Short Communication

Outbreak of *Aeromonas hydrophila* associated with the parasitic infection *Ichthyophthirius multifiliis* in pond of African catfish (*Clarias gariepinus*) fingerlings at Sebeta, Ethiopia

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Abstract

Outbreak of a disease was observed on African catfish (*Clarias gariepinus*) fingerlings manifested by white nodules all over the body and hemorrhage in the skin that occurred on June 20, 2011 in an earthen pond at Sebeta, Ethiopia. The outbreak was investigated by using a combination of methods that included clinical observations, gross and histopathology examination and bacterial isolation. On histopathological examination co-infection of *Aeromonas hydrophila* with *Ichthyophthirius multifiliis* a holotrichous ciliate, was found to be the cause of the outbreak. In order to control the outbreak, the fish density was reduced and the fish were removed and treated with sodium chloride (3%) and moved to another properly disinfected pond that contains fresh and good quality water. The former pond was drained and left empty for two weeks to dry and then lime was added over it before filling it with water. The sick fish got cured after three weeks and no new case was observed; which may be due to development of immunity or the intervention measures taken to control the problem. This intervention protocols need to be further investigated in a properly designed experiment as a possible control of co-infection of these two pathogens in catfish fingerlings.

Keywords: *Aeromonas hydrophila,* Co-infection, Ethiopia, *Ichthyophthirius multifiliis*

Introduction

Aeromonads are essentially ubiquitous in the microbial biosphere. The relative environmental distributions of *A. hydrophila* make it as the predominant bacteria in vertebrates and fresh water, common in saline water and foods and less in invertebrates (Janda and Abbott, 2010). *A. hydrophila* as a cause of fish

disease (hemorrhagic septicemia or ulcer disease) both in experimental and natural infection is documented elsewhere in the world (Yesmin *et al*., 2004; Al-Dughaym, 2000; Aydin and Ciltas, 2004). Turutoglu and his colleagues (2005) tried a pathogencity study in rabbits using a crocodile isolate which died as the result of *A. hydrophila* infection and observed local abscess in subcutaneously inoculated ones and death in those inoculated intraperitoneall*y*. *A. hydrophila* infection could be accelerated in the presence of parasitic infection including *Ichthyophthirius multifiliis,* a ciliated parasite which parasitizes the epithelial surface of fish, and the mechanical trauma caused by the parasite may act as a portal of entry for pathogens present in the water including *A. hydrophila* and *Edwardsiella ictaluri* (Liu and Lu, 2004*;* Xu *et al.*, 2012). In human beings *A. hyrophila* cause infection of different body systems including skin and soft tissue and is also zoonotic (Janda and Abbott, 2010; Aslani and Alikhani, 2004; Abraham, 2011). In Ethiopia survey of bacterial and parasitic fish pathogens has been carried out by EshetuYimer (2000) in Lake Ziway and *A. hydrophila* was not among the reported ones. However, Anwar Nuru and his colleagues, (2012) isolated *A. hyrophila* from Lake Tana and *A. hyrophila* was the most frequent isolate in *Clarias gariepinus,* second from kidney and intestine samples and common in immature compared mature stages. The bacteria were also isolated from water samples collected at different fish habitats. This and other previous studies elsewhere clearly indicated the importance of *A. hyrophila* as a fish and zoonotic pathogen and most importantly when combined with the parasite *Ichthyophthirius multifiliis*. We do not believe that there is enough information in Ethiopia on the co-infection of the two pathogens. The aim of this report is therefore to describe a case of skin lesion associated with co-infection of *A. hydrophila* and the parasite (*Ichthyophthirius multifiliis*) in pond catfish (*Clarias gariepinus*).

Study pond description

The former Sebeta Fish Culture Station and now National Fishery and Aquatic Life Research Center (NFALRC) was established in January 1977 and is doing research on fish and aquatic fauna and flora. As part of its research facility, the center owned a total of 38 ponds, of which 12 are concrete walled ponds and the remaining 20 are earthen ponds. The size of ponds varies from 50 m^2 to over 900 m^2. The water supply for the ponds comes from a borehole with a capacity of 19 liters per second. The center propagates and maintains five different exotic and indigenous fish in these ponds mainly for research, namely Nile tilapia (*Oreochromis niloticus*), Tilapia (*Tilapia zilli*), African catfish (*Clarias*

gariepinus), Common carp (*Cyprinus carpio*), and Gold fish (*Carassius auratus*) (NFALRC, 2012). An outbreak of a disease occurred in earthen pond that was stocked with African catfish (*Clarias gariepinus*) fingerlings of 4 months of age on June 20, 2011. Some of the water quality parameters like pH of 8.83 and temperature of 18.2°C at the time of the outbreak were normal and dissolved oxygen (DO) level of 4.5mg/l were also recorded.

Case history and clinical observations

Nodular swelling was observed all over the body of *Clarias gariepinus* fingerlings on June 20, 2011 in earthen pond at NFALRC, Sebeta, Ethiopia. Approximately 70% of the pond fish were affected and fingerlings were the ones most affected (4 months of age). As a result the growth rate of the fish was retarded but mortality was not observed. Externally, there was hyperemia, paleness on the skin and nodular swellings on the skin.

