for the biochemical tests are the catalase test, Triple Sugar Iron Agar (TSIA), Simmon's Citrate Agar (SCA), Urease, Sulfide Indol Methility (SIM), and Methyl Voges Proskauer (MR—VP), which will be incubated for 24 hours. After 24 hours, the changes observed were observed (Figures 3 and 4).

Haematology examination

Based on the results of blood tests in the clinical pathology laboratory, it was discovered that Tilapia with protocol number F-19 was anemic, macrocytic, hypochromic, hypoproteinemic, hyperfibrinogenemic, neutrophilia, lymphocytopenia, monocytopenia and basophilia. On blood smear or hematology examination,

there are abnormalities in erythrocytes such as echinocytes, teardrops and sickle cells (Table 2).

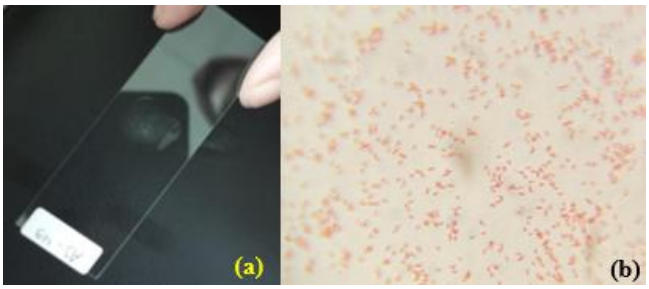

Figure 3: (a) Preparation of gram staining (b) Microscopic examination with gram staining.

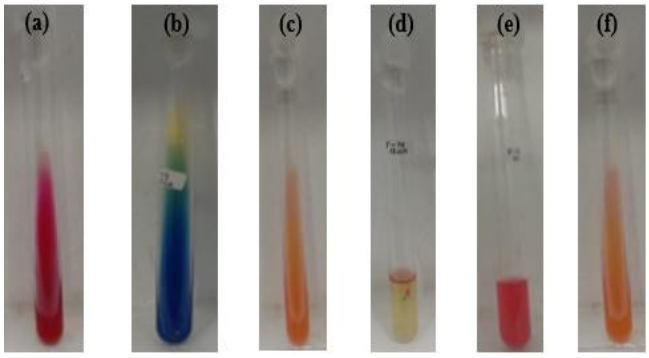

Figure 4: Biochemical tests (a) TSIA, (b) SCA, (c) Urease, (d) SIM, (e) MR, (f) VP.

Table 2: Tilapia hematology examination results

Item	Result	Unit	Reference	Notes	Interpretation
RBC	0,51	10^6 /µL	0,2-3*	Normal	Normal
Hb	4	g/dL	6-10*	Low	Anemia
PCV	22	%	22-60*	Normal	Normal
MCV	431,37	fL	80-100*	High	Macrocytic
MCH	78,43	Pg	26-32*	High	-
MCHC	18,18	%	25-35*	Low	Hypochromic
TPP Awal	2,4	g/dL	1,8-3,2*	Normal	Normal
TPP Akhir	2	g/dL	1,5-3,0*	Normal	Normal
Fibrinogen	0,4	g/dL	0,1-0,2*	High	Hyperfibrinogenemia
WBC	9,35	10^3 /µL	5-13*	Normal	Normal

Discussion

Histopathological examination showed gill rupture in the secondary lamella. If there is a rupture or damage to the lamella, it will disrupt blood circulation and gas exchange. Ion exchange in the lamellae can transfer 60-80% of oxygen from water into the blood (13). If damage like this occurs over time, it will disrupt the circulation system, which can cause the fish to lack oxygen supply, which in turn will cause a lethal effect on the fish due to disruption of the fish's respiratory system (13). Histopathological examination of the gill organs also found telangiectasis. Telengiectasis is the dilatation or widening of blood vessels in fish's gills. In other conditions, it was dilated postcapillary venules in the papillary and superficial reticular dermis (14). Telengiectasis is a condition where the capillaries in the gills become swollen. This condition can be caused by various factors, including infection with the *Aeromonas hydrophila* bacteria. In *A. hydrophila* infection, the bacteria invade the gill epithelial cells (15). These bacteria then release toxic substances that cause damage to epithelial cells. The injury to these epithelial cells causes swelling of the capillaries. Thus, swelling of these capillaries disrupts blood flow to the gills. This can cause hypoxia, a condition where the gills do not get enough oxygen. Hypoxia is a significant threat because it can cause fish to die (15).

