Histopathological changes in the intestine of *Channa micropeltes* infected with the cestode *Senga rostellarae*

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**Abstract.** *Channa micropeltes* or *toman* is a native freshwater fish species and not commercially species in Malaysia. The fish become one of the anglers’ attractions to Kenyir Lake, especially for recreational fishing purpose. However, parasitic diseases are one of the most common problems for this fish population. Studies on parasites of *C. micropeltes* is essential to our knowledge for the maintenance of natural resources. So, the objectives of this study were to identify the cestode and observe the histopathological changes on gastrointestinal tract of infected *C. micropeltes*. About 15 *C. micropeltes* were caught by using fishing rod. The fish were pithing, dissected and gastrointestinal tract was removed, then placed on petri dish. The stomach and intestine were fixed in 10% buffered formal in and proceed for preparing histology slides by using standard method for histology. Then, the tissues were stained by using hematoxylin and eosin (H & E) stain. The tissue changes were described. The cestodes were collected, fixed in 70% ethanol, stained with aceto-carmine and prepared the permanent slide. The cestodes morphology were observed under microscope for identification. In this study, only *Senga rostellarae* was identified. The histopathological changes showed the intestine with severe villus damage with destruction of villi epithelium and necrosis. Some areas with cross section of cestode showed increase of goblet cells and generated necrosis. Based on this study, *S. rostellarae* was a dominant species, and its activities caused severe damage to the fish intestine. The conditions can cause death to the fish due to hemorrhage and malabsorption of nutrient.

1. **Introduction**

*Channa micropeltes* are native to the fresh waters of Southeast Asia, Malaysia, Thailand, Indo China, Myanmar, and India [1]. The fish are inhabiting all parts of Malaysia and usually, they prefer lakes, reservoirs, canals, rivers and most of it commonly deep, standing or slow-flowing water [2]. It is a common species found in Kenyir Lake. The *C. micropeltes* is a daytime predator, feeding on fishes, frogs, and birds. They are very aggressive and even attacking humans. The aggressiveness and strong swimmers attracted anglers seek for recreational fishing purpose.
In Kenyir Lake, *C. micropeltes* commonly are infected with ectoparasites such as *Argulus* sp and Monogenea (Unpublished data). Shaharom (2012) [3] reported the cestode infection in the intestine of *C. micropeltes*. However, adult cestode is common parasites in digestive tract of fishes [4]. The parasitic infections are sometimes very fatal and cause high mortalities when their life cycles are well supported by intermediate hosts [5]. The serious mechanical injuries occurred with high numbers of cestodes and inflammatory reactions may occur in association with mature worm and plerocercoid larvae [4]. The depth of penetration of some species various and depended on fish hosts [6]. This study is important because, in wild, the rate of infection and effect of the cestode are unknown. Thus, this study aimed to identify the species of cestode and describe the severity of pathological changes of intestine caused by the cestode infection.

2. Materials and methods

2.1. Fish sample
Fish samples of *Channa micropeltes* (n=10) were collected from Lake Kenyir, Terengganu and their weights (g) and lengths (cm) were measured using a measuring board. Next, pitching method was applied by cutting the fish’s spinal cord to make it paralyzed. After that, the fish was cut open using scissors up from the anus to the bottom of the jaw and the gastrointestinal tract was taken out and placed on petri dish. Gastrointestinal was divided into 3 part; stomach, proximal intestine, and mid intestine. Each end portion was fastened using a thread and was injected by 3 ml syringe with needle that contained 10% buffered formalin solution. Fixed sample in urine bottle contained 10% buffered formalin solution for histology. Sample of cestode was collected and fix in 70% ethanol.

2.2. Identification of cestode
Cestodes were fixed in AFA solution; alcohol, formalin, and acetic acid. It then being stained with aceto-carmine and put on the slide, covered with a coverslip to prepare the permanent slide [7]. The structure/illustrations of the Cestodes were drew by using Lucida camera which fitted on Olympus Microscopes under 4X magnification.

