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Histopathological Changes in Gills of Wild Snakehead Murrel, *Channa striata* (Bloch, 1793) Infected with *Ichthyophthirius multifiliis* Fouquet, 1876 from Surabaya River

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ABSTRACT
The present study purposed for the identification of the effects of histopathology of natural infection of the ciliated protozoa, *Ichthyophthirius multifiliis* on the wild snakehead murrel *Channa striata* gills, collected from Surabaya River in Surabaya, East Java Province, Indonesia. This is because *I. multifiliis* is a ciliated protozoan that often infects and causes serious problems in freshwater fish, including snakehead fish. On the one hand, histopathology devotes a well mode for discover the irritants and pathogens effects in various organs including gills which are vital organs of fish. Live fish were immediately transferred to the Parasitology laboratory and examined within 24 h. The gill smears were tested for the parasite *I. multifiliis* and ciliates were identified microscopically sans staining. The second step was performed a histopathological examination of the gills. We found a white spot with different diameters on the gill filaments, and the colour changed. The study of histopathology showed heavy damage to the primary lamellae and secondary lamellae, including: increased cell size (hypertrophy) and cell number (hyperplasia), fusion of secondary lamellae and blood congestion from the base of the primary lamella. We concluded that the changes of histopathological became an indicator of gill damage caused by *I. multifiliis* will ultimately conduct to reduced surface area for effective breathing, then cause hypoxia, lethargy and death.

Keywords*: histopathological changes, gill, Ichthyophthirius multifiliis, wild snakehead*

1. INTRODUCTION

Fish is one of the important sources of protein needed by humans. One type of fish as a source of protein and is often used by the people of Indonesia is freshwater fish including snakehead fish is one of them. *Channa striata* is one of snakehead fish type that freshwater fish are in demand in the country due to their high nutritional value, low cost and availability in the local market. In Indonesia, the population of *Channa striata* is quite large and Indonesian people usually get it wild in nature such as rivers or lakes. These fishes are often infected by parasites, which reduce the growth rate as well as the reproductive rate of the host fish. Among parasitic infections, *Ichthyophthirius multifiliis* is the most crucial and overflow ciliated protozoan parasite affecting freshwater fish. It has significant economic impact and infects a broad spectrum of wild and cultured fish species in most parts of the world. *Ichthyophthirius multifiliis* characterized by the appearance of white spots on the gills and skin. the white spot usually occure in the skin, fins and gill. [1]. The structure of gill is strongly influenced by external factors, and may be an indicator of many problems. The histology of gill of fish is used as an identification of infectious and non-infectious diseases. In regard to the gill pathology, earlier studies have focused on the histologic effects of parasite infectious fish pathogens in wild-type fish gills and damage due to of them, especially protozoan [2] and metazoan [3]. As one of the organs that is often infected with *I. multifiliis*, the gills can often be used as one of the main organs of examination for the diagnosis of

ichthyophthiriasis. Therefore, *I. multifiliis* multiplication can occur in various organ tissues, the resulting tissue histopathological changes can be used to determine the status of infection and the impact on the host. The present study was conducted to find the histopathological gill lesions type related to external parasitic protozoan, *I. multifiliis* of *C. striata*.

2. MATERIALS AND METHODS

The wild snakehead murrel *C. striata* were collected in Surabaya River in Surabaya, the capital city of East Java Province, Indonesia. Live fish samples were immediately transferred to the Parasitology Laboratory, Faculty of Veterinary Medicine, Airlangga University. The samples were observed within 24 h. The gills were smeared and tested for the ectoparasites *I. multifiliis* were identified microscopically under 200x and 1000x magnification without need staining [1]. Second step includes histopathological examination of the gills. Normal and infected gill tissue specimens were rinsed with saline solution and stabilized in 10% formalin for 24 h. After that, the tissues were dehydrated in an alcohol series of increasing concentrations (70%, 80%, 90%, and 100%, respectively), then embedded in paraffin wax and cut at 5µm. Tissue sections were stained with hematoxylin-eosin (H&E). Every slide was observed with the microscope at 200x, 400x, and 1000x magnifications and captured with an installed Nikon DS-Fi2, Nikon H600L HTC-one camera.

3. RESULTS AND DISCUSSION

Based on necropsy results of wild snakehead fish (Fig.1A) seen there was necrosis area marked by fusion of the filament edge (Fig. 1B). The smear examination without staining of gill showed the morphology of ciliate protozoan, *I. multifiliis* was recorded infest *Channa striata*. Despite, *I. multifiliis*, is one of the ectoparasites that were found in this study (Figs. 1C and 1D).