Histopathological examination of the gill organs also revealed necrosis and erosion of the gills. As a result of the hypoxic condition or other toxins, the mitochondria in the cells are damaged so that they cannot be repaired, and swelling of the cell organelles (in Yunani known as"Oncosis"), rupture of the plasma membrane and then necrosis occurs (16). Necrosis is irreversible or irreversible cell death. Necrosis can occur in cells in all body tissues, including fish. Necrosis is often associated with inflammatory reactions. Necrosis can be caused by hypoxia, physical factors, chemical agents, immunology and infectious or microbial agents such as *A. hydrophila* (17). The histopathology of the gills showed necrosis and was characterized by dead and destroyed gill cells. These dead gill cells will look pale or white and have an irregular shape. Apart from that, A. hydrophila bacteria can also be seen in the infection of gill cells. In severe infections, necrosis can cause extensive damage to the gills. This can cause the fish to have difficulty breathing and eventually die. *Aeromonas hydrophila* bacteria are Gram-negative bacteria that can cause various diseases in fish, including gill necrosis. These bacteria can enter the fish's body through wounds or injured

skin. After entering the fish body, these bacteria will attack the gill cells and cause them to die. Necrosis The microscopic picture of erosion on the gills of fish infected with *A. hydrophila* bacteria shows damage to the gill tissue. This damage can include cell detachment, white blood cell infiltration, and ulcer formation. Erosion of gills infected with *A. hydrophila* bacteria can cause various health problems in fish. Infected fish can experience difficulty breathing, loss of appetite, and death (18)

Echymoses hemorrhage of the abdomen and microscopic examination of the liver showed congestion, hemorrhage and hemosiderosis. The area around the liver portal vein also experiences hemorrhage characterized by an accumulation of red blood cells (erythrocytes) coming out of the lumen of the blood vessels. Hemorrhage is an outcome of blood channel disruption and is a symptom of severe physical impairment (19). The occurrence of congestion and hemorrhage in the liver indicates that the blood vessels in the liver are damaged. Congestion can be interpreted as a condition where red blood cells (erythrocytes) collect in the lumen of blood vessels. Other hemorrhage, vacuolization, patchy degeneration, blood congestion, and hypertrophy nucleus can also occur in the liver because the liver is an organ directly affected by toxic materials or other infectious agents (19). Apart from that, the histopathology of the liver also experiences hemosiderosis, a condition characterized by a buildup of hemosiderin in the fish liver. Hemosiderin is a protein that contains iron, and a buildup of hemosiderin in the liver can cause liver damage. Microscopically, fish liver hemosiderosis is characterized by hemosiderin pigment in the liver cells. This pigment can be seen as black or brown spots in the cytoplasm of liver cells (20).

On microscopic examination, the fishtail organ shows inflammatory cell infiltration, which can be caused by various factors, such as bacterial, viral or parasitic infections. Infection can cause histological changes such as hepatocyte vacuolization, glycogen depletion, inflammation, changes in the shape of sinusoidal vessels, and neoplasms, which can be interpreted as a response to environmental stress or other pathological processes (21). On microscopic observation, inflammatory cell infiltration is characterized by a collection of inflammatory cells in the organ tissue. The inflammatory cells most often found in this case are neutrophils, which are white blood cells that play a role in fighting infection. In the examination carried out on the case of *A. hydrophila* tilapia fish with protocol number F-19, the symptoms were red spots on the body surface, rotting fins and tail, peeling scales and protruding eyes. These symptoms were similar to the research (22).

The results of hematology test, the erythrocytes (RBC), 0.51 x 10/µL, are within the normal range. Erythrocytes in Tilapia infected with *A. hydrophila* bacteria experienced a significant decrease in erythrocytes. Usually, a decrease in erythrocytes is also followed by a decrease in hematocrit (23,24). The erythrocyte value of Tilapia infected with Aeromonas hydrophila bacteria ranges from 0.02-3 x 106 cells/mm3. This decrease in erythrocytes is caused by hemolysis, which breaks down red blood cells. Hemolysis can be caused by toxins produced by the bacteria *A. hydrophila*.

