Histopathology of gill, muscle, intestine, kidney, and liver on *Myxobolus* sp.-infected Koi carp (*Cyprinus carpio*)

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Abstract This research aims to investigate the clinical symptoms of *Myxobolus* sp.-infected Koi carp (*Cyprinus carpio*) and to observe fish histopathology including histological alteration in the intestine, kidney, and liver organs. Descriptive method was employed in this research. The fish sample was obtained from Fish Farmer Group at Nglegok Village in Blitar City. The main observed parameters were the alteration of intestine, kidney, and liver of Koi infected with *Myxobolus* sp. parasite. The observation result of the histopathology of the parasite-infected fish indicated that necrosis and cyst occurred in the intestine. Necrosis also occurred in kidney, while hyaline degeneration occurred in tubule cells. Furthermore, necrosis and inflammation were also found in liver. It is obvious from the result that *Myxobolus* sp. infection resulted in the damage of Koi carp vital organs.

Keywords Koi carp · *Myxobolus* sp. · Histopathology · Intestine · Kidney · Liver

Introduction

Koi carp (*Cyprinus carpio*) is one of fishery commodities that attracts the attention of both local and international ornamental fish hobbyists due to its unique color

composition captivating ornamental fish hobbyists. Koi carp commodity has a business prospect which can be developed through pond cultivation. However, many water quality problems and various diseases can disrupt the cultivation.

The causes of the disease can be from the fish body itself or from outside. The internal causes of disease in fish are heredity (genetics), internal secretion, immunodeficiency, and neurological or metabolic disorders. While the external causes of disease and histology organs include pathogen attack due to environmental influences, miss management of fish feed and antibiotic application (Maftuch et al. 2016). One of the pathogens is *Myxobolus* sp. which is found in Koi (Oumouna and Hoffmann 2011). *Myxobolus koi* is one of *Myxobolus* sp. species which can cause parasitic diseases in fish called *Myxobolus*. In general, this disease-causing organism is systematic and recognized from the spore morphology along with the number and location of polar filaments (Camus and Griffin 2010).

This research aims to investigate the clinical symptoms that occurred in the several organs of *Myxobolus* sp.-infected Koi carp (*C. carpio*). Furthermore, the histopathology of gill, muscle, intestine, kidney, and liver were also examined.

Materials and methods

Fish sample

The sample of Koi fish were obtained from Fish Farmer Group of Nglegok Village in Blitar city. The sampling was carried out in March–April 2017. It was collected from the population of Koi carp which was infected with *Myxobolus* sp. parasite. Health Koi fish from the pond of Laboratory of

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Fish Reproduction at faculty of Fisheries and Marine Science was used as a negative control. They represented normal organs histology. This study has been approved by ethic committee of Brawijaya University, and all activity of this research already follow the international.

Histologic analysis

Fish organs, gill, muscle, intestine, liver, and kidney preparation for histologic analysis was based on Stoskopf's method (1993). The samples experienced fixation process for 24 h using 10% formalin solution. The next steps were dehydrating, clearing, paraffin infiltration, blocking, cutting/sectioning, staining, and mounting. The histology analysis followed Allen (1992). A light microscope was used to analyze the samples. The histopathology analysis based on the book of Genten et al. (2009).

Data analysis

All Histopathological condition of the fish organs was calculated using score semiquantitative protocol. The detail of the analysis was provided elsewhere (Bernet et al. 1999).

Result and discussion

Fish infection

One of Koi carp samples that were suspected to be infected with *Myxobolus* sp. can be seen in Fig. 1. The gill had swell and pale color, and its gill cover (operculum) could not close completely (Fig. 1a). Then, white spots also appeared on the edge of the lamella. Figure 1b is the picture of gill taken from a fish which presumably experienced *Myxobolus* sp. infection. Figure 1b indicates the swollen and pale red gill, along with some spots on the gill. The observed primary and secondary lamellae are merged in this figure.

Based on the characteristics obtained from Fig. 1a, b, the infected Koi carps are recognized by the appearance of clinical symptoms found on the gills. One of the symptoms for *Myxobolus* sp.-infected Koi carp is the apparent parasite cyst in the gills (Skirpstunas et al. 2006; Kallert et al. 2009).

