# Histopathological changes in the intestine of sharptooth catfish, *Clarias gariepinus* naturally-infected with the cestode, *Tetracampos ciliotheca*

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**Abstract**

The aim of this study was to describe the histopathology of the intestine of the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) naturally infected with the bothriocephalidean cestode, *Tetracampos ciliotheca* Wedl, 1861. A total of 45 fish (10-54 cm) were collected from the Vaal Dam, (Gauteng Province, 26°52.249'S, 28°10.249'E), South Africa. A total of 39 (86.7%) of the catfish examined were infected with the cestode. Heavily infected fish (> 20 worms per individual host) were chosen for this study. Intestinal tissue with parasites *in situ* were fixed and processed for routine histological investigation. The worms penetrated deep into the mucous membrane with their scolex, leaving the proglottids nestled between villi and extending into the lumen. This attachment resulted in acute mechanical effacement of the epithelium, disruption of the lamina propria and desquamated cells mixed with mucus. In addition, mild fibrosis was occasionally evident in the lamina propria, in regions adjacent to the scolex. In some cases, the scolex fully penetrated the mucosal layer and extended to the level of the submucosal layer resulting in ulceration and oedema in the lamina propria. Coagulation necrosis was evident on the ulcer, which presented a granular substance that had a characteristic amorphous material. Furthermore, the nuclei of epithelial cells adjacent to the parasite displayed chromatin clumping and clearing. Several conspicuous granulomas of unknown etiology were observed in the muscularis of the intestine. There was no evidence for the presence of secondary microbial infections in this study. The histopathological changes caused by this parasite on these wild fish were mild and did not seem to cause any serious threat to infected hosts. In addition, no dead tapeworms were encountered during dissection.

## Introduction

*Tetracampos ciliotheca* Wedl, 1861 is a common parasite of clariid catfish and it is widely distributed throughout Africa, with most published reports from Egypt, Nigeria, Senegal, South Africa, Zimbabwe, Ethiopia and Kenya (Kuchta et al., 2012). Taxonomic history of this bothriocephalidean parasite has been problematical because conspecific tapeworms were reported under different species names and were placed in several genera (Kuchta et al., 2012). The cestode has also been reported from Israel (Paperna, 1964) and Turkey (Soylu and Emre, 2005) as a result of introductions of African catfish species to these countries (Kuchta et al.,

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2012). Although *T. ciliotheca* is a widely distributed tapeworm, the host-parasite relationships of this worm and its fish host have only been studied by a few authors (e.g. Tadros, 1979; Akinsanya and Otubanjo, 2006; Ibrahim et al., 2008).

The sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) is valued in subsistence fisheries, aquaculture and angling (Skelton, 2001). With a growing interest in the development of aquaculture in both developed and developing countries, a full understanding of the diverse effects of fish parasites on their hosts is therefore central to the development and maintenance of aquaculture and fisheries worldwide (Barber et al., 2000). *Tetracampos ciliotheca* is a dominant endoparasite of *C. gariepinus* in the Vaal Dam, suspected to be pathogenic; therefore, it was in the interest of this study to investigate the histopathological changes caused by this parasite in the intestine of this economically important catfish.

## Materials and methods

A total of 45 sharptooth catfish were sampled from the Vaal Dam (Gauteng Province, 26°52.249'S, 28°10.249'E). Fish were collected with gill nets in October 2011, January 2012 and April 2012 respectively. The fish were killed by severing the spinal cord, after which the standard length and weight were measured. The digestive tract was removed and opened longitudinally and examined for cestodes with the aid of a dissection microscope. Only heavily infected fish (> 20 worms per host) were chosen for histopathological investigations. In these cases, a 20 x 20 mm piece of tissue that surrounded the site of tapeworm attachment was excised and then fixed in Alcohol-Formol-Acetic acid (AFA) for 20 minutes. Thereafter, the fixed tissues were preserved in 70% ethanol until they were processed for histology. The tissues were dehydrated in graded ethanol concentrations, cleared in Xylene, impregnated with paraffin wax, sectioned at 5 microns and stained with Haematoxylin and Eosin. For comparative purposes, intestinal tissues from uninfected fish were also processed simultaneously. A few of the undetached cestodes were put into Petri dishes containing dam water and observations on the movement of the tapeworms were made with the aid of a dissection microscope. Detached cestodes were transferred to a clean sampling bottle, containing 0.9% physiological saline solution. This was then shaken to remove debris and induce muscle fatigue; which in turn deters strong contraction of the scolices and relaxes them. Whilst swirling the sampling bottle, an equal amount of hot AFA solution was added to kill and fix the specimens. The specimens were then mounted on glass slides to study the morphology of the cestode.

