

## Incidence of ulcer disease in African catfish (*Clarias gariepinus* Burchell) and trial for its chemotherapy

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### Abstract

Young *Clarias gariepinus* cultured in an artificial tank were severely affected by an ulcer type of disease where 77% fish died within 5 weeks. From the lesions and kidney of affected fish *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Micrococcus* and *Staphylococcus* were isolated where *Aeromonas* was observed as the dominant bacteria. Among them, an *A. hydrophila* isolate AGK 34 was detected as a pathogen by the experimental challenge test. In order to find out a suitable remedial measure of the disease, four different chemotherapeutants were applied to the affected fish in 6 different ways under laboratory condition. Affected fish were recovered from the disease in different treatments. But the best result was obtained by a successive bath in 1-2% NaCl and subsequent oral treatment with commercial oxytetracycline at a dose of 75 mg/kg body weight of fish.

**Key words :** Ulcer disease, *Aeromonas hydrophila*, Chemotherapy

### Introduction

African magur, *Clarias gariepinus* is one of the fast growing exotic fish in Bangladesh. It has been widely cultured both in ponds and artificial tanks in this country since 1989. Like all other fishes, they are not free from the threatening of various types of diseases in Bangladesh.

Chowdhury (1993) reported that farmed carps and catfishes including African magur have been suffering from diseases like ulcer type of disease including EUS, various types of lesions, tail and fin rot, bacterial gill rot, fungal and parasitic diseases in a number of fish farms of Bangladesh. Among these, ulcer type of disease is the most important one where bacteria were found to be involved with the disease (Rahman and Chowdhury 1996). No systematic works on the diseases of *Clarias gariepinus* were reported. Proper diagnosis of any disease is very important to the context of Bangladesh. Moreover, our fish farmers have no proper knowledge of fish health management, use different chemicals as an irregular practice of chemotherapy. In most cases farmers fail to control the incidence of disease, which need research based information and its extension.

Considering the importance, present research works were planned to find out the mortality patterns of fish suffering from ulcer type of disease under a case study, to

isolate and identify bacteria from the affected fish, to perform the challenge test with some suspected bacterial isolates, and to perform trials of chemotherapy to control the disease.

## Materials and methods

### *Outbreak of ulcer disease in an artificial tank*

A total of 500 African magur, *Clarias gariepinus* ( $43 \pm 7$  g) was stocked to grow up in an artificial tank (11.7 m x 6.65 m x 1.1 m) situated at the site of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Under ground water was used in that tank and changed in every 3 or 4 weeks. The fish were fed prepared food using rice bran (45 %), fish meal (15 %), mustard oil cake (30%) and flower (10%) in irregular basis.

An ulcer type of disease was observed in that fish from the second week of November to the middle of December, 1996. The disease was grossly detected by the appearance of lesions on the body surface of fish. Investigation of the incidence of lesions and mortality of fish was considered as a case study. It was performed on the basis of observation and sampling of fish 2-3 times in a week from different parts of the tank. Some physico-chemical parameters viz.,  $P^H$ , temperature and dissolved oxygen of tank water was also recorded during the study period.

### *Bacteriological examination*

Moribund and freshly dead fishes were collected randomly from different parts of the tank with a scoop net in a plastic container with the same water and brought immediately to the Fish Disease Laboratory for bacteriological examination.

Tryptone Soya Agar (TSA, Oxoid), Cytophaga agar (Anacker and Ordal, 1959) and Aeromonas Agar Base (Oxoid) supplemented with ampicillin SR 136 E were used for culture and isolation of bacteria in the present study.

Swabs from external lesions and kidney of the affected fishes were taken aseptically and inoculated into different bacterial culture media according to the standard decimal dilution method. The culture plates were incubated aerobically at 25° C for 48 h. Then some representative colonies were further cultured on fresh agar plates to obtain pure cultures.

### *Characterization of the bacterial isolates*

The bacterial isolates were characterized and identified following the standard morphological and biochemical tests stated in the Cowan and Steel's Manual for Identification of Medical Bacteria edited by Barrow and Feltham (1993). However the aeromonad isolates were further identified up to species level according to the methods described in the Bergey's Manual of Systematic Bacteriology (vol. 1) edited by Krieg and Holt (1984). The results of the tests were also confirmed with a known strain of *Aeromonas hydrophila* (Thai Strain).

### ***Bacterial challenge test***

For bacterial challenge test, 4 identified aeromonad isolates were selected which were recovered from the kidney of infected fish. Healthy young *C. gariepinus* (20g) and an exotic carp, *Puntius gonionotus* (15g) were used as experimental fish. The fish were acclimatized in the laboratory earlier. The test was performed following a water-borne infection method (contact by immersion) described by Rahman and Chowdhury (1996).

### ***Drug Sensitivity test***

Sensitivity test was performed to know the sensitivity of the selected aeromonad isolates to different drugs. The test was conducted according to the method described by Chowdhury *et al.* (1997).