Bacterial isolation

Fish with the lesions were submitted to National Animal Health Diagnostic and Investigation Center (NAHDIC) in a bucket directly from the pond. Samples were collected from the nodular swellings and hyperemic skin lesions aseptically by disinfecting the surface with 70% ethyl alcohol to remove the normal flora. Isolation was conducted following standard procedures described by Quinn *et al.* (1999). The surface of the samples was first decontaminated by hot scalpel application and then an incision was made with a sterile scalpel blade. After the incision an inoculum was taken from interior of the skin using inoculating wire loop and cultured on Blood and MacConkey agar and incubated at 37°C for 24 hours. Grey, flat and mucoid colonies with haemolysis were observed on blood agar and colonies were non lactose fermenter. Primary tests including Gram's reaction, catalase, oxidase, oxidation-fermentation (O-F), motility, glucose, growth on Blood and MacConkey agar were employed. In addition, secondary biochemical tests including growth on 6.5% NaCl, Indole production and, sucrose, maltose and mannitol fermentation were conducted. Clinical pictures and characteristic growth on Blood agar (haemolysis) and MacConkey (bile salt sensitivity) were used to differentiate *A. hydrophila* from other groups of motile aeromonads like *A. sobria and A. caviae.*

Gross lesions and histopathological findings

Grossly white nodules were observed all over the body and hemorrhage in the skin of catfish were observed (Figure 1). During necropsy skin and muscle with nodular lesions were collected and fixed in 10% buffered formalin for 48 hours. They were trimmed and subsequently dehydrated in a series of different alcohol concentrations, cleaned with xylene and embedded in paraffin wax. The tissues were then sectioned at about 4µm thickness on microtome and mounted on glass slides, dewaxed and stained with hematoxylin and eosin (HE) (Bancroft *et al*., 1996). The tissue sections were examined under microscope and encysted parasite with horse shoe shaped nucleus was observed in the muscle of the fish (Figure 3). This parasite looks like *Ichthyophthirius multifiliis*.

Figure 1. White nodules all over the body of catfish fingerlings

Figure 2. Hemorrhage in the skin of catfish

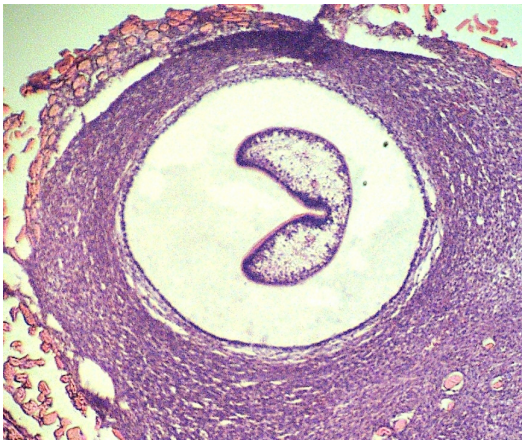

Figure 3. Histopathological section (40x) showing encysted parasite having horse shoe shaped nucleus in the muscle of catfish fingerlings

Discussion

Previous studies have showed that *A. hydrophila* could be a primary or secondary pathogen in causing disease in fish and other animals (Yesmin *et al*., 2004; Al-Dughaym, 2000; Aydin and Ciltas, 2004; Turutoglu *et al.*, 2005). *I. multifiliis* is also a long-time-recognized parasite occurring in tropical, subtropical and temperate zones causing Ichthyophthiriasis or 'white spot disease' (Scholz, 1999). In experimental study of co-infection of Channel catfish with *I. multifiliis* and *A. hydrophila,* parasitized catfish showed higher mortality (80.0%) than non-parasitized fish (22.5%) after exposure to *A. hydrophila* by immersion (Xu *et al.*, 2012). There are several possible roles of Ich parasitism in contributing to fish death when co-infection with *A. hydrophila* occurred. The parasite

first directly damages fish skin/gills and cause fish death, second damages fish first line of defense and helps *A. hydrophila* gain entry into fish host and third causes stress and reduces fish's immune protection thus increasing the ability of *A. hydrophila* to infect fish (Sitja-Bobadilla, 2008; Jorgensen and Buchmann, 2007 cited in Xu *et al.*, 2012).

In the present outbreak although approximately 70% of the pond fish were affected and the growth rate retarded, mortality was not observed. This may be due to the early intervention taken after clinical signs were noticed. The recommended stoking density of a pond is 3 to 6 fish per square meter (Diana *et al*., 1995; Diana *et al.,* 96). But the fish stock in the pond was up to 9 per square meter. Therefore, the fish density was reduced to the recommended level to avoid overcrowding and stress. The fish in the pond were removed and disinfected with 3% sodium chloride and transferred to another well disinfected and fresh and good quality water filled pond. The former pod was drained and left for two weeks to dry and then lime was added over it before filling the water. Lime was added to kill bacteria, fish parasites and their intermediate hosts by its toxic and caustic action, to neutralize and buffer the pH to an acceptable alkaline level and to reduce potential of oxygen depletion (Boyd, 1979; Yamada, 1986; Dittrich *et al*., 1997). The sick fish were cured after three weeks and no new case was observed which may be due to the development of immunity or the intervention measures taken. The intervention needs to be further investigated as a possible control of the co-infection of these two pathogens.

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