The catalase test results show a positive reaction as indicated by bubbles (gas) forming in the tube or glass object. Bacteria can produce catalase and oxidase enzymes in the catalase and oxidase test. A positive sign is obtained because the bacteria can produce catalase and oxidase enzymes (25), and the genus *Aeromonas* has gram-negative characteristics, fermentative, oxidase-positive and catalase-positive (26). The TSIA (Tryple Sugar Iron Agar) test results include sucrose, lactose and glucose. The working principle of this test is to detect bacteria that can ferment lactose, sucrose and glucose. In the TSIA test results, the Alk/Alk and H2S (-) results were obtained. The TSIA test results on *Aeromonas* bacteria can be observed based on changes in the colour and shape of the bacterial colony. The colour changes that occur in TSIA media can indicate the ability of bacteria to ferment carbohydrates and produce acid. Interpretation of TSIA test results on Aeromonas bacteria with red colonies indicates that the bacteria are positive for fermenting glucose and producing acid (27).

The SCA test showed the colour change from green to blue. SCA is a slant agar medium with a solid surface, making it easy to determine the significance of growth. Because the use of citrate requires oxygen, this slant pattern can increase the amount of growth. The increase in pH of the medium after the decomposition of citrate can be detected by the colour change of BTB (Brom Timol Blue) that co-occurs (28). The result of the SCA biochemical test was positive. The SCA test results showed that the fish samples were positive. This is indicated by a change in the colour of the SCA media from green to blue. The colour change of the SCA media from green to blue is caused by the formation of citric acid from the citrate metabolism by bacteria. The results of this study indicate that the bacteria *Aeromonas*. contained in tilapia fish can utilize citrate as a carbon and energy source. This shows that *Aeromonas* bacteria can cause disease in fish.

The urease test shows negative results because positivity is indicated by a change in the colour of the media from yellow to pink (29). A negative urease test result indicates that the bacterial isolate does not produce the urease enzyme, which breaks down urea into ammonia and carbon dioxide. Negative results on the urease test indicate that the bacterial isolate cannot break down urea (30).

In the Sulfide Indole Motility Test (SIM), the results showed a red ring after being dropped on the Kovacs reagent, showing a positive result where the bacteria formed indole and there was no motility (31). Biochemically tested *Aeromonas hydrophilia* showed positive results. The motility test is carried out to determine the movement of a microorganism. Motility is seen by the spread of germ

growth at the puncture site or the appearance of cloudy media. The results of this study indicate that the SIM test is an effective method for identifying *Aeromonas hydrophila* bacteria in fish. Other research results support this by showing that the SIM test has high sensitivity and specificity for identifying *Aeromonas hydrophila* bacteria (32).

A positive Methyl Red (Mr) test result indicates a change to red after methyl red is dropped. The research results show that the *Aeromonas* bacteria isolate is positive in the MR test. MR media containing *Aeromonas* bacterial isolates changed colour to red after incubation for 24 hours. This colour change is caused by the production of acetic acid by *Aeromonas* bacteria from glucose fermentation. A positive result indicates that there is no mixed fermentation in the isolate. The bacteria ferment glucose and produce various acidic substances (33,34)

The results of the Voges Proskauer (VP) biochemical test after planting bacteria and incubating them for 24 hours in an incubator and adding 10% KOH reagent and Alpanapthol showed no change, the results of the VP test on *Aeromonas* bacteria found in fish showed negative results. The bacteria cannot ferment glucose by producing acetic acid and pyruvic acid. These results are consistent with the results of other studies, which show that *Aeromonas hydrophila*, one of the most commonly found Aeromonas species, has negative VP test results (35).

The results also showed low hemoglobin (Hb). Erythrocytes produce hemoglobin during the maturation process. Heme synthesis occurs in mitochondria, while globin is produced in ribosomes (36). Other factors that can influence Hb concentration include the adequacy of iron in the body. Iron is needed to produce hemoglobin, so anemia due to iron deficiency will cause the formation of smaller erythrocytes with a low Hb content. Iron is an important substance for the formation of hemoglobin (Hb). The function of iron as a medium for transporting, storing, and utilizing oxygen as hemoglobin, myoglobin, or cytochrome is to meet the needs of hemoglobin formation (37). Hemoglobin levels also affect erythrocyte and hematocrit levels. The relationship between hemoglobin and hematocrit is that erythrocytes contain Hb, which binds oxygen and is used for the catabolism process to produce energy. The fewer erythrocyte cells, the lower the hemoglobin levels in the blood (38). In fish, *A. hydrophila* bacteria produce hemolysin, a toxin that can break down red blood cells. Hemolysin damages the red blood cell membrane, releasing hemoglobin and entering the blood plasma. Low hemoglobin levels in fish blood can cause fish to experience hypoxia, a condition of lack of oxygen. Hypoxia can cause various health problems in fish, such as reduced growth, decreased appetite, and even death (39).