## Results

Thirty-nine (86.7%) of the 45 catfish examined were infected with *T. ciliotheca.* The intensity of infection ranged from 5 – 35 tapeworms (mean = 15). Infected fish were 10 – 54 cm (mean = 39 cm) long. The mean intensity of *T. ciliotheca* was greatest in the larger fish examined (40 to 54 cm) and lowest in the smaller fish (10 to 24 cm). Statistical comparisons could not be made to ascertain infection differences because of the unequal representation of the different groups. More females were infected (prevalence = 51.1%, mean intensity = 21) than males (prevalence = 33.3%, mean intensity = 9). However, Student's *t*-test revealed a lack of significant differences ($p$ values > 0.05) in the prevalence and intensity of infection between sexes.

Specimens of *T. ciliotheca* encountered were 26 – 45 mm (mean = 32 mm) long and were all located in the anterior part of the intestine, with their scolices embedded within the intestinal wall. The elongate scolex, armed with 25 – 40 hooks arranged in two lateral semicircles, assumed a narrower and tapering end during movement, while at other times it contracted into a mushroom shaped structure. The worms penetrated to the deep layers of the mucous membrane with their scolex, leaving the proglottids nestled between villi and extending into the lumen (Figure 1a). This penetration resulted in acute mechanical effacement of the epithelium, disruption of the lamina propria and desquamated cells mixed with mucus cells (Figure 1a). Mild fibrosis was occasionally evident in the lamina propria, in regions adjacent to the worm. In areas more distant from the point of entry the gut wall was still covered by an intact epithelium (Figure 1a).

In some cases, the scolex fully penetrated the mucosal layer and extended to the level of the submucosa, giving rise to a shape-conforming mucosal ulcer (Figure 1b). The ulcer exhibited coagulation necrosis. The lamina propria had marked oedema with homogenous amorphous and eosinophilic material (Figure 1b). Furthermore, the nuclei of epithelial cells adjacent to the parasite displayed chromatin clumping and clearing (Figure 1c).

In the present study inflammatory changes around the worm and in the gut lumen were not observed, and examinations did not confirm the appearance of granuloma around the site of attachment. However, several granulomas of an unidentified etiology were prominent in the muscularis of twelve infected fish (Figure 1d). This chronic host reaction was associated with infiltration of the mucosa, submucosa and muscularis layer with inflammatory tissue (Figure 1d). Fibroblasts were prominent in the periphery of the granulomas (Figure 1d) while monocytes, eosinophilic granulated cells and lymphocytes filled most of the inner region of the granulomas (Figure 1d).

## Discussion

The bothriocephalidean tapeworm, *T. ciliotheca* is a common parasite of *C. gariepinus* in the study area and exhibits a preference for the anterior section of the intestine (Madanire-Moyo and Avenant-Oldewage, 2013). The attachment of *T. ciliotheca* in the intestine resembles the mechanism of attachment of the caryophyllidean, *Atractolytocestus huronensis* as previously described by Molnár et al. (2003). The scolex morphology of *T. ciliotheca* varies from a tapering spear shape to a mushroom-like broad structure. The former shape enables penetration into crypts of the intestinal mucosa while the latter eases attachment in the deep layers of the intestinal wall (Molnár et al., 2003). By extending its scolex into a mushroom-like shape and with the aid of its hooks, the worm lodged deep into the intestinal crypts. The scolex, which is much wider than the crypts, mechanically disrupted the crypt epithelium, and consequently initiated mechanical effacement of the epithelium. The changes observed were consistent with forceful penetration observed by Molnár et al. (2003), as evidenced by compression of epithelial layer. This attachment resulted in injury as demonstrated by the presence of desquamated cells mixed with mucus and the disruption of the lamina propria. These pathological changes consequently incited fibrosis, an occurrence also observed by Ibrahim et al. (2008) on *C. gariepinus*