Gills alteration

The alteration of *Myxobolus* sp.-infected Koi carp can be seen in Fig. 2. Edema and congestion can be observed in Fig. 2b, c.

Normal gills of the healthy fish indicated a neat order of primary and secondary lamellae which could be differentiated easily. The damaged gill with congestion could be recognized with blood accumulation which caused swell (Fig. 2b). Thrombocytes which form a colony have nothing in common with normal gills as shown in Fig. 3a. Congestion, as a passive process, was caused by venous blood stream decrease (increased blood volume in a vessel located in gill lamellae). Congestion shows the red color change that the degree of red color depends on blood oxygenation level. Hence, in the marginal channel (telangiectasia), erythrocyte congestion usually occurs (Hadi and Alwan 2012).

Edema appeared in the damaged gill as the result of fluid infiltration into tissues. Corrosion occurred in secondary lamella and thrombocytes did not seem to exist in the lamella. Thus, the lamella looks empty, yet it contains a fluid; the gill color is red pale. Sukarni et al. (2012) stated that a gill with edema is irritated as the result of foreign matter infiltration which causes swollen cell. A continuous edema precipitates necrosis or cell death in the epithelium.

Myxobolus sp. specifically attacks the gills on the arch and filaments gill. According to some studies, arch and gill filaments which are infected by *Myxobolus* sp. experience discrete blobs Puth (cyst) with irregular shapes (Ozak et al. 2012). In some cases, Plasmodium is found in gill filament

Fig. 1 Koi infected with *Myxobolus* sp. parasite in open operculum state (a), fish gill infected with *Myxobolus* sp. parasite in swollen state (b)

Fig. 2 Gill histologic structure. Normal gill structure (a), congestion on gill (b), edema in gill (c), $\times 400$ magnification

Fig. 3 Muscle cell structure. Normal muscle (a), necrosis damage in Koi muscle (b), myositis damage in Koi muscle with $\times 400$ magnification (c)

as a type of intralamellar-vascular. It is present within the entire length of the filament (Kaur et al. 2015).

Muscle cell alteration

Histopathology of Koi carp muscle is depicted in Fig. 3.

In the normal fish, the muscle layer composing of skeletal muscle was lateral muscle layer. While the necrosis occurred in the infected muscle was characterized by cell fading (Fig. 3b). Furthermore, the damaged muscle caused by myositis which was characterized with a defect in the muscle can be seen in Fig. 3c.

Necrosis is the death of cells caused by acute cell damage. It is characterized by the death of muscle tissue that still attached to the fish body surface. Prince and dan Wilson (2006) also explained that necrosis is a cell which has very low activity and then experiences the death of tissue cell, causing loss of function in areas with necrosis. According to Molnár and Kovács-Gayer (1985), *Myxobolus* sp. is a typical intracellular parasite of muscle cells, which their spores are found in other organs. The spore will spread to the organs throughout blood circulation. Histopathologically, necrosis is characterized by the appearance of an unclear cell membrane and cell nuclei, which even disappear (Wikiandy and Rosidah dan Titin 2013). It is supported by the opinion of Plumb (1994) saying that necrosis is characterized by the death of cells or tissue that accompanies each cell degeneration in animal life and it is the final stage of irreversible degeneration.

Muscle band on the lateral muscle fiber of Koi became unclear (Fig. 5c), which is a sign of Myositis damage. This symptom will finally damage the muscle. The change is in the form of cytoplasm muscle (sarcoplasm) which turns into fine granules or granules that are acidophilic in the sarcolemma. According to Takashima and dan Hibiya (1995), infiltration of inflammatory cells shows a pathological reaction that occurs in the muscles.

Intestine cell alteration

Intestine cell alteration of infected Koi carp could be seen in Fig. 4.

In the normal fish intestine, intestine tissue structures could be stained entirely, and its structure of the villi, mucosal epithelial cells, goblet cell and lamina propria were still visible. Meanwhile, intestine cells damage in the form of necrosis occurred in infected fish (Fig. 4b and cysts emergence in Fig. 4c).