The decrease in PCV values in Tilapia infected with *A. hydrophila* is thought to be caused by several factors, namely, hemolysis in red blood cells, decreased production of red blood cells by the bone marrow and increased capillary permeability due to infection with the *Aeromonas hydrophila* bacteria (40). The increased MCV calculation results (431.37 fL) show that the volume of erythrocytes or large erythrocyte size will affect the viscosity of blood fluid, thus disrupting the activity and smooth circulation of blood (41). The normal average MCV value is 80-100 fL. Mean cell volume (MCV) is the average volume of red blood cells. It is calculated from hematocrit (HCT) and erythrocyte (RBC) count. Low MCV indicates microcytic (small RBC size), but high MCV indicates macrocytic (large RBC size) (42). The increase in the MCV value of 431.37 fL from the normal value. So, we can interpret it as indicating macrocytic anemia. It is caused by excessive production of red blood cells, accumulation of immature red blood cells and changes in red blood cell metabolism (43). At the same time, MCHC is decreased in fish with *A. hydrophila* bacteria and is usually caused by iron or protein deficiency. This is because *A. hydrophila* is a facultative anaerobic bacterium. These bacteria can grow in both aerobic and anaerobic conditions. Under aerobic conditions, these bacteria will use oxygen for respiration. However, under anaerobic conditions, these bacteria will use iron or protein as a substitute for oxygen (44).

WBCs, known as leukocytes, are a heterogeneous population of cells, including lymphocytes, monocytes and granulocytes consisting of neutrophils, eosinophils and basophils. In the differential counting calculation, F-19 tilapia experienced an increase in lymphocytes and a decrease in eosinophils. Neutrophils decrease in fish infected with *Aeromonas hydrophila* bacteria because these bacteria releases toxins that can damage white blood cells, including neutrophils. Neutrophils are white blood cells that play an important role in the fish's immune system. The main function of neutrophils is to fight bacterial infections (45). A decrease in the number of lymphocytes will cause fish to become more susceptible to infection. Fish infected with *A. hydrophila* will usually experience symptoms such as skin wounds, bleeding, and death (46). A decrease in monocytes in fish infected with *A. hydrophila* bacteria can be an early indicator of infection. A decreased number of monocytes can cause fish to be more susceptible to secondary infection and death. The bone marrow produces eosinophils in fish and then circulates in the fish in the blood. When fish are exposed to parasites, eosinophils will move to the site of infection and release granules containing antiparasitic substances. Eosinophils have been traditionally associated with initiating and propagating inflammatory responses, particularly in allergic diseases and helminth infections (47). Basophils increase in fish infected with *A. hydrophila* bacteria. This is an immune response from the fish body to fight the bacterial infection.

Reticulocytes are young red blood cells originating from the bone marrow's normoblast maturation process. Reticulocytes will enter the peripheral blood circulation and survive for approximately 24 hours before finally maturing into erythrocytes. The increase in *A. hydrophila* bacterial

reticulocytes in Tilapia is caused by the fish's immune response. Reticulocytes are young erythrocytes that still contain a nucleus. An increase in the number of reticulocytes is a sign of the fish body's nonspecific immune response to bacterial infections (48). Thrombocytopenia can occur in the bacteremia phase, and long-term disorders of bone marrow reproduction can cause platelet deficiency. Anemia can cause a decrease in platelet production when the bone marrow no longer produces enough red blood cells for the body. Factors that cause internal and external hemorrhage can also cause thrombocytopenia (49-51).

Conclusion

Nile Tilapia is from traditional fish farms in Surabaya, which are infected by *A. hydrophila* bacteria. Comprehensive pathological analysis is important for examining infected fish. Through microbiological examination to find and grow bacteria in the media, gram staining and biochemical tests prove the presence of these bacteria. During macroscopic and microscopic examinations, there were abnormal lesions such as necrosis, hemorrhage, and inflammation of the fish's gills, liver, and tail skin. In addition, the hematology results showed macrocytic anemia, hypochromia, hyperfibrinogenemia, neutropenia, lymphocytopenia, monocytopenia and basophilia.

Acknowledgment

The authors thank to the University of Wijaya Kusuma Surabaya for supporting this research.