Gills are respiratory organs that have a very important role. It is also one of the most vulnerable organs of fish due to its outer location and inner most layer in contact with water [4], therefore it is often infected and damaged by causative agents, including ectoparasites. *I. multifiliis* (Figs. 1C and 1D) is the most crucial and overflow ciliated protozoan parasite that affects the white spot disease (Fig. 1B) on observed fish

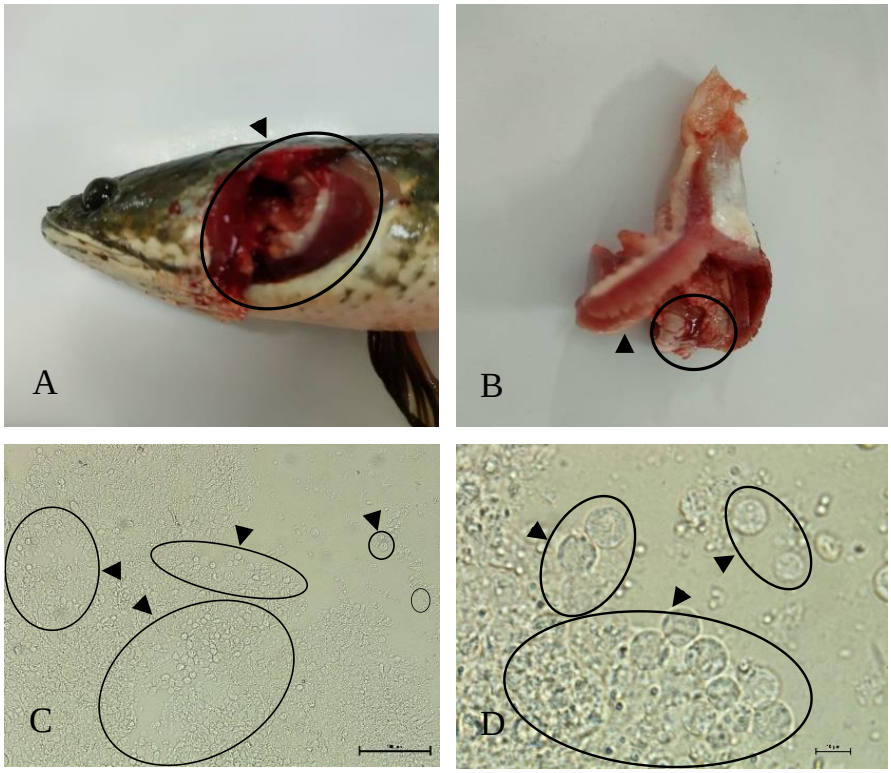

Figure 1. A, Wild snakehead murrel, *Channa striata* infected *I. multifiliis.* B, The gills of infected *I. multifiliis* give rise to the appearance of white areas or white spots. C (200x) and D (1000x), Many trophonts stage of *Icthyophthirius multifiliis*, in which the trophont looks macronucleus and micronucleus (1000x).