Cells with necrosis can be recognized from shrinking nucleus (pyknosis) and enlarged, blurred or disappear karyolysis. Woo and Buchman (2012) said that necrosis describes the condition of tissue activity decrease which is characterized by the gradual loss of some parts of the cells from a tissue. Therefore, cell death will happen in anytime soon.

Myxobolus sp. cyst (in the circle) had been found in the intestine. Spore of *Myxobolus* sp. is usually detectable in the mucus drawn off the gut wall. This occurs as solitary spores or as small clusters of spores embedded in yellow bodies (Molnar 2002). *Myxobolus* sp. cyst infestation in the intestine can damage villi and mucosal epithelium causing myolitic. Marjoram and Bagnat (2015) explained that intestine infection leads to myolitic on the intestinal wall.

Fig. 4 a Normal intestine cell structure. b Necrosis and c *Myxobolus* sp. cyst infestation with $\times 400$ magnification

Kidney cell alterations

Kidney cell damage in Koi carp due to the infection of *Myxobolus* sp. parasite can be seen in Fig. 5. The damages were necrosis (Fig. 5b) and hyaline degeneration (Fig. 5c).

In infected kidney cells, nucleus looks blurred so the membrane cannot be seen clearly. Hong et al. (1998) stated that necrosis is the death of tissue cells. Necrosis may occur as the result of toxic materials, microorganism's activity, and metabolic disorders. Necrosis will cause an inflammatory response in active tissues around the necrosis. The inflammatory response is indicated by the red colored tissue as the effect of excessive erythrocytes.

Kidney damage in the form of hyaline degeneration is characterized by swollen tubular cell, nuclear membrane, shrinking tubular lumen, the appearance of hyaline objects in some tubular lumen, and pink color in hematoxylin–eosin staining. In myxosporean infection, Kidney tissue damages, such Glomerular destruction, hemorrhagic and leucocytic infiltration are the main histopathological effect (Alvarez-Pellitero and Sitja-Bobadilla 1993).

Liver cell alteration

Liver damages in the infected Koi (*C. carpio*) are depicted in Fig. 6. The damages found in infected fish were necrosis (Fig. 6b) and inflammation (Fig. 6c).

Even though the area which experienced necrosis was not wide, it is obvious that *Myxobolus* infection caused liver necrosis (Fig. 6b). Necrosis in the liver tissue caused hematoxylin stain could not be absorbed. Necrosis in the fish liver can be caused by several factors such as bio-agents (viruses, fungi, bacteria, and parasites), and blood transport disorder that affects the blood supply to particular tissues. All of these disturbances can trigger specific damage to the cell (Kotob et al. 2016).

In the analysis of liver cells, red blood spots were also found as the result of excessive erythrocytes out of the vessel. According to Novoa et al. (2010), inflammation is characterized by red lump of blood because there are many erythrocytes found out of the blood vessels. This inflammatory response aims to recover the tissue and suppress the causative agent of necrosis.

On the parasite infection, in general, cell alteration such as hepatic coccidiosis, can also occur. The symptom is agglomerated enlargement of oocysts with granulomas development from connective tissue or fibrotic capsules which partially replace the host liver parenchyma. This condition leads to the fish weight loss and even death due to the defective functional activity of the liver (Iwanowicz 2011).

Fig. 5 a Normal kidney structure. b Necrosis and c hyaline. $\times 400$ Magnification

Fig. 6 Liver cell histology. **a** Normal liver structure. **b** Necrosis and **c** inflammation. $\times 400$ Magnification

Conclusion

Based on the result of this research, the researcher can conclude that *Myxobolus* sp. parasite—infected Koi shows a clinical symptom such as a reddish cyst (lump). The infection of *Myxobolus* sp. parasite damages fish gills, muscle, intestine, kidney, and liver.

Author's contribution MM designed the experiment and wrote the manuscript. ES, IF, BAS, LR, SH, and NF conducted experiment and data analysis. AAP wrote draft and finalization of the manuscript.

Compliance with ethical standard

Conflict of interest All authors declare no conflict of interest for publication this article.

Ethical standard The research has been approved by ethical committee of Brawijaya University. All the research procedure has followed international guide for animal research.

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