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## Research Article

# Severe Metacercarial Invasion of the Gills of Farmed Clariid Catfish Juveniles

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### Abstract

**Background and Objective:** Mass mortality of earthen-pond reared *Clarias gariepinus* and hybrid catfish juveniles were reported to the Veterinary Teaching Hospital of the University of Ibadan, Nigeria. The incidence of the disease condition, especially with the presence of aquatic snails in the culture facility that is stream-fed necessitated clinicopathological and epizootiological investigations to determine the causal factor and the course of the disease. **Materials and Methods:** Five samples of each clariid catfish species were clinically examined, sacrificed and dissected for autopsy. Microscopy of gill tissue, skin, and intestinal scrapings, as well as crush preparations of the liver, the kidney and the eye lens, was conducted. Also, 5 aquatic snail samples were cracked open and snail tissues were subjected to microscopy. Experimental infection was conducted by making 10 healthy catfish juveniles cohabit with 20 infected snails. **Results:** Numerous cysts were observed in the gills of the fish samples submitted for clinical examination, with severely disrupted structures. Microscopy of the snails revealed the presence of numerous cercaria, and that of the gills revealed the presence of cysts identified to be metacercaria of a digenea trematode. Experimental infection confirmed transmission of the trematode from snail to fish. Mass fish mortality reported was occasioned by decreased respiratory tolerance due to severe gill damage caused by the invasion of the digenea metacercaria. **Conclusion:** The aquatic snail observed in the affected ponds is the first intermediate host of the worm. Aquatic snails should therefore be eliminated from ponds as part of routine pond preparation to reduce the risk of digenea infection and possible zoonosis.

**Key words:** Digenea, metacercaria, infection, aquatic snails, clariid catfish, microscopy, heteroclerias

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**Competing Interest:** The author has declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Digenea trematode infection is said to be commonplace in wild fish, with encysted metacercaria being more common than the adult worm<sup>1</sup>. The metacercaria is said to be a typically quiescent larva stage though, depending on the predilection site, heavy infection of young fish accompanied by severe organ damage can result in high mortality<sup>2,3</sup>.

Incidentally, there is no therapeutic control of the metacercaria stage of digenea infestation in fish, hence prevention of infection by eliminating the snail intermediate host becomes pertinent. More importantly, some digenea are of zoonotic importance, especially where raw or undercooked fish is consumed<sup>4</sup>. This condition, therefore, constitutes a serious economic and public health concern when incidence is observed in commercially cultured food fish.

Clinico-pathological investigation of the incidence of mass mortality of juvenile *Clarias gariepinus* and hybrid catfish (*Heteroclaris*) stocked in earthen ponds on a teaching and research farm in Ibadan, Oyo State, Nigeria was conducted using microscopy and experimental infection trial to detect and identify the causal factor as well as the course of the disease incidence that culminated in the mass mortality.

## MATERIALS AND METHODS

**Study area:** This study was conducted at the Veterinary Teaching Hospital and the Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, in June, 2021. The study was conducted over 3 weeks.

**Clinical and pathological examination:** Five samples each of moribund juveniles of *Clarias gariepinus* and *Heteroclaris* (hybrid cross of female *C. gariepinus* and male *Heterobranchus longifilis*) collected from a stock of 2000 growing fish in each of two earthen ponds were submitted to the Aquatic Animal Clinic of the Veterinary Teaching Hospital of the University of Ibadan, Ibadan, Nigeria, for clinical examination. Mortality recorded in the affected ponds for *C. gariepinus* was more than 500 juveniles, while that observed in the hybrid stock was about 35 juveniles.

The rearing ponds were said to receive water supply from a nearby stream and there were numerous aquatic snails in affected ponds (Fig. 1a-b). Clinical and post-mortem examinations were carried out as described by Noga<sup>3</sup>.

**Microscopy:** Microscopic examination of skin scrapings, gill filament, crushed preparation of the liver, the kidney, the eye lens and the scrapings of the intestinal lining was carried out on fish samples. Five samples of the aquatic snails were cracked open and crush-preparation of the tissues from the snails was also examined under the microscope. Microscopy was performed using a biological microscope (Olympus, China) connected by Scopephoto<sup>R</sup> (×64) to a laptop to enable the capture of images for possible detection and identification of causal agents based on morphological features.

