

Histology of Selected Organs of *Clarias Gariepinus* Infected With The Metacercaria of A Digenean Trematode

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Running title: *Clarias* spp organ histological changes due to trematode infection

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Abstract

Fish have become a major source of protein and essential fatty acid for fish lovers in the world. Thus, many farmers are venturing into fish farming but have challenges due to fish diseases and parasites. This study examined the possible pathological lesions associated with digenean trematode infections in the *Clarias gariepinus*.

Clarias gariepinus juveniles reared in fibre-glass tanks under intensive, flow through system were obtained from a commercial farm. Aquatic snails were obtained from earthen ponds on three commercial fish farms. The presence of cercaria was established in these snails, which were reared in laboratory conditions with *Clarias gariepinus* juveniles, for two weeks, to cause infection. Age matched un-infected *Clarias gariepinus* served as controls. Presence or absence of metacercaria after histological examination was used to determine infected and controls, respectively. Fish were sacrificed after two weeks and organs re-examined for the presence and absence of metacercaria.

Preliminary examination using wet mount revealed the presence of metacercaria only in the gills of juveniles reared with infected snails. Histological staining and examination using haematoxylin and eosin stain, revealed histopathological lesions in the gills, kidneys, liver,

spleen, brain and skin. Lesions included loss of secondary filaments in the gills, tubular degeneration and necrosis in the kidneys, vacuolations and spongiosis in the cerebellum and liver, and loss of pigmentation in the skin. There were no lesions observable in the intestine and stomach.

Findings obtained from this experiment indicate that the presence of parasites in the gills alone is cause for concern, especially in commercial fish farming. It is postulated that the lesions in other organs may be partly due to the disruptive effect on the gills resulting in oxygen deprivation.

In conclusion, there was significant disruption of these organs due to massive invasiveness of metacercaria in the gills, which may lead to economic losses and possible zoonosis. Measures targeted at snail control in earthen ponds with fishes, will break the transmission chain, and prevent digenea infestation in cultured fish.

Keywords: Aquatic snails, *Clarias gariepinus*, organ damage, parasite, histology

Introduction

The world-wide increased demand for fishery products has served as a stimulant for the increased growth and development of marine and freshwater aquaculture (FAO, 2005).

The African catfish (*Clarias gariepinus*) is an omnivore freshwater fish with a fairly impressive commercial potential (Bruton, 1979; Olojo et al., 2005). It is a well-accepted source of protein as well as a popular delicacy for local consumers in Africa (Adedeji & Okocha, 2011; Awuor & Karugu, 2014). It is widely cultured due to its fast growth rate, hardiness (Olojo et al., 2005) and efficient feed conversion (Rosa et al., 2007).

Currently in Nigeria, cultivation and sales of this fish provides employment to a very large number of people, making it a very viable venture economically.

Parasites are common parts of biological systems, which have evolved in diverse and independent lineages, resulting in great diversity in taxa. They have been reported to affect the host physiology and morphology, reproduction, behaviour, and are gaining recognition on the impact they have on individual hosts populations, communities and ecosystems (Timi & Poulin, 2020).

Digenean trematodes (trematodes, Class – Digenea), are parasites belonging to the phylum Platyhelminthes, and possessing a complex lifecycle. They are distributed worldwide (Feliu et al., 2006). Although they often use micromammals as definitive hosts (Feliu et al., 2006), they are also present in fish (Sohn, 2009; Taglioretto et al., 2018; Klemme & Karvonen, 2016).

Fish-borne trematodes are of zoonotic importance, as infections from

them are said to affect more than 18 million people globally (Sohn, 2009). Humans can be infected when they consume raw or insufficiently cooked fish containing the infective larvae, or metacercaria. The presence of these parasites results in great damage and economic loss to aquaculture, which is a key source of protein and also employment in the developing world (Sohn, 2009).

This study is aimed at highlighting the histopathological changes observable in different organs of the African catfish in a case of digenean trematode infection.

