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Diagnosis and Management of Coccidiosis in a *Clarias gariepinus* Catfish Farm: A Case Report

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ABSTRACT

Coccidia are spore-forming obligate intracellular protozoan parasites that cause disease in several animal species, including fishes. In this report, we present the clinical diagnosis and therapeutic management of a case of coccidiosis in a Clarias gariepinus catfish farm with a high morbidity and mortality rate. Following the report of an increasing mortality on the farm, fish showing nondescript signs were sampled, and subjected to parasitological evaluation. Dissection of the small intestines revealed small white to grayish raised nodules in portions of the anterior intestines, the mucosa of the small intestine also showed congestion, hemorrhage, and presence of brownish blood-mixed contents in the intestinal lumen. Histological examination of sections of the small intestines showed the presence of cystic structures full of spores (sporocyst of about 580 µm in size) developed in portions of the anterior intestine of the affected fishes. These were identified as Coccidian sp. because of their similar morphology to those of the parasites in the Coccidian group. Based on history, clinical symptoms, gross, feacal and histopathological findings, the present case was diagnosed as coccidiosis. Treatment was instituted with amprolium delivered through 'top-dressing' of the feed at 0.05% for 5 days. Effectiveness of the instituted treatment could not be ascertained, as the owner sold of the entire flock to cut further losses on the farm.

Keywords: Fish, *Clarias gariepinus*, Coccidiosis, Coccidia parasite, Catfish, Oocyst.

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INTRODUCTION

The African Sharp tooth catfish (*Clarias gariepinus*) is an important aquaculture fish species and a staple in Nigeria and West Africa. The increase in the culture of this fish species has led to a rise in the economic value of the fish in Nigeria and in West Africa [1, 2]. According to the Federal Department of Fisheries (FDF), there was an increase of about 41% in the production of *Clarias gariepinus* in Nigeria, representing about 237.8 million tons between the year 2000 and 2007 [3]. However, the increase in the intensification of *Clarias gariepinus* production has resulted in an increase of the occurrence of diseases in fish farms across the country [2, 4].

According to [5, 6], emerging diseases of economic significance in catfish farms in Nigeria includes furunculosis, aeromonas infections, haemolytic syndromes caused by *Enterobacter* sp, *Staphyllococcu* sp; White spot disease caused by the protozoan, *Ictyophthirius multifilis* [7,8]. Also of significance, are other parasitic diseases caused by helminths infestations and the protozoan infections caused by *Eimeria sp*. [6, 7, 9].

Typically, piscine coccidia follow the pattern of merogony, gamogony, oogony, and sporogony that has been observed in coccidian infections in terrestrial animals [10, 11]. Coccidia of terrestrial vertebrates undergo exogenous sporogony, in contrast with fish coccidians that generally, undergoes endogenous sporogony [12, 13, 14]. Infections with coccidia may be direct or indirect. Direct infection is generally caused by ingestion of contaminated feces, which could explain higher infectivity rates with intensive culture under poor water and management conditions.

Intestinal coccidiosis in fish is a disease caused by the common piscine apicomplexan parasite genera, including *Eimeria* spp., *Goussia* spp., and *Cryptosporidium* spp. [13, 14, 15]. The pathogenicity of the disease depends on individual fish species sensitivity [15, 16], immune status [17], bacterial co-infection, water quality parameters, and the sites of the intestine affected [18, 19]. Pathological changes seen in the intestinal tissues includes denuded intestinal epithelium, intense inflammation, focal necrosis, and the presence of mucoid casts containing parasite stages [17, 18, 19]. The disease is treated by the application of anticoccidials line amprolium in the feed for five to seven days.

Materials and Methods

Case Report and Clinical findings

A fish farmer reported to the Veterinary Teaching Hospital, University of Abuja with a complaint of increasing mortality in his catfish farm. This farm is an integration of a grow-out and a hatchery operation with a stock of over seventy thousand *Clarias gariepinus* catfish stocked in earthen ponds of varying dimensions (Figure 1).

Figure 1: *Clarias gariepinus* catfish farm with outbreak of eimeria infection

The client further revealed there had been mortalities that cut across all age groups in the past. The present mortality pattern however appears to be more amongst the juveniles and has been sustained over the last 2-3 week. Two brood fish (adult female average weight 2.70 kg) were presented for necropsy (Figure 1b).