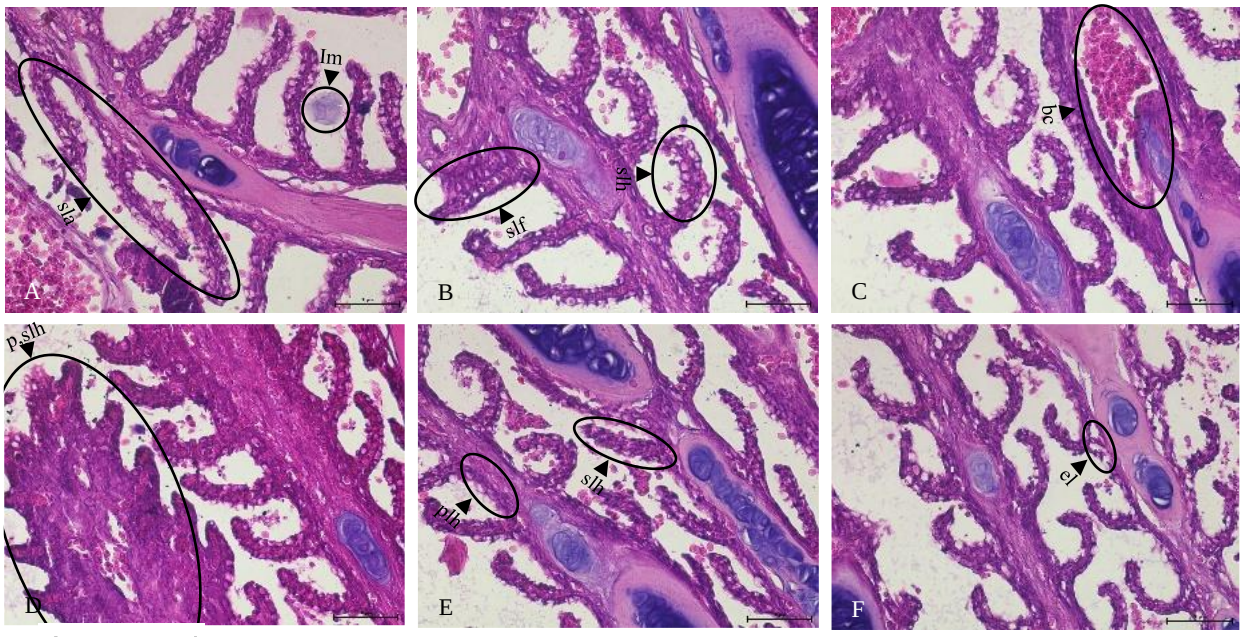

Figure 2. The extensive pathological changes consistent with proliferative gill inflammation (PGI) was seen on gill. Some lamellae were moderately to highly apically thickened due to epithelial changes, deformation of the lamellae, the margins were thickened due to the basal infiltration of the epithelium with inflammatory cells. Enlarged epithelial cells were also observed in some of lamellae. A: secondary lamellar apposition (sla), *Icthyophthirius multifiliis* (Im); B: secondary lamella fusion (slf), secondary lamella hyperplasia; C: The base of the primary lamella was seen blood congestion (bc); D: primary and secondary lamella hypertrophy (p,slh); E: primary lamella hyperplasia (plh), secondary lamella hyperplasia (slh); F: epithelial lifting (el). Magnification (400x).

[5]. *Ichthyophthirius multifiliis* is a ciliate pathogen affecting freshwater fish and initiated the ichthyophthiriosis infection (white spot diseases). This parasite can survive in fish as a low subclinical (enzootic) infection and in the environment turning into an epizootic clinical infection. This infection will be easier for fish under stress conditions due to poor management practices (e.g., overcrowding, poor sanitation, and poor feed). Pathogens are not host specific and recovery from disease provides resistance to reinfection [6]. It was proven in this study that the snakehead fish sample did not show any clinical signs in the form of white spots on the gills which is a characteristic feature of this parasitic infection even though *I. multifiliis* was found in the smear test carried out. This condition can reflect two possibilities, the first is that the infection status is still at a subclinical level and the second is that the host is immune to re-infection because it has been infected before. Commonly, standard gill filaments (primary lamellae) of the snakehead fish, *C. striata* generate rows of secondary lamellae (sl) including interlamellar regions and primary lamellae, and secondary lamellae were coated by epithelium, which includes different types of cells, pavement, mucus, chloride and pillar cells [7]. Histopathological observations revealed heavy damage to primary lamellae and secondary lamellae because the bounding and absorbance of this parasite (trophont stage). The gill filaments in the infected fish showed a colour changes in the form of white spots with different diameters (Fig.1B).

Figure 2A-F showed the epithelial hypertrophy and hyperplasia in the primary and secondary lamellae as moderate to heavy infection marks. Lamellar apposition, defined as the initial phase of hyperplasia, was observed in the gills of snakeheads infected with *I. multifiliis* (Fig. 2A). Hypertrophy of the gill lamella cells occurred as a host response to infection, whereas hyperplasia occurred as an increase of cells count [8]. Figure 2B showed a fusion of secondary lamellae, that is, two or more lamellae fused. Figure 2C showed blood congestion at the base of the primary lamellae. In addition, an epithelial lifting was also observed on the gills infected with *I. multifiliis* (Fig. 2F). Our results are in agreement with those reported by previous studies [1, 9]. All of the above serious damage is caused by trophonts stage parasitic activity which contributes to osmoregulation problems and ultimately fish death [10].

4. CONCLUSION

In conclusion, the changes of histopathological became an indicator of gill damage caused by *I. multifiliis* will ultimately conduct to reduced surface area for effective breathing, then cause hypoxia, lethargy and death.

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