Materials and Methods

Fish and snail samples and infection

The *Clarias gariepinus* juveniles were obtained from an intensive, water flow-through facility in a commercial fish farm, while the snails used were obtained from three commercial fish farms using earthen ponds. Snails were detached from their shells and examined microscopically, for the presence of cercaria, using wet mount. Presence of cercaria was confirmed in the snails (Fig. 1A). Fish were also dissected and all organs crushed and microscopically examined likewise for metacercaria. Since metacercaria were not observed in fish, ten healthy fish juveniles weighing 9 – 11 g were raised with the snails for two weeks to induce infection. They were reared in 20 L aquarium with the

snails. Another group of ten juveniles reared under similar condition served as controls after microscopic examination, to rule out presence of metacercaria. Fish were re-examined for the presence of metacercaria after two weeks of co-habitation with the snails.

Histology

After confirmation of presence or absence of metacercaria in fish organs, organs were fixed in 10% formaldehyde and thereafter processed for hematoxylin and eosin (H & E) staining (Igado et al., 2020).

All microscopic examinations and obtaining of photomicrographs were carried out using a light microscope with inbuilt camera (Model YJ-2005, China, software SXview).

Results

Based on the use of identification keys (Yousif et al., 2019), the aquatic snails were identified to be most likely *Melanoides*.

Wet mount: In the infected fish, a preliminary examination using wet mount revealed the presence of metacercaria (Fig. 1B) only in the gills and no other organ. The gills showed blood spots with clumping and loss of gill filaments (Fig. 1C).

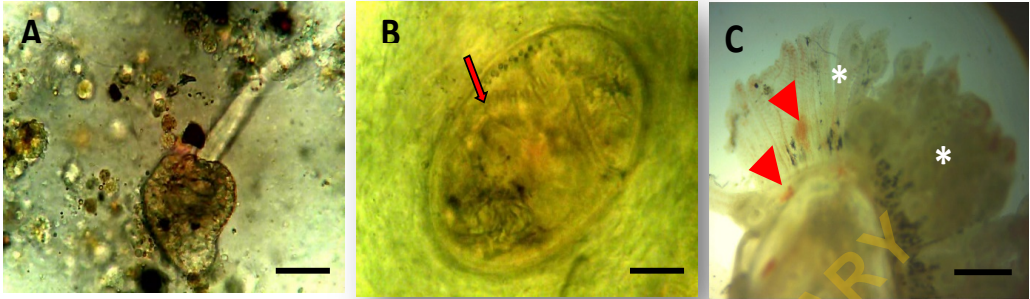


Fig. 1: Wet mount. A – Microscopic view of a cercaria. Note the eye spot (white arrow head); B – Microscopic view of a metacercaria showing the sucker (red arrow); C – wet mount of infected gills; note the blood spots (arrow heads) and clumping and loss of gill filaments (*). Scale bar: A and B = 50 μ m, C = 100 μ m.

H & E staining

Gills: There was loss of secondary filaments, coupled with a blunt, plump appearance of the primary filaments, which appeared infiltrated, with some

foci completely eroded. There was complete erosion of secondary filaments with cellular infiltration. Inner core appeared degenerated and the hyaline cartilage appeared expanded (Fig. 2).

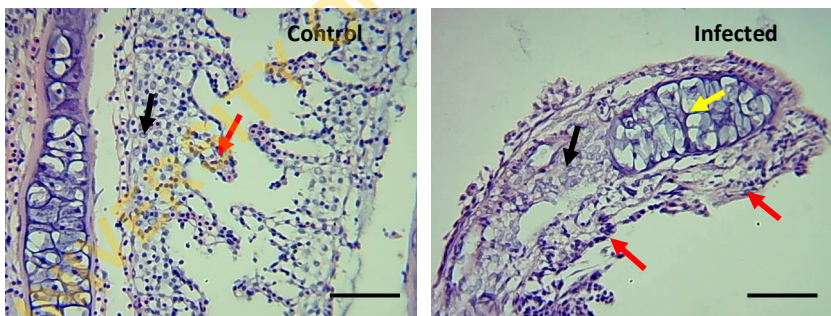


Fig. 2: Gills, H&E. Note the blunt, plump appearance of the primary filament in the infected (black arrows) and the complete erosion of the secondary filaments (red arrows). The hyaline cartilage appears expanded relative to control (yellow arrow). Scale bar = 50 μ m.

Kidney: These showed severe diffuse tubular degeneration and necrosis, with sparse and disrupted lymphoheamopoietic regions (Fig. 3).

Liver: The liver of the infected group showed generalized vacuolations, necrosis and loss of cellular outline (Fig. 4).