**Experimental infection trial:** Ten healthy *C. gariepinus* juveniles weighing 9-11 g, collected from healthy stock reared under a flow-through system in fibre-glass tanks, in a commercial farm in Ibadan, Nigeria, were kept in 10 L of water, in a 20 L plastic aquarium together with twenty aquatic snails collected from the digenea infected fish ponds in the affected farm. Another group of 10 healthy fish of similar size range was kept without snails under similar conditions (control). Water was changed daily in all treatments and fish were observed daily for signs of disease. Gill tissue from all the fishes in both treatments was examined via autopsy 1 week post-cohabitation with snails.

## RESULTS

The snails collected from the affected ponds were small with a size range of 1.2-2.2 cm in total length. They possess thick, hard, operculated shells with long spires and pointed apex (Fig. 2). Based on the observed morphological features and the identification keys provided by Peso *et al.*<sup>5</sup>, the aquatic snail is most likely a melanoides. Species identification will require molecular characterization.

During clinical examination, the fish samples were observed to be lethargic, hanging vertically in the water column and piping at the water-air interface. On close examination, it was observed that there was prolapse of the gills in all samples of the two species, as gill filaments protruded out of the branchial cavity (Fig. 3a).

A post-mortem examination revealed matted and pale gills as shown in Fig. 3b, indicating the need for microscopy. No other lesion was observed in any other organ except that the liver also appeared pale.

Microscopy of the gills revealed the presence of multiple, round-to-ovoid cysts in the gill filaments resulting in severe gill swelling and loss of gill structure as shown in Fig. 4a.

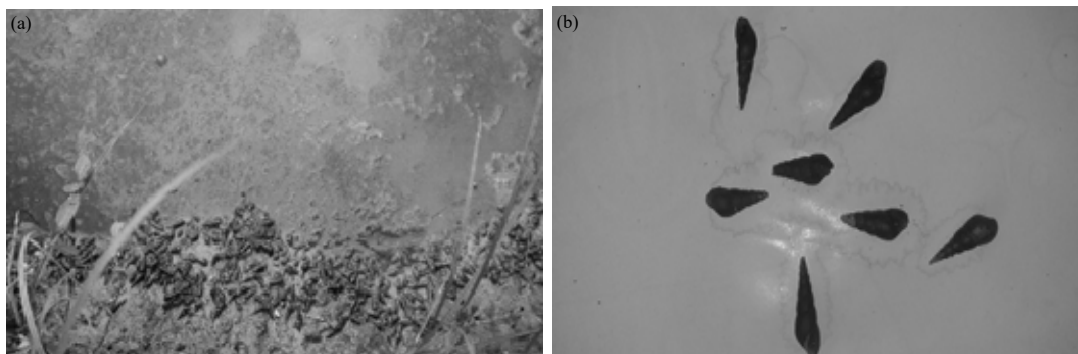


Fig. 1(a-b): Massive infestation of the (a) Earthen pond by (b) Aquatic snails

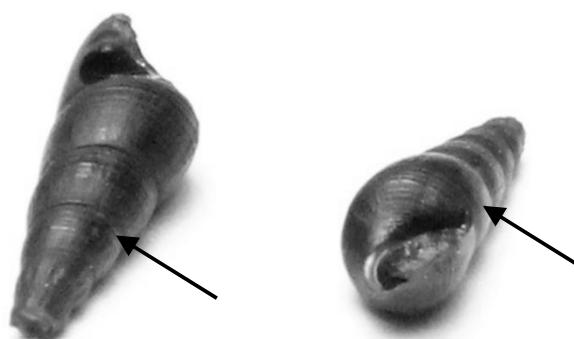


Fig. 2: Aquatic snails suspected to be melanoides based on observed morphological features  
Features like the thick and hard shell, long spire which tapers to a pointed apex and successive transverse lines of growth (arrow)

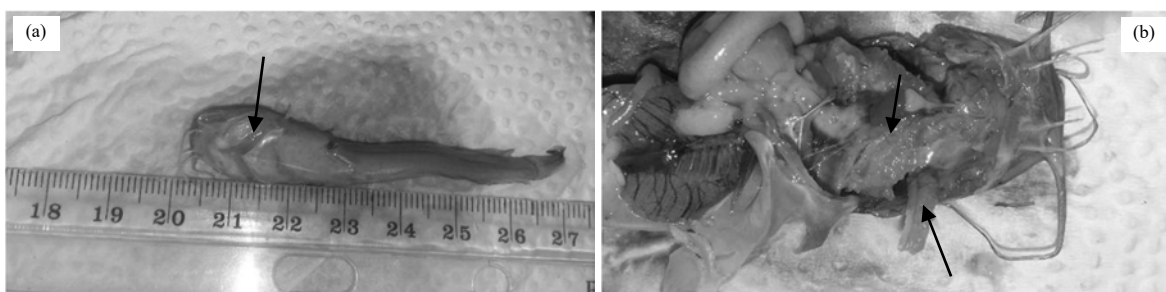


Fig. 3(a-b): Juvenile *C. gariepinus* showing (a) Gill prolapse (arrow) with matted and (b) Pale gills at post-mortem