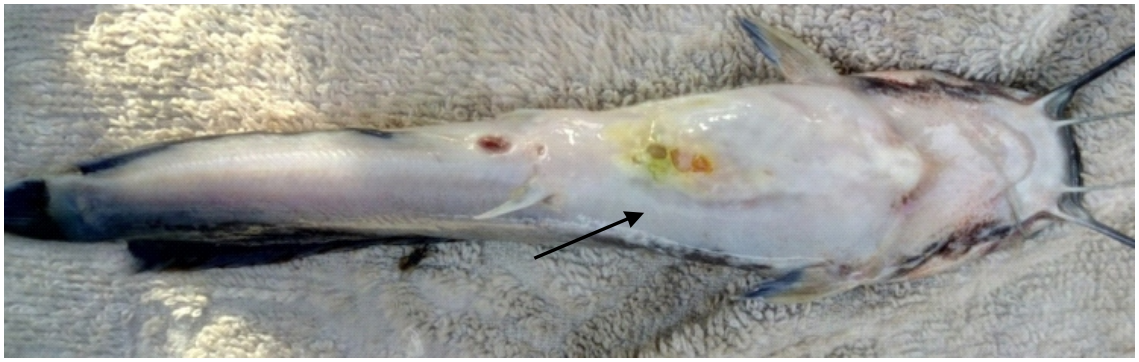


Figure 2a: *Clarias gariepinus* presented for necropsy (ventral view). Notice the bloating of the abdomen and the red-green discoloration and ulceration of the abdomen (black arrow).

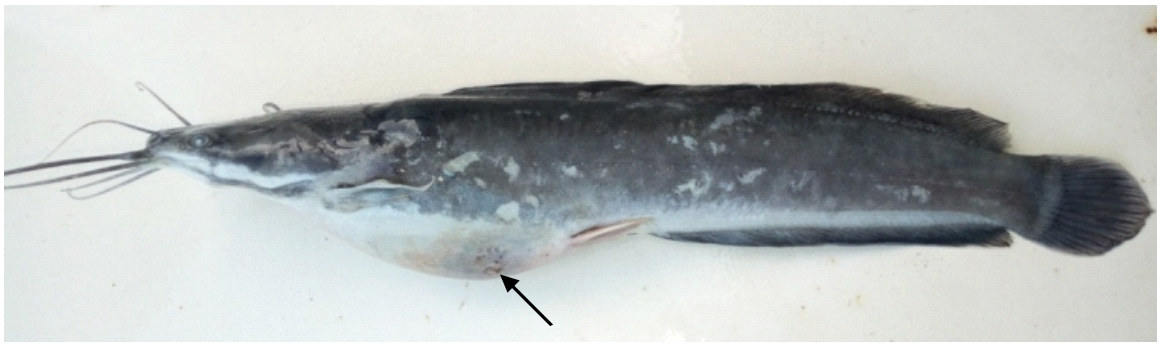


Figure 2b). *Clarias gariepinus* presented for necropsy (lateral view). Notice the bloating of the abdomen and the blue-green discoloration and ulceration of the abdomen (black arrow).

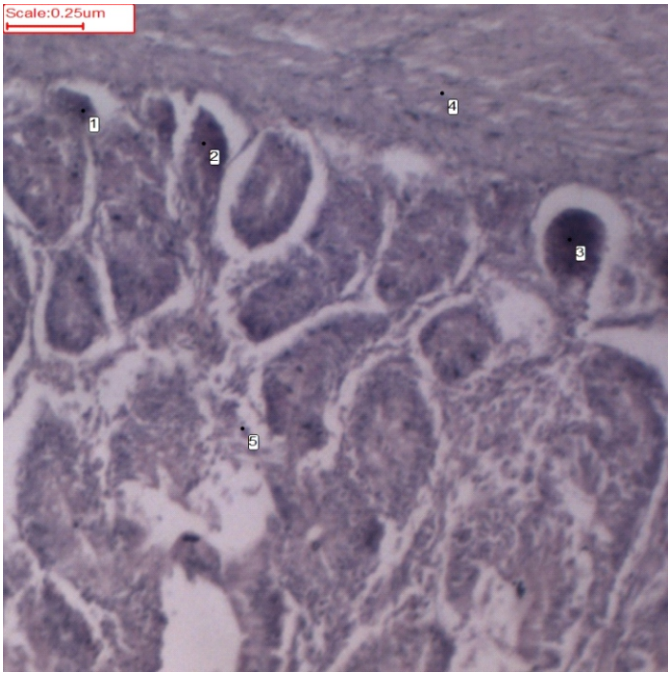
1.1 Procedures and Diagnosis

Fish were euthanized with eugenol 0.2 mL, as previously described [20]. Briefly, 0.05 ml of clove oil was mixed with 500 ml of dechlorinated water. Individual fish was placed into the container for 30 minutes, to develop deep anesthesia. A lateral incision was made on the abdominal region, and the stomach, small intestine, and large intestine were separated and collected in 10% buffered formalin. The intestines were turgid and contracted, with a corrugated appearance, and contained intraluminal amorphous debris. The small intestine was dissected into three parts: anterior, mid, and posterior intestine. The intestinal samples were subsequently subjected to routine histological techniques using Hematoxylin and Eosin stains, and cut into 3-5µm sections that were collected on microscope slides.