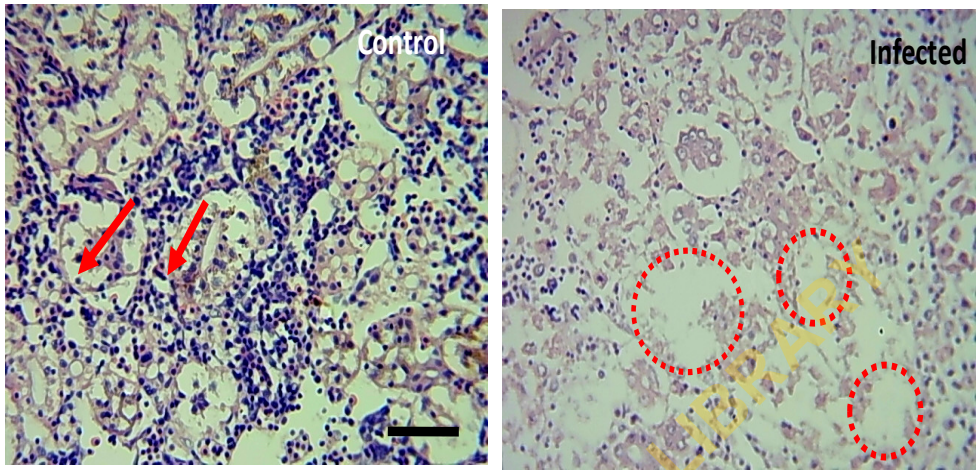


Fig. 3: Kidney, H & E. Note the vacuolations, with severe and diffuse tubular degeneration and necrosis, with sparse and disrupted lymphohaemopoietic regions (red circles). Red arrows indicate normal tubules. Scale bar = 150 μ m (consistent for both panels).

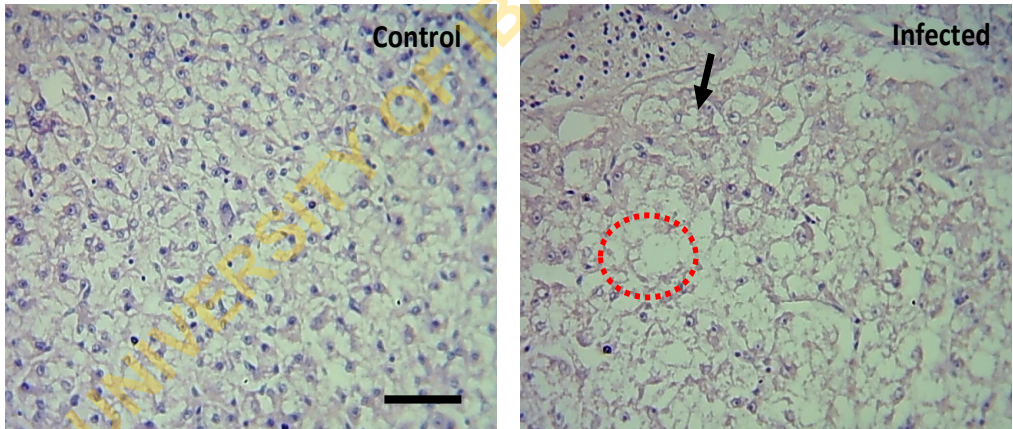


Fig. 4: Liver, H & E. Note the vacuolations (red circles), necrosis and loss of cellular outline (arrow) in the infected. Scale bar 150 μ m (consistent for both panels).

Brain: Vacuolation and spongiosis were observed in the cerebellum, at the junction between molecular and granular layers (Fig. 5).

Skin and muscle: The infected group showed a complete ablation of the entire epidermis; there was loss of pigmentation (Fig. 6).

No lesions were observed in the stomach and intestines.