The secondary lamellae were completely obliterated giving rise to the cyst-filled, club-shaped gill filaments. The amoeboid movement was observed inside each cyst which sometimes revealed the presence of a sucker on the worm in the cyst (Fig. 4b). All fish samples submitted for clinical examination had their gills infected with metacercaria, which was not found in any of the other organs examined. Even the arborescent organ which is also located within the branchial cavity and is attached to the gills was not infected.

Two of the five aquatic snail samples examined were found to be infected with highly motile cercaria (Fig. 5a). The cercaria (Fig. 5b) has an ovoid to oblong body shape inside

which there is a constant amoeboid movement. It possesses a long tapering tail and two eye spots.

The cysts were identified as metacercaria based on their morphological features and those of the cercaria observed in the aquatic snail. The identification keys provided by Hechinger<sup>6</sup> were used to arrive at a tentative diagnosis. Proper identification will require molecular characterization.

Only the gills of all fish that cohabited with infected snails were observed to be invaded massively with metacercaria 1 week post-cohabitation. No metacercaria was found in the control group.

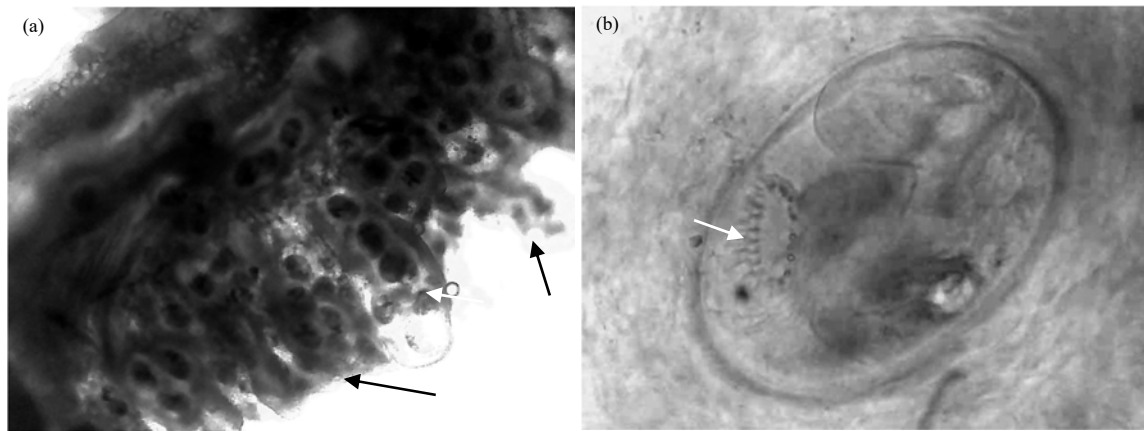


Fig. 4(a-b): Microscopic view of the gills, (a) *Clarias gariepinus* gills, showing filaments (black arrows) invaded by numerous encysted metacercaria (white arrows) and (b) Single, encysted metacercaria ( $\times 400$ ) observed in the gill filament of *Clarias gariepinus*

Note the sucker (white arrow)

