Profile of *Myxobolus* infection in koi fish (*Cyprinus carpio*) gill tissue from Land Pond, Nglegok, Blitar Regency

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**Abstract.** Koi (*Cyprinus carpio*) hatchery and aquaculture sector is highly beneficial as it is one of the Indonesian and international superior commodity. However, Koi are susceptible to pests and diseases. One of the diseases is Myxobolusis which is caused by the *Myxobolus* sp. parasite. The clinical symptom of Myxobolusis are lumps (boils) on the gills and cloudy reddish fluid secretion similar to pus. This research aims to determine the infection profile of Myxobolus through histology observation on gill tissue damage of koi cultivated in a ground pond. This research uses a descriptive method and a quantitative approach. This research observes the gill tissue damage of koi collected from the ground pond of Nglegok, Blitar Regency. The researcher observes the type of damage using a microscope with a magnification of 400x. This research uses a scoring method to assess the damage. Based on the result of histopathological observation, the infected fish exhibits oedema, haemorrhage, hyperplasia, lamellar fusion, and vacuolization. The percentage (%) of tissue damage in Myxobolus infected koi are 72%, 33%, 77%, 75% and 52% respectively. The average score shows moderate to severe damage levels. The observation result on water quality parameter (temperature, pH, DO, and CO2), as supporting parameter in water quality management, shows a tolerable condition and is not the primary cause of *Myxobolus* sp. infection. However, the transmission of infection may occur due to the presence of other organisms that act as *Myxobolus* sp. host.

1. Introduction

Ornamental fish has high export value and tend to increase from year to year. One of the popular ornamental fish is koi (*Cyprinus carpio*). Koi is a type of freshwater fish, possessing high economic value in the national and international market. Koi has an attractive colour and ideal shape, therefore it has a good sales prospect [1]. The high demand for koi encourages fish farmers to increase koi cultivation. For instance, the fish farmers in the Nglegok District, Blitar Regency, East Java Indonesia. The majority of ornamental koi fish farmers use traditional cultivation ponds in the form of the ground pond using the river as a water source. However, the main drawback of ornamental fish is the health and environmental factors. The failure of ornamental koi cultivation is caused by parasite attacks such as *Myxobolus koi*. Based on Lu’lu’in et al. [2], *Myxobolus* attacked 3-5 cm fish seeds with a mortality rate of 90% in Blitar between 2009 to 2012 period. Koi fish are susceptible to diseases due to unbalanced interaction between fish as host, water as environment, and disease agent (pathogen). The unbalanced interaction causes stress, decreases body cdefence mechanism, and increases susceptibility to disease.
Myxobolus has a shape similar to a watermelon seed encased in a nodule containing thousands of spores [3]. The nodule grows in fish tissue such as gills. It spreads and disturbs the oxygen circulation system in the gill lamellae, causing the fish to lose strength and die. Out of ninety recorded Myxobolus sp. species, thirty-six species are the parasite. Nineteen species attacks gills and seventeen species attacks several organs including gills [4]. During the spore phase, the definitive host releases Myxobolus sp. The spore spreads in the bottom part of calm waters. In the triactinomyxon phase, worms consume and secrete the spore [5]. Based on Azmi et al. [1], Myxobolus infects koi on pH 7, the temperature of 29°C and ammonia of 0.3 ppm. In addition, Myxobolus infects koi on DO 4.0 ppm and NH$_3$ 0–0.25 ppm with pond water brightness above 20-35 cm.

Histological analysis is a disease study on functional and morphological changes, as well as reactions that develop in organisms due to infection [6]. In addition to external and internal clinical signs observation, the histopathological examination can provide early diagnosis of infected fish as biomarkers. The diagnosis is adequately efficient to determine the health condition of fish through changes in tissue structure [7]. This research aims to study and determine the infection profile of Myxobolus parasites through histological observations on gill tissue damage of koi (C. carpio) maintained in-ground ponds.

2. Methods

2.1. Research Methods and Materials
This research was conducted at the Fish Biological Resources Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University. This research was conducted in January-February 2020. The research used a descriptive method through histopathological observations to determine the infection profile in the gill tissue. The researcher observed the infection profile of koi by observing clinical symptoms and conducting a histological analysis of gill tissue. In addition, the researchers measured water quality parameters of koi ponds as supporting data. The water quality parameters are temperature, pH, DO, and CO$_2$.

2.2. Fish Sample Collection
The researcher obtained fish samples from traditional ground ponds in Nglegok Village, Nglegok District, Blitar Regency, East Java, Indonesia. A sampling of fish consisted of 10 healthy carp and 10 carp infected by Myxobolus, each of which had a juvenile size of 7–12 cm. The selection of infected fish used observations of external clinical signs based on the characteristics described by Yanuhar et al. [8]. The fish samples were taken to the laboratory for dissection and gill tissue preparation using the Raskovic et al. [9] method.