Conflict of interest

There is no conflict of interest.

References

1. Munguti MJ, Nairuti R, Iteba JO, Obiero KO, Kyule D, Opiyo MA, Abwo J, Kirimi JG, Outa N, Muthoka M, Githukia CM, Ogello EO. Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) culture in Kenya: Emerging production technologies and socioeconomic impacts on local livelihoods. Aquac Fish Fish. 2022;2(4):265-276. DOI: 10.1002/aff2.58
2. Kaleem O, Sabi AF. Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. Aquac Fish. 2021;6(6):535–547. DOI: 10.1016/j.aaf.2020.07.017
3. Abwao J, Jung'a J, Barasa JE, Kyule D, Opiyo M, Awuor JF, Keya GA. Selective breeding of Nile tilapia, *Oreochromis niloticus*: A strategy for increased genetic diversity and sustainable development of aquaculture in Kenya. J Appl Aquac. 2021;35(2):237–256. DOI: 10.1080/10454438.2021.1958728
4. Bentsen HB, Gjerde B, Nguyen NH, Rye M, Ponzoni RW, de Vera MP, Bolivar HL, Velasco RR, Danting JC, Dionisio EE, Longalong FM, Reyes RA, Abella TA, Tayamen MM, Eknath AE. Genetic improvement of farmed tilapias: Genetic parameters for bodyweight at harvest in Nile tilapia (*Oreochromis niloticus*) during five generations of testing in multiple environments. Aquac. 2012;338:56–65. DOI: 10.1016/j.aquaculture.2012.01.027
5. Magouz FI, Moustafa EM, Abo-Remela EM, Marwa RH, Passant M, Amira AO. Summer mortality syndrome bacterial pathogens infarmed Nile tilapia (*Oreochromis niloticus*). Open Vet J. 2024;14(1):53–69. DOI: 10.5455/OVJ.2024.v14.i1.7
6. Dias C, Mota V, Martinez-Murcia A, Saavedra MJ. Antimicrobial resistance patterns of *Aeromonas spp.* isolated from ornamental fish. J Aquac Res Dev. 2012;3(3). DOI: 10.4172/2155-9546.1000131
7. Semwal A, Kumar A, Kumar N. A Review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. Heliyon. 2023;9(3):e14088. DOI: 10.1016/j.heliyon.2023.e14088
8. Rashid MM, Hossain MS, Ali MF. Isolation and identification of *Aeromonas hydrophila* from silver carp and its culture environment from Mymensingh region. J Bangladesh Agric Univ. 2013;11(2):373–376. DOI: 10.3329/jbau.v11i2.19943
9. Greiner M, Anagnostopoulos A, Pohl D, Zbinden R, Zbinden A. A rare case of severe gastroenteritis caused by *Aeromonas hydrophila* after colectomy in a patient with anti-Hu syndrome: A case report. BMC Infect Dis. 2021;21(1):1097. DOI: 10.1186/s12879-021-06784-3
10. Wahjuningrum D, Solikhah EH, Budiardi T, Setiawati M. Control of *Aeromonas hydrophila* infection in dumbo catfish (*Clarias sp.*) with a mixture of Meniran (*Phyllanthus niruri*) and garlic (*Allium sativum*) in feed. Indonesian J Aquac. 2010;9(2):93-103. [available at]
11. Markey BK, Leonard FC, Archambault M, Cullinane A, Maguire D. Provides information on the materials and methods of bacteriology, mycology, and virology. In: Markey BK, Leonard FC, Archambault M, Cullinane A, Maguire D, editors. Clinical veterinary microbiology. UK: Elsevier; 2013. 920 p.
12. Windarti and Simarmata AH. 2015. Buku Ajar Struktur Jaringan. Pekanbaru, Indonesia: Penerbit Unri Press.
13. Hoole D, Bucke D, Burgess P, Wellby I. Diseases of carp and other cyprinid fishes. UK: Blackwell Science Ltd; 2001. 531–535 p. DOI: 10.1017/S0031182001228984
14. Walker JG, Stirling J, Beroukas D, Dharmapatni K, Haynes DR, Smith MD, Ahern MJ, Thomson PR. Histopathological and ultrastructural features of dermal telangiectasias in systemic sclerosis. Pathol. 2005;37(3):220-225. DOI: 10.1080/00313020500033262
15. Yang Y, Wang Z, Wang J, Mu W, Lyu F, Xu K. Histopathological, hematological, and biochemical changes in high-latitude fish *Phoxinus lagowskii* exposed to hypoxia. Fish Physiol Biochem. 2021;47(4):1-20. DOI: 10.1007/s10695-021-00947-4
16. Kumar V, Abbas AK, Aster JC, Cotran RS, Robbins SL. Robbins and Cotran. Pathologic Basis of Disease. 9th ed. USA: Elsevier; 2015.
17. Fathima SD, Gururaj N, Sivapathasundharam B, Vennila AA, Lavanya MKK, Sarayushivani U. Histopathological significance of necrosis in oral lesions: A review. J Oral Maxillofac Pathol. 2023;27(2):340-347. DOI: 10.4103/jomfp.jomfp_39_23
18. Giancarlo A. The effect of the degree of argulus infestation on the histopathological picture of the gills, fins, and skin of koi fish (*Cyprinus carpio*) (case study in Mungkid and Muntilan district, Magelang regency, central Java [Ph.D. dissertation]. Indonesia: Faculty of Fisheries and Marine Affairs, Universitas Airlangga. 2017. [available at]
19. Hasan J, Ferdous SR, Rabiya SA, Hossain MF, Hasan AM, Shahjahan M. Histopathological responses and recovery in gills and liver of Nile tilapia (*Oreochromis niloticus*) exposed to diesel oil. Toxicol Rep. 2022;9:1863-1868. DOI: 10.1016/j.toxrep.2022.10.005
20. Azis SP. Molecular markers in the selection of catfish resistant to *Aeromonas hydrophila* infection. Indonesia: Jakad Media Publishing; 2019. [available at]
21. Araújo FG, Gomes ID, Nascimento AA, Santos MJ, Sales A. Histopathological analysis of liver of the catfish *Pimelodus maculatus* in a tropical eutrophic reservoir from Southeastern Brazil.