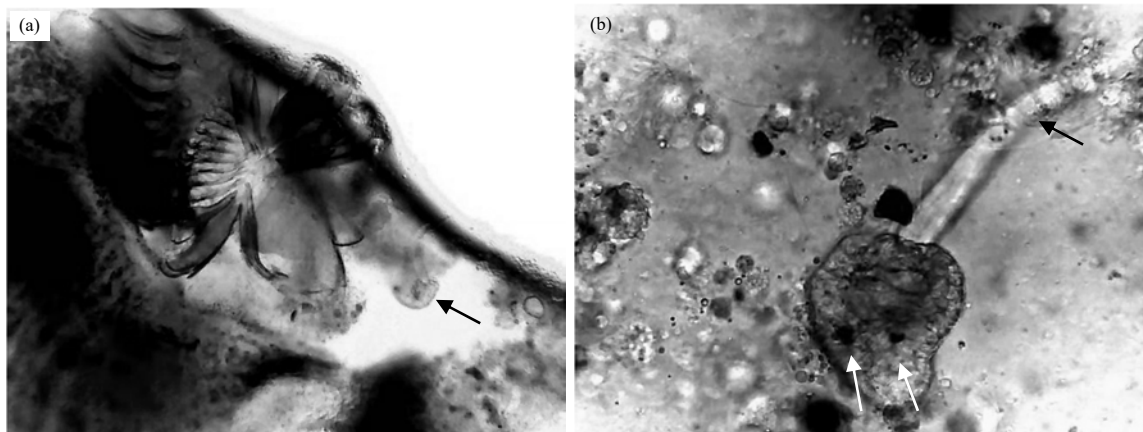


Fig. 5(a-b): Microscopic view of the aquatic snails, (a) Tissues extracted from aquatic snail, showing cercaria (black arrow) note the long tail ( $\times 40$ ) and (b) Single cercaria, showing two eye spots (white arrows) and the long, tapering tail (black arrow)

## DISCUSSION

Fish culture holds the promise of bridging the huge gap in animal protein demand and supply. However, considering public health in the production of food-fish by culture has become very important, especially with the possibility of zoonosis<sup>4,7,8</sup>.

Aquatic snails are known to be the first intermediate host for pathogenic helminths of fish, which are the second intermediate or even final host of such helminth pathogens<sup>4,6</sup>. In this study, aquatic snails were observed in earthen ponds which were fed with stream water, indicating the likelihood that they were introduced when the ponds were impounded.

The presence of a heavy load of metacercariae of a digenea in the gills of *Clarias gariepinus* and its hybrid (Heteroclarias), stocked in ponds where aquatic snails were found, is a pointer to possible transmission from the aquatic snails to the fish. It has been observed that massive metacercarial invasion with numerous cysts causes severe destruction of normal gill structure, reducing the surface area of respiratory epithelium and may also compromise osmoregulation<sup>8</sup>. The resultant effect of all these is mass mortality especially in young fish, as observed in the affected ponds in this study. Numerous metacercariae cysts were observed in the gills of all fish affected, obliterating the secondary lamellae and thus severely reducing the respiratory surface area, forming a distorted club

gill. The prolapse of the gills observed is an indication of gill enlargement due to metacercaria infiltration with the possibility of gill hyperplasia.

The features of the cercaria observed in the tissues of the melanoid snail and the metacercaria found in the gills of the infected fish is suggestive of a digenean infection. The presence of the two eye spots on the cercaria which possess a long tapering tail and a metacercaria which possesses a sucker lend credence to this observation, as they agree with the identification keys provided by Hechinger<sup>6</sup>. The features of the digenean are similar to what was described for *Clonorchis sinensis* (Chinese liver fluke) said to infect 15-20 million people in China, Korea and Vietnam where raw or undercooked freshwater fish is a delicacy<sup>4</sup>. The possibility of poorly cleaned and under-cooked 'barbecued catfish' is there in Nigeria, where the fish head (that houses the branchial cavity) is also a delicacy, making the zoonotic transfer of this digenea a possibility.

The mortality observed in the *C. gariepinus* stock (over 25%) is very high and can be considered to be economically significant. The hybrid catfish (Heteroclarias) stock on the other hand suffered low mortality (1.75%), though metacercarial gill invasion was observed to be heavy in both species. This observation tends to support the purported disease-resistance trait of the hybrid catfish<sup>9</sup>.

Since the treatment of metacercaria gill invasion is said to be very difficult, breaking the life cycle of the helminth by eliminating the snail's primary host as earlier suggested becomes attractive. This can be accomplished during routine pond preparation via pond desiltation and the application of appropriate chemicals. Furthermore, consumers should be enlightened on the danger of eating raw or undercooked fish to avoid zoonosis.

## CONCLUSION

The mass mortality observed in the affected stock of the clariid catfish species was due to the severe gill distortion in the affected fish. This was occasioned by massive infiltration of the gill tissues by digenea metacercaria, affecting the respiratory and osmoregulatory functions. Cercaria was transmitted to the fish through the aquatic snails that invaded the fish ponds impounded from the nearby streams. The condition has economic and public health significance and should be prevented through the elimination of aquatic snails from fish ponds.

## SIGNIFICANCE STATEMENT

Mass mortality in farmed, juvenile clariid catfish species due to metacercarial invasion not only have serious economic implications but is also of possible public health significance. Clinicopathological and epizootiological investigation will aid effective prevention and control of this zoonotic condition.

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