2.3. Histopathological Observation of Gill Tissues
Histopathological observations of gill tissue can be performed as an early diagnosis of infection. The gill preparations were observed under an Olympus light microscope with a magnification of 400x. The researchers observed the specified field of view 3 times. Assessment of tissue damage for each organ used the semi-quantitative scoring method based on Pantung et al. [10]. Assessment provisions are based on the percentage of damage per field of view (%). The assessment score is as follows: score of 0 defined as no damage (0%), a score of 1 defined as minor damage (<30%), a score of 2 defined as moderate damage (30-70%), and score of 3 defined as severe damage (> 70%).

2.4. Water Sampling and Measurement of Water Quality Parameters
The measurement of water quality parameters as supporting data covers temperature, pH, DO, and CO$_2$. The researchers conducted PH and DO temperature measurements directly in the fish pond, and CO$_2$ measurements in the laboratory. Water temperature measurement used a thermometer, salinity measurement used an Atago refractometer, DO value measurement used a DO meter, and the pH value
measurement used a pH meter. CO₂ measurement referred to Prasetyawan et al. [11]. Water quality parameters were taken at the inlet and outlet of the ground pond of koi cultivation.

3. Results and Discussion

3.1. Clinical Symptoms of Myxobolus Infected Koi (Cyprinus carpio)
The researchers selected infected fish samples using visual observation on external clinical signs of Myxobolus infection symptoms. One of the most prominent factors is the growth of nodules similar to boils in the gill. The nodules cause breathing difficulty, limpness, mucus production, and pale skin colour. Yanuhar et al. [8] stated that common clinical symptoms of Myxobolus infected fish are slow swimming speed, tendency to stay at the bottom of the water, low feeding response, and death. In addition, the operculum is open or unable to close completely due to the swelling Myxobolus sp., ultimately disrupts the respiration process. Furthermore, fish health is determined from the body and behavioural condition. Healthy fish do not have wounds on their bodies, brightly coloured, actively moving, and do not swim on the surface of the water.

3.2. Histology Observation on Gill Tissue of Koi
Based on observation results using a light microscope, healthy fish (not infected with Myxobolus) showed little damage compared to infected fish. In addition to being easily observed, the gill organs are the main target of Myxobolus infection [12]. Gills have structured filaments and a large surface area. The thin filaments and gill lamellae (respiratory epithelium) serve as a place to exchange respiratory gases [6]. The researcher assessed gill tissue damage using the Pantung et al. [10] scoring method based on observations per field of view (%). Figure 1 shows the histopathological analysis result of the gill tissue.

The histopathological analysis results showed damage to the gill lamella tissue in the form of oedema, haemorrhage, lamellar fusion, and vacuolation. The percentage of damage found in healthy fish tissue is less compared to infected fish. The damage may occur in healthy fish despite the absence of nodules on the gills. Oedema (3%), hyperplasia (3%), and hemorrhage (2%) indicate stress on non-infected fish. This research found minor damage to non-infected fish (<5%). The infected fish exhibited edema damage of 72%, haemorrhage of 33%, hyperplasia of 77%, the lamellar fusion of 75%, and vacuolization of 52%. The infected fish showed a moderate to the severe percentage of tissue damage due to Myxobolus infection spreading to nearly the entire tissue.

![Figure 1. Gill histology. (A1) healthy carp with 400x magnification; (A2) healthy carp with 100x magnification; (B1) carp infected by Myxobolus with 400x magnification; (B2) carp infected by Myxobolus with 100x magnification. Edema (E), Hemorrhage (HM), Hyperplasia (HY), Lamellar Fusion (FL), and Vacuolization (V).]
Gill lamellae exhibit oedema and haemorrhage in both infected and non-infected koi. Oedema is the swelling of cells caused by the entry of parasites into the gills or the accumulation of excess fluid in the narrowed body tissues. Oedema causes a functional deficiency on gills and disrupts the respiration process and body metabolism [13]. Oedema causes the secondary lamellae to release epithelium. In addition, epithelial cell necrosis can disrupt the epithelial function in dissolved gas trapping. Furthermore, the disrupted respiration and osmoregulation process ultimately lead to death. The increased intravascular hydrostatic pressure causes oedema and enlarged blood plasma fluid circulating the interstitial space [14]. Oedema is caused by direct contact between the myxosporean and the epithelial cells which spread and accumulate, inducing irritation.