Acta Sci Biol Sci. 2019;41:e41039. DOI: 10.4025/actascibiolsci.v41i1.41039
22. Tantu W, Tumbol RA, Longdong SJ. Detection of the presence *Aeromonas sp* on Nile tilapia cultured in floating net cage in lake Tondano. Budidaya Perairan. 2013;1(3):74-80. [available at]
23. Yazdanpanah GL, Rokhbakhsh ZF, Zorriehzahra MJ, Kazemipour N, Kheirkhah B. Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss*. Iran J Fish Sci. 2020;19(5):2422-2436. DOI: 10.22092/ijfs.2020.122060
24. Janda JM, Dixon A, Raucher B, Clark RB, Bottone EJ. Value of blood agar for primary plating and clinical implication of simultaneous isolation of *Aeromonas hydrophila* and *Aeromonas caviae* from a patient with gastroenteritis. J Clin Microbiol. 1984;20(6):1221-1222. DOI: 10.1128/jcm.20.6.1221-1222.1984
25. Holt JG, Krieg NR, Sneath PA, Stanley JT, William ST. Bergey's manual of determinative bacteriology. USA: Williams and Wilikins; 1994. 786-788 p.
26. Anwar ME, Tugiyono T. Isolation and identification of *Aeromonas hydrophila* bacteria in dumbo catfish (*Clarias gariepinus*). J Pertan Agros. 2023;25(3):2073-2078. [available at]
27. Anggraini R, Aliza D, Mellisa S. Identification of *Aeromonas hydrophila* bacteria using microbiological tests on dumbo catfish (*Clarias Gariepinus*) cultivated in Baitussalam district, Aceh besar regency [Ph.D. Dissertation]. Indonesia: Syiah Kuala University; 2016.
28. Williams MM. Citrate Test Protocol. USA: American Society for Microbiology; 2016.
29. Ulfa A, Suarsini E, Al Muhdhar MH. Isolation and mercury sensitivity test of bacteria from gold mining waste in West Sekotong, West Lombok Regency: Preliminary research. Proc Biol Edu Conf Biol Sci Environ Learn. 2016;13(1):793-799. [available at]
30. Ikhsan MN, Hidayati N, Mulyadi SH. Karakterisasi Aeromonas Hydrophila Yang Diisolasi Dari Ikan Nila (*Oreochromis Niloticus*). Jurnal Ilmiah Mahasiswa Kelautan Dan Perikanan Unsyiah. 2016;1(2):270-286.
31. Eissa N, Hussin K, El-Mabrok A. Isolation, identification and antibiogram of *Pasteurella multocida* isolated from apparently healthy rabbits in Al-Bayda, Libya. Benha Vet Med J. 2019;36(1):227-33. [available at]
32. Syahrizal M, & Sulistyowati I. Identifikasi Bakteri *Aeromonas hydrophila* Pada Ikan Lele Dumbo (*Clarias Gariepinus*) dengan Uji SIM. J Perikanan Dan Kelautan Tropis. 2022;15(2):101-108.
33. Dwi NR, Mulia DS, Suwarsito S, Purbomartono C. Isolasi, Karakterisasi, dan Identifikasi Bakteri Aeromonas sp. pada Lele (Clarias sp.) di Kabupaten Banyumas, Jawa Tengah. Sainteks. 2023;20(2):189-204. DOI: 10.30595/sainteks.v20i2.19557
34. Brenner DJ, Krieg NR, Staley GM. Bergey's Manual of Systematic Bacteriology. USA: Department of Microbiology and Molecular Genetic, Michigan State University, East Lansing; 2005.
35. Nurhayati S, Wulandari NI, Susanti S. Isolasi Dan Identifikasi Bakteri *Aeromonas sp*. Pada Ikan Lele (*Clarias Sp.*) Di Kabupaten Banyumas, Jawa Tengah. J Nasional Sainteks, 2023;10(2):270-286.
36. Nurfitri M, Syawal H, Lukistyowati I, Riauwaty, M. Profile Erythrocytes of *Oreochromis niloticus* Fed with Herbs Fermented and Infected with *Aeromonas hydrophila*. Asian J Aquat Sci. 2023;6(3):422-431. DOI: 10.31258/ajoas.6.3.422-431