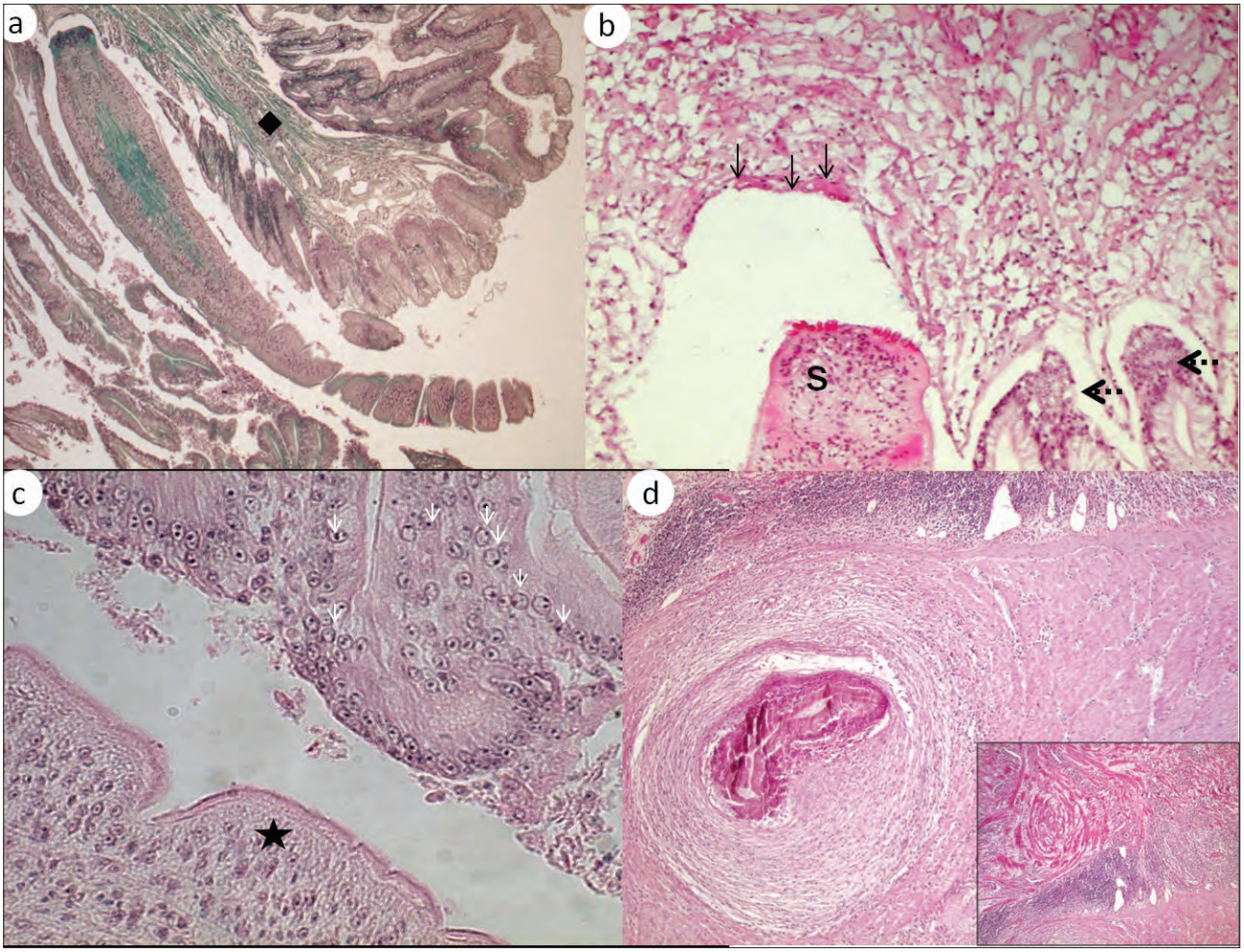


**Figure 1.** (1a) (1b-g) Histological sections of infected intestine of the sharptooth catfish, *Clarias gariepinus* showing (a) acute mechanical effacement of epithelium, desquamated cells mixed with mucus, disruption of lamina propria and fibrosis (diamond shape). Intact epithelial cells (E) were evident in areas more distant from the point of entry, *Haematoxylin and Eosin Y staining.* (b) Apical portion of scolex with a shape-conforming mucosal ulcer (arrows), lamina has marked oedema with eosinophilic amorphous material and normal intestinal crypts (broken arrows). *Haematoxylin and Eosin staining.* (c) Nuclear chromatin clumping and clearing (arrows) of intestinal epithelial cells in areas adjacent to the worm (star); Haematoxylin *and Eosin staining.* (d) Granulomatous reaction of an unidentified agent in the muscularis of *T. ciliotheca* infected fish. Insert shows that collagen deposition is more pronounced in the periphery and leukocytic infiltration is restricted to regions immediately surrounding the granuloma. Scale bars: 10 μm.

infected with *T. ciliotheca*. Thus, proliferation of fibroblasts was a possible response to trauma caused by this attachment.