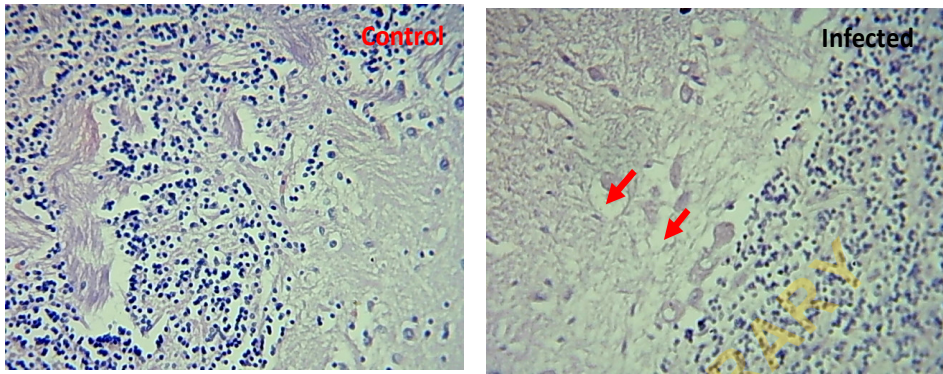


Fig. 5: Cerebellum, H & E. Note the vacuolations (red arrows) between the molecular and granular layer, in the infected group. Scale bar 150 μ m (consistent for both panels).

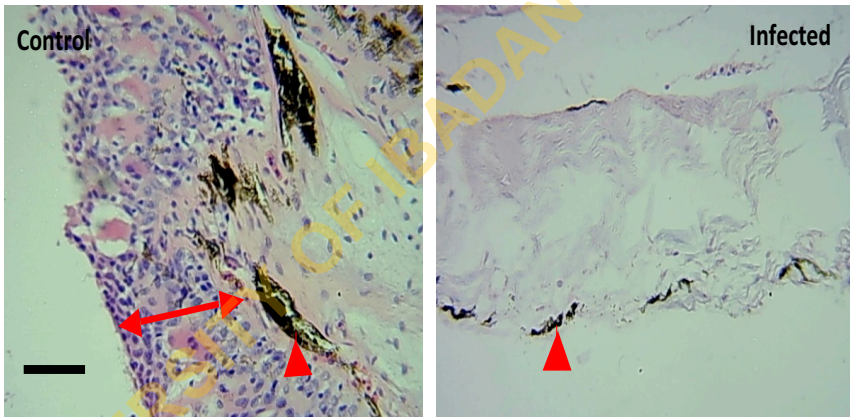


Fig. 6: Skin, H & E stain. Note the complete ablation of the entire epidermis, present in control (red arrow) and the loss of pigmentation, note reduced melanin (red arrowheads). Scale bar 150 μ m (consistent for both panels).

Discussion

Parasites can impose great demands on hosts, affecting morphology and on the long run survival (Marcogliese, 2004). In this way, they regulate host populations (Frainer et al., 2018). While regulating host population may work well in the wild, it causes economic loss in commercial fish farming.

Aquatic snails are reportedly the initial host of pathogenic helminths of fish (Hechinger, 2012; Hong, 2014).

Pathologies observed in the gills of *Oreochromis andersonii* obtained from random health assessment sampling in commercial farms in Botswana (van Dyk et al., 2009) were a bit similar to that observed in this study, vis a vis, epithelial hyperplasia in some regions

of the gills. Although the authors did not report loss of secondary filaments. Vacuolar degeneration and loss of tubule structural integrity were reported in the liver and kidneys of sea bass in some other commercial farms. This is similar to what is reported in this study and could probably be due to helminths (Saraiva et al., 2015). However, contrary to our report, they reported pathologies in the stomach and intestines. This contrary report may be due to the length of time (period) of infection, as our study probably was for a shorter period.

The severe depletion of the lymphohemopoietic compartment seen at histology is worth further investigation to prove if it has similar actions seen in Enteromyxosis, caused by *Enteromyxum scophthalmi*. This myxozoan parasite of fish was found in a study to cause leukocytic depletion along with other morbidity (Ronza et al., 2019).

The authors postulate that the incidence of and response of fish to parasites may vary based on a number of factors, ranging from location, breed and

species and nature of helminth the fishes are exposed to. This may result in a wide variety of responses, but ultimately, still a loss to the commercial farmer.

Interestingly, previous literature citing histological assessment of fish organs did not include the brain. The pathologies observed in the brain in this study were probably due to hypoxia caused by the effects of the metacercaria on the gills, resulting in reducing oxygen uptake. This may be responsible for the pathologies observed in other organs as well, especially since the presence of metacercaria was not observed in any other organ apart from the gills. Although, other tests were not carried out, cognition may have been affected in the affected fish.

In conclusion, to prevent unwanted economic loss due to infestation of cultured fish by pathogenic digenea, and to avoid possible helminth zoonosis, preventive measures should be put in place to break the transmission chain by preventing the incursion of aquatic snails in earthen ponds on commercial fish farm.

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