37. Nuryati S, Kuswardani Y, Hadiroseyani Y. Effect of bee resin on blood profiles of infected *Carassius auratus* by *Aeromonas hydrophila*. J Akuakultur Indonesia. 2006;5(2):191-199. [available at]
38. Kurniawan R. Hematological and Physiological Profiles of *Pangasianodon hypophthalmus* catfish fed with the addition of herbal supplements [master thesis]. Indonesia: Faculty of Fisheries and Marine Science, Universitas Riau; 2019. 85 p.
39. Andrianti DN, Rahmawati A, Satria IB, Tarmizi A. Analysis of the resilience of dumbo Catfish (*Clarias Gariepenus*) infected with *Aeromonas hydrophila* bacteria with different concentrations. Al-Aqlu J Matematika Teknik Dan Sains. 2023;1(2):72-76. DOI: 10.59896/aqlu.v1i2.21
40. Jamal L, Wahjuningrum D, Hasan A. Use of Chitosan to Prevent *Aeromonas hydrophila* Infection on Catfish *Clarias sp.* J Akuakultur Indonesia. 2008;7(2):159-69. DOI: 10.19027/jai.7.159-169
41. Londok JR, Manalu W, Wiryawan IG, Sumiati S. Hematological Profile of broiler fed lauric acid and *Areca vestiaria* Giseke as a source of natural antioxidant. Jurnal Veteriner. 2018;19(2):222-229. DOI: 10.19087/jveteriner.2018.19.2.222
42. Al-Zahaby MA, Shalaby AM, Abd-El-Rahman GF, Ayyat MA. Impact of water quality on the blood parameters of Nile tilapia in different fish farms. Anim Poult Prod. Zagazig J Agric Res. 2017;44(2):571-581. DOI: 10.21608/zjar.2017.53875
43. Rahmi A. Respon Hematologis Ikan Bandeng, Chanos Chanos (Forskall, 1755) yang Dipapar Timbal (Pb) Pada Konsentrasi Subkronik [Ph.D. Dissertation] Indonesia: Uin Ar-Raniry Banda Aceh; 2020.
44. Nainggolan TN, Harpeni E, Santoso L. Non-specific immune response and growth performance of *Clarias gariepinus* (Burchell, 1822) feeded with *Moringa oleifera* leaf flour suplementation (Lamk, 1785). J Perikanan dan Kelautan, 2021;26(2):102-114. DOI: 10.31258/jpk.26.2.102-114
45. Hidayat S, Saptiani G, Agustina. Isolates of lactic acid bacteria to control *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus*). Nusantara Trop Fish Sci J. 2023;2(1):41-49. DOI: 10.30872/jipt.v2i1.250
46. Pattipeiluhu SM, Laimeheriwa BM, Lekatompessy AP. Infeksi *Aeromonas hydrophila* Dan Dampaknya Pada Parameter Darah Ikan Nila Oreochromis Niloticus. J Fish Marine Res. 2022;6(3):6-13. DOI: 10.21776/ub.jfmr.2022.006.03.2
47. Alves A, Dias M, Campainha S, Barroso A. Peripheral blood eosinophilia may be a prognostic biomarker in non-small cell lung cancer patients treated with immunotherapy. J Thorac Dis. 2021;13(5):2716-2727. DOI: 10.21037/jtd-20-3525
48. Yunus YE. Pengaruh Pemberian Ekstrak Tanaman Lidah Buaya (Aloe Vera) Melalui Pakan Terhadap Performa Hematologi, Respon Imun Dan Efek Anti Parasit Pada Ikan Nila (Oreochromis Niloticus) [Ph.D. Dissertation]. Indonesia: Universitas Hasanuddin; 2021.
49. Ezzraimi AE, Nadji H, Antoine M, Jean-Marc R, Laurence CJ. Platelets and *Escherichia coli*: A complex interaction. Biomed. 2022;10(7):1636. DOI: 10.3390/biomedicines10071636
50. Haryo A, Nurhidayati R. Pathology change of dropsy syndrome in Koi Fish (*Cyprinus carpio*) at IBAT Punten Batu. J Phys:Conf Ser. 2020;1430012011. DOI: 10.1088/1742-6596/1430/1/012011
51. AlYahya SA, Ameen F, Al-Niaeem KS, Al-Sa'adi BA, Hadi S, Mostafa AA. Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus*. Saudi J Biol Sci. 2018;25(1):182-185. DOI: 10.1016/j.sjbs.2017.10.019