**Table 1.** Score and Percentage of Gill Tissue Damage on Non-infected fish

| Damage Type      | Average (%) | Score* | Damage Level |
|------------------|-------------|--------|--------------|
| Edema            | 3           | 1      | Minor        |
| Haemorrhage      | 2           | 1      | Minor        |
| Hyperplasia      | 3           | 1      | Minor        |
| Lamellar Fusion  | 0           | 0      | No Damage    |
| Vacuolization    | 0           | 0      | No Damage    |

*(Pantung et al., 2008)*

The process of gill defence system against parasitic infections can cause bleeding. The lamella defence reaction will rapidly stimulate gill epithelial cell growth. The lamellar haemorrhage (bleeding) is due to direct contact with pathogens during the respiration process. The irritation increases the osmotic power of blood vessels, causes the fluid to exit blood capillaries, and enters the surrounding tissue, ultimately increasing the size of cells [15]. On the other hand, hyperplasia is excessive tissue formation due to an increase in the number of cells. Hyperplasia is a physiological response to protect the network from toxic substances. Hyperplasia can reduce the surface area of the secondary lamellae used in the gas exchange of erythrocytes [16]. Lamellar hyperplasia causes the thickening of the epithelial tissue at the tips of the filament or the epithelial tissue located near the base of the lamellae. Hyperplasia increases the number of secondary lamella epithelium. In turn, the secondary lamellae become enlarged and stuck to each other, encouraging secondary lamellar fusion. The tissue cells will degenerate over time to form a vacuolization indicated by the appearance of abnormal sized space [17].

**Table 2.** Score and Percentage of Gill Tissue Damage on *Myxobolus* Infected Koi

| Damage Type      | Average (%) | Score* | Damage Level |
|------------------|-------------|--------|--------------|
| Edema            | 72          | 3      | Severe       |
| Haemorrhage      | 33          | 2      | Moderate     |
| Hyperplasia      | 77          | 3      | Severe       |
| Lamellar Fusion  | 75          | 3      | Severe       |
| Vacuolization    | 52          | 2      | Moderate     |

*(Pantung et al., 2008)*

Lamellar fusion occurs due to hyperplasia that extends to the basal cells and epithelium, encouraging secondary lamellae fusion. Lamellar fusion, which occurs due to lamella cell hyperplasia, continuously fills the space between secondary lamellae with new cells, causing adhesion between secondary lamellae [18]. Lamellar fusion disrupts the respiratory process and expiration of respiratory gases [19]. The lamellar fusion is a severe level of damage as it is late-stage damage following hyperplasia damage. Changes in tissue structure can partially disrupt gill function, reducing respiratory capacity and ion exchange.
3.3. Water Quality Measurement Results

The low water quality and the presence of microbes or parasitic animals in the environment increases disease infection rate. The infection initially occurred due to the high intensity of fish cultivation activities that generally destabilize the physical, chemical, and biological environment. Fish under stress is very susceptible to microorganisms. For instance, parasites, bacteria, fungi, and viruses [20].

| Water Parameter Quality | Unit | Inlet | Outlet |
|-------------------------|------|-------|--------|
| Temperature             | °C   | 25    | 27     |
| pH                      | -    | 7     | 7      |
| DO                      | mg/L | 7,1   | 8,2    |
| CO₂                     | mg/L | 5,1   | 5,2    |

Table 3. Water Quality Data

Water temperature, pH, and DO are important water parameters associated with the disease as these parameters fluctuate rapidly. Narantaka [21] stated that the optimum temperature for goldfish growth is in the range of 25 °C-30 °C. Tjahjahningsih et al. [22] stated that the pH range between 6.5 - 7 tends to be neutral and good for koi growth. The pH value is very optimal for the continuity and growth of koi. Fish aquaculture requires at least 5 mg/l dissolved oxygen and 4mg/L CO₂. Fish can live well at CO₂ concentrations below 10 mg/l. At a concentration of 20 mg/l, CO₂ can affect fish metabolism. In addition, a concentration of 20 mg/l can be toxic and increases the mortality rate of domestic fish [23].

4. Conclusion

Types of tissue damage found in the gill tissue of non-infected koi are oedema and haemorrhage. Types of tissue damage found in Myxobolus infected fish are oedema, haemorrhage, hyperplasia, fusion, and vacuolization. The average score for the infected fish is classified as moderate to severe damage. The observations result of water quality parameters (temperature, pH, DO, and CO₂), as supporting parameters in water quality management, are classified as tolerable. The water quality parameter is not the primary cause of Myxobolus sp. infection. However, the transmission of infection may occur due to the presence of other organisms that act as Myxobolus sp. host.

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