The tapeworm penetrated deeply into the mucosa of the intestinal wall, causing mechanical injury by the attachment of the apical crown of hooks on the scolex. This is consistent with observations made on the same cestode in the intestine of *C. gariepinus* (Tadros, 1979; Akinsanya and Otubanjo, 2006; Kuchta et al., 2012). The hooks of the cestode incited the formation of an ulcer; which exhibited coagulation necrosis characterised by the presence of numerous shrunken nuclei with condensed chromatin and proteinaceous debris staining intensely with eosin (Cheville, 2006). The shallow ulceration at the attachment site and a moderate eosinophilic

infiltration of the area adjacent to the scolex correspond to previous descriptions by Gjurčević et al. (2012) from common carp infected with the caryophyllidean, *A. huronensis*.

The scolex of *T. ciliotheca* was not observed to reach any muscle layer in this study, whereas Faisal et al. (1989) observed complete penetration of this tapeworm through the intestinal wall, with consequent attachment in the liver, spleen and ovary. In another study, the scolex occasionally reached the muscularis mucosa, sometimes perforating the gut wall, where intestinal contents were observed to fill the peritoneal cavity (Wabuke-Bunoti, 1980). This tapeworm has been found in the gall bladder (Faisal et al., 1989; Barson et al., 2008), where it caused nodular outgrowths in the mucosa (Wabuke-Bunoti, 1980). Thickening of the lining epithelium resulting in a feature of squamous-like epithelium and goblet hyperplasia with lymphocytic infiltration have been observed as a result of *T. ciliotheca* attachment in the gall bladder (Eissa et al., 2010; Eissa et al., 2012). Extra intestinal localisation of the cestode was not observed in this study, despite the high number of examined hosts and the heavy infections.

The epithelial cells around the worm showed a nuclear anomaly with most cells exhibiting extensive clumping of chromatin into blocks separated by clear zones. This phenomenon of chromatin clumping and clearing is a retrogressive and degradative process that leaves behind abnormally cleared areas of parachromatin devoid of the finely divided chromatinic threads (Frost, 1997). It is evident that *T. ciliotheca* was an inciting agent of chromatin clumping and clearing as this was most observable at the host-parasite interface.

Wabuke-Bunoti (1980) reported inflammation around bothria of *T. ciliotheca* attached to gut mucosa in infected Lake Victoria *C. gariepinus*. Such inflammatory response at the host-parasite interface was not observed in this study, and examinations did not confirm the appearance of granuloma formation around the site of attachment. However, several granulomas of unknown etiology were prominent in the muscularis. In all the granulomas observed in this section of the intestine, the outline of the worm could not be discerned and these are unlikely to have been caused by *T. ciliotheca* since no sections of the cestode were detected in the deeper layers of the gut wall. These inflammatory responses could be attributable to a simultaneously existing proteocephalid infection of catfish from the same study area as previously observed by Madanire-Moyo and Avenant-Oldewage (2013). Data on fish granulomas by helminthes, such as acanthocephalans, larval nematodes and cestodes have been reported (e.g. Taraschewski, 1988; 1989; Karanis and Taraschewski, 1993; Dezfuli et al., 2009). Further studies are required to establish the real causative agent of these conspicuous inflammatory responses in the intestine of *C. gariepinus*.

## Conclusion

There was no evidence for the presence of secondary microbial infections in this study. No mortalities and signs of fish ill health were observed. The histopathological changes caused by this parasite on these wild fish were mild and do not seem to cause any serious threat to infected hosts. In addition, no dead tapeworms were encountered during dissection. On the other hand, the tegument of the tapeworms remained intact without any observable disintegration of the body. Results herein suggest

that an evolutionary balance has been achieved between the host and the parasite. Perhaps this is because cestodes possess a diverse range of glands within their scolices; many of these secretions are histolytic in nature, protecting the cestode from the host's immune response (Hayunga, 1979).

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