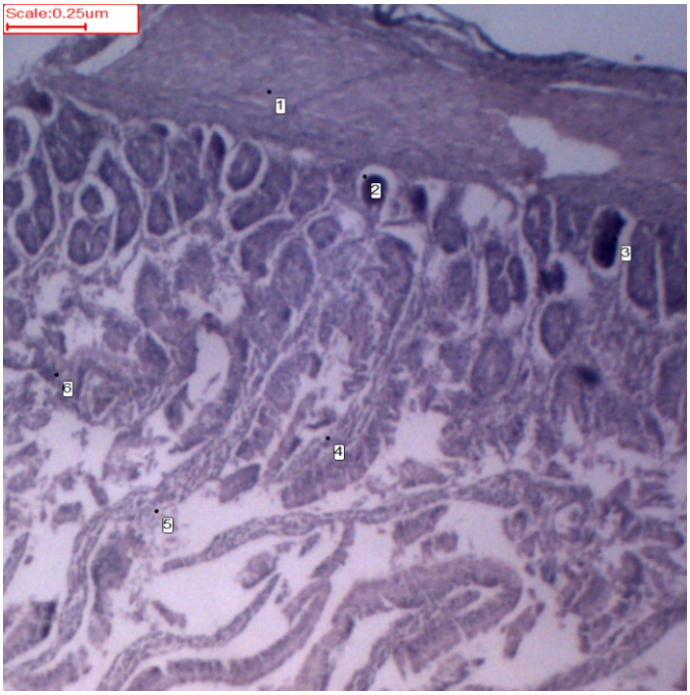
The coccidian infection, distribution and histopathological changes were investigated in the stomach and in the three different portions of the small intestine and in the large intestine. In these portions, the epicellular, intracytoplasmic, and extracellular (subepithelial, submucosal) were evaluated for the presence of coccidian parasites following the methods of [19, 20]. The morphological identification of the coccidian parasite was performed and confirmed by a parasitologist using [15].

3.0 **Laboratory Results, Treatment and Discussions**

Light microscopic evaluation of the HE-stained sections revealed randomly distributed spaces within the intestinal mucosal epithelium that often contained 1-8 crescent-shaped to elliptical, eosinophilic structures suggestive of sporulating coccidian oocysts (Microplates 1 and 2). No coccidia or other significant lesions were noted in the liver, stomach and spleen.



Microplate 1: Crescent-shaped to elliptical, eosinophilic structures suggestive of sporulating coccidian oocysts



Microplate 2: crescent-shaped to elliptical, eosinophilic structures suggestive of sporulating coccidian oocysts and denuded debris in the lumen of the intestine.

1.1 Confirmatory Diagnosis

Both fishes examined were positive for coccidian parasites marked by the presence of sporolated oocysts in the intestinal lumen and merozoites in the intestinal epithelium (Plate 1 & 2).

No lesions were observed in the liver, stomachs and large intestines of both fish. The intestinal lesions and coccidia parasites seen were present predominantly in the small intestine, with the highest parasite proportion detected in the anterior gut, followed by the mid and posterior gut (Figure-2a). The detection of the coccidian stages at three sites was observed in both fish sampled. The observed parasites were seen in the epicellular, intracytoplasmic, and extracellular or subepithelial layers of the affected intestines. There were inflammatory cell infiltration and vascular congestion in the intestines. Although the inflammation observed were limited to the mucosal and submucosal intestinal layers inhabited by the coccidian stages.

One of the presented fish exhibited extensive inflammation, which was illustrated by the presence of invasive lymphocytes and eosinophil infiltration into the muscular layer and gut wall (Plate 1). In this same fish, there was severe active congestion or hyperemia of the affected intestine due to blood vessel dilatation with accumulation of red blood cells at the site of inflammation. Desquamated epithelia were also seen in areas with a higher degree of active inflammation and this was dependent on the intestinal location (Plate 2). Epithelial desquamation in the inflamed anterior intestine was more severe than in other locations.

Treatment

Amprolium 200 mg/kg feed for 5 days (Drug was applied as 'top dressing', using corn gluten as binder).

3.3 Discussion

Piscine apicomplexan parasites exhibiting epicytoplasmic development in host cells are reported to be members of the genera *Cryptosporidium*, *Eimeria*, *Epieimeria* or *Goussia* [9, 10, 12,]. Infections of fish by Eimeria species has been reported in Asian seabass, *Lates calcarifer* [20], Bluegill, *Lepomis macrochirus* [12] and in *Chrysichthys auratus* [18]. Intestinal coccidiosis has also been reported to be caused by Gaussian species in Alewives, *Alosa pseudoharengus* [21] and in Carp *Cyprinus carpio* [19].