2.3. Histopathological studies
The infected tissue of *C. micropeltes* was taken out and fixed in 10% buffered formalin solution. Then it being dehydrated, clarified with xylene and processed for preparation of paraffin wax blocks [7]. Next, the tissue was cut at 4 - 5μm thickness rotary microtome and was put on slides. The tissue was stained with hematoxylin and eosin (H & E) for histopathological analysis and being observed under a light microscope. Lastly, the photo of the tissue was captured and being described if any damage caused by Cestode on *C. micropeltes* gastrointestinal tract presented.

3. Results and discussion

3.1. Result

3.1.1. Identification of cestode. The morphology of the present cestode consist structure of scolex, hook and bothria at the head (Figure 1) and mature proglottid structure consist testes and ovary (Figure 2). All proglottids are much broader than long. Immature proglottids have no trace of any reproductive organs. Mature proglottids are medium in size with almost straight lateral margin, quadrangular in shape and slightly broader and longer. Mature proglottids have developing ovary, testes, and vitelline follicles and arranged in a cluster.

The ovary is medium size, distinctly bilobed, transversely situated in the posterior region. Ovarian lobes are irregular margin, big size, and lobulated. Isthmus is connecting the two ovarian lobes, slightly curved, transversely placed and near the posterior margin. Testes are medium in size, round in shape, crowded together and some overlapping on each other. The vitellaria are follicular.
Figure 1. *Senga rostellarae*. Head region. (A) The head region stained with aceto-carmine (B) drawing by camera lucida.

Figure 2. *Senga rostellarae* showed mature proglottid. (a) Ovary (b) Isthmus (C) testes. 100x total magnification  Scale bar 200 µm.

3.1.2. Histopathological studies
The cestode was found lying in the lumen of the intestine. The intestinal layer pale and filled with mucus in the lumen. The lumen of intestine full with the body of cestode and most of the head embedded in the intestinal wall (figure 3).
Figure 3. Cestode in the lumen of the intestine.

The pathological effect exhibited ruptured serosa layer, inflammatory infiltration at the infected area, shortened and irregular shaped of villous. However, in the heavy infestation with the cestodes showed the breakage, hemorrhage, and separation of villous processes with large space (figure 4). There were also highly distribution of goblet cells in the villous (Figure 5). The epithelial necrosis was noticed (figure 4).

Figure 4. Cross-section of cestode (●) and destruction of villi and necrosis (arrow) (H & E; x10
Figure 5. The infected intestine showed highly distribution of goblet cells, infiltration of inflammatory cells, and hemorrhage. (H & E; x40)

3.2. Discussion
The internal and external organ of the S. rostellarae was described to compare other Senga spp, which is important for identification. However, the Senga spp found in C. micropeltes in this present study were different from species S. parva and S. filiformis [8]. Based on the morphological descriptions, the present cestode is Senga rostellarae [9].

The type of scolex is covered with numerous hooks and has related to severe damages of the intestinal wall. The hemorrhage occurred when the scolex reached and damaged to the blood vessel. In the case of helminths infection, Haque and Siddiqi (1978) [10] reported that the surface desquamation of mucosal epithelium, infiltration of eosinophils and plasma cells.

The severe infection was evidenced by the total eruption of villi from the mucous membrane which resulted to a major disruption of the structural organization of the intestine which might have a profound influence on the nutrition and digestion process of the fish. The degree of pathogenicity and damages were depended on the intensity of infection [11,12]. The S. rostellarae blocking the intestinal lumen and disrupt the overall absorption efficiency of the intestine which affects fish’s growth by restricting its food intake.

4. Conclusion
The cestode found in C. micropeltes was only S. rostellarae. The severity of tissue changes or damages were related to the intensity of cestode infection. The severe damage to the intestine of fish showed that S. rostellarae is pathogenic. The damages might cause the fish has digestive disturbance and lead to physiological problems.

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