التحليل العياني والمجهري لعدوى بكتيريا Aeromonas hydrophila في سمك البلطي النيلي في مزارع الأسماك التقليدية في سورابايا

كورنيا ديسياندورا[1]، روندیوس سولفين[1]، إندرا رحماواتي[2]، أرياتي أريفين[3]

[1]قسم علم الأمراض البيطرية، [2]قسم علم الأنسجة البيطرية، [3]طالب برنامج المساعدة المشتركة، كلية الطب البيطري، جامعة ويجايا كوسوما، جاوة الشرقية، إندونيسيا

الخلاصة

اجريت هذه الدراسة على الأمراض البكتيرية في سمك البلطي النيلي من خلال تحليل مرضي شامل. يعد سمك البلطي النيلي أحد أسماك المياه العذبة التي يتم تربيتها بشكل شائع ولديه إمكانات كبيرة لتطويرها لمشاريع صيد الأسماك في إندونيسيا لأنه يتمتع بقيمة اقتصادية عالية. ومع ذلك، فإن هذا العمل ليس خاليا من المشاكل المختلفة، مثل الآفات والأمراض. ولهذه المشاكل آثار سلبية، مثل انخفاض الإنتاج، وانخفاض جودة المياه، أو موت الكثير من الأسماك، أو الموت الكلي. يأتي سبب المرض من العوامل المسببة للأمراض، مثل الفيروسات والطفيليات والبكتيريا. وأظهرت الأسماك، التي يملكها مزارع تقليدي في سورابايا، أعراضا سريرية مثل الخمول، والسباحة على السطح، ونزيف في الزعانف والبطن، وجحوظ العين، واليرقان. وشملت الفحوصات المخبرية التقييمات العيانية (علم الأمراض التشريحي) والمجهرية (علم الأمراض النسيجي)، وتحليلات علم الأحياء الدقيقة وعلم الأمراض السريرية (علم الدم). وبنتيجة تحليل علم الأحياء الدقيقة، أصيبت الأسماك ببكتيريا Aeromonas hydrophila sp. النتائج العيانية والنسيجية المرضية هي شحوب الخياشيم وتمزق الصفيحة الثانوية مع توسع الشعيرات الدموية وتآكل الصفيحة الأولية والنزيف والنخر وتسلل الخلايا الالتهابية. عضو الكبد أصفر شاحب، التهاب الكبد النخري والنزفي وداء هيموسيديريا، والزعنفة الصدرية عبارة عن نزيف وارتشاح للخلايا الالتهابية. النتائج المرضية السريرية هي فقر الدم الناقص الصباغ كبير الكريات، وفرط فيبرينوجين الدم، وقلة العدلات، وقلة اللمفاويات، وقلة الوحيدات، والقاعدية.