Aside from the report of [9], [16] and the experimental study on the tissues response in *Clarias gariepinus* following challenge with *Eimeria subepithelialis* [17], to the best of our knowledge, there are no reports of intestinal coccidiosis in farmed *Clarias gariepinus*. This report may be a first case report of clinical intestinal coccidiosis in this important fish species.

Infections with *Eimeria spp* leading to the disease condition termed coccidiosis is likely to occur only under conditions of high stocking densities which favours the build-up of potentially pathogenic populations of the parasites [14, 19, 21], or in situations of challenged health conditions with poor immune competence [13, 15]. Apart from causing disease, subclinical infections of Eimeria Spp causes impaired feed conversion and hence low thrift thereby increasing the final cost of production and reducing the economic value of the final product [12, 15]. Other predisposing factors to this disease includes environmental (poor water quality and or nutritional stress, as seen in this case, where poultry wastes were used as supplementary feeding on the fish farm).

The life Cycle of a typical *Eimeria spp*, comprises of a parasitic and a non-parasitic phase. Although, we could not determine the origin of the infection in this report, coccidial infection is acquired through the ingestion of sporulated oocysts. Motile sporozoites emerge from the oocyst wall and attach to the intestinal epithelial cells. In the host epithelial cells, the sporozoites induces the fusion of microvilli so

that it becomes surrounded by a double membrane of host origin referred to as being 'extracytoplasmic' [14, 15]. Where the parasite undergoes a trophic period in which it probably derives nutrients from the host cell [14, 15].

The trophozoite then undergoes an asexual replication, termed either merogony or schizogony, resulting in the production of 4-8 merozoites. These Mature merozoites are released into the intestinal lumen from where they infect new intestinal epithelial cells (as observed in plate 1). Merozoites are morphologically similar to sporozoites. The merozoites can either undergo additional rounds of merogony, resulting in the production of more merozoites, or undergo a sexual cycle known as gametogony (or gamogony) [15].

Some of the merozoites will develop into macrogamonts (also called macrogametocytes) which mature into macrogametes. The microgamont (or microgametocyte) will undergo several rounds of replication producing numerous microgametes which are released into the intestinal lumen (plate 2). The infectious oocysts are excreted in the feces, thus completing the life cycle which may result in an autoinfection in which ex-cystation takes place within the same host. If the sporulated oocyst is ingested by a susceptible fish and through the agencies of mechanical and chemical factors in the gut (bile salts and trypsin), on the sporocysts, the sporozoites contained therein are released in the duodernal lumen [18]. Where they invade the mucosa, resulting in the phases of intracellular growth and asexual multiplication with periodic release of merozoites back into the intestinal or gut lumen (Plate 1 and plate 2), [10].

After a period of this cycle (termed the schizogonous cycles), sexual forms termed the gametocytes develops intracellularly. These gametocytes differentiate into the micro and macrogametocytes. The microgametocytes releases several microgametes which are flagellated and motile and migrates to find macrogametocytes (not seen in this report), [15, 17]. The macrogametocytes develops into a single macrogamete which, after fertilization, develops into a zygote and thence an oocyst with a prepatent period of four to five days which may vary with species, but ultimately results in hundreds of thousands or even millions of oocysts produced from one ingested oocyst [14].

The oocyst thus formed contains an undifferentiated spherical body, and it only becomes potentially infective after undergoing multiple sub-divisions into four sporocysts each of which contains two sporozoites a process termed sporulation [13, 22]. Sporulation is favoured by two environmental parameters: Warmth (water temperature between 25^0C-30^0C) and available dissolved oxygen (D.O. $\geq$ 2%); both conditions readily prevalent in pond systems on the farm.

4.0 Conclusion and Recommendation

The pathological changes accompanying coccidian infection in farmed *Clarias gariepinus* were observed predominantly, in the anterior intestinal area. These are severe mucosal inflammation extending to the deeper muscular layer, severe villi denudation, and mucosal necrosis. This emphasized the importance of the effect of coccidia on the health and vitality of the fish. It is therefore of importance to prevent the introduction of the coccidian parasites into the farm, by way of proper screening of new fish, and the non utilization of poultry wastes as feed or supplementary feed in fish farms. There is also, a need to include anti-parasitic drug protocols in catfish cultured in earthen pond system. Unfortunately, follow-up samples were unavailable for laboratory studies to attempt to evaluate or demonstrate the effectiveness of the treatment protocol adopted as the entire stock was sold off by the farmer.

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