### ***Trials for Chemotherapy***

In order to find out suitable remedial measures of the disease 4 different chemotherapeutants were applied in 6 different ways on the affected fish in laboratory condition. The application methods and doses of the chemotherapeutants were used according to the standard methods and doses described by Herwig *et al.* (1979) and Roberts *et al.* (1989) for the treatment of fish suffering from various diseases. The chemotherapeutants were commercial oxytetracycline, commercial cotrim, table salt (NaCl) and quick lime [Ca(OH)<sub>2</sub>].

At a time, 28 affected fish were collected randomly from the tank and kept in 7 different aquarium (60 cm x 15 cm x 15 cm) containing preserved tap water where, the stocking load was maintained 10 g/l. Then different treatments of drugs were applied on these fish. The trial was continued for 20 days and artificial aeration was maintained in that time. The aquarium water was changed every after two days. All the treatments were repeated 4 times. After the treatment the recovered fish were observed in the laboratory with normal pelleted feed prepared with rice bran, fish protein concentrate and flour at a rate of 3% of the body weight.

### ***Drug application***

#### ***Treatment I***

The affected fish were successively bathed in 1%, 1.5% and 2% NaCl solution for 1 hour for each step and transferred to an aquarium containing tap water. The treatment was repeated for 5 days.

#### ***Treatment II***

Commercial oxytetracycline capsule (Pharmadesh Lab. Ltd.) containing 250 mg oxytetracycline were used in this treatment. Everyday small pellets were prepared using rice bran, fish protein concentrate and flour and required amount of OTC was mixed with the feed. The rate of OTC applied to the affected fish was 75 mg/kg body weight /day for 5 days. The feeding rate was 3% of the body weight.

#### *Treatment III*

Commercial cotrim tablets (Square Pharmaceuticals Ltd.) were crushed into powder form and mixed with the pellets. The dose of cotrim supplied to the affected fish was 100 mg/kg body weight/day for 5 days.

#### *Treatment IV*

At first the fish were successively bathed in 1%, 1.5% and 2% NaCl solution for 1 hour each and kept in an aquarium containing tap water. The fish were fed with OTC at 75 mg/kg body weight /day for 5 days.

#### *Treatment V*

In this treatment the fish were simultaneously bathed in 1%, 1.5% and 2% NaCl for 1 hour at each step and maintained in an aquarium with normal tap water. Then the fish were fed cotrim at 100 mg/kg body weight/day using the pellet feed for 5 days.

#### *Treatment VI*

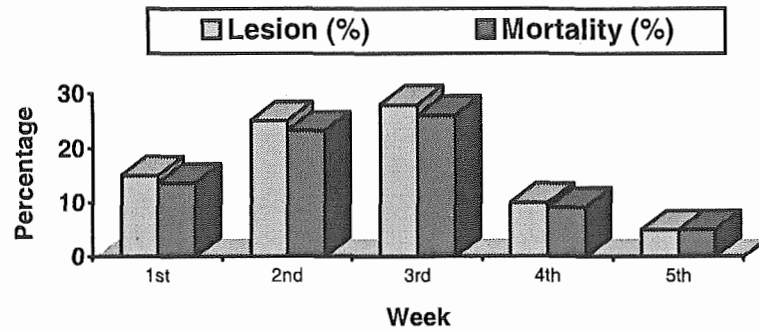
The fish were successively dipped in NaCl (1%) plus Ca(OH)<sub>2</sub> (0.1%), NaCl (1.5%) plus Ca(OH)<sub>2</sub> (0.15%) and NaCl (2%) plus Ca(OH)<sub>2</sub>(0.25%) for 5 minutes at each step. The fish were then maintained in an aquarium containing tap water and observed for recovery.

#### *Treatment VII (control)*

No drugs were applied on the affected fish. Only pelleted feed was supplied to the fish and water was changed in every 2 days.

### **Results and discussion**

A severe ulcer type of disease in young *Clarias gariepinus* occurred in an artificial tank with the expression of irregular lesions on the body surface of fish which gradually turned into ulcers. The affected fish started to die within 3-4 days after appearance of the disease. In the first week, out of 500 stocked fish 15.0% showed lesion on the body surface and 13.6% died (Fig. 1). However, the disease became severe in the 3rd week when mortality recorded 26.0%. In the 5th week of the experiment the affected fish were found to recover, when mortality became 5.0%. During this period cumulative mortality was 77.0%. The results were correlated with the reports of Rickards (1978) where he observed 80% mortality of Japanese eel fingerlings caused by an ulcer type of disease in Japan. Fotis *et al.* (1994) also observed 80% mortality of common carp (*Cyprinus carpio* L.) in an ulcer type of disease in Greece. However, during the study period P<sup>H</sup> of the tank water was recorded as 7.78. Average temperature and dissolved oxygen was recorded 21.5°C and 7.6mg /l respectively.



**Fig. 1.** Prevalence of the occurrence of lesion and mortality in *C. gariepinus* in the artificial tank.

T-1 : Bath in NaCl, T-2 : Oral treatment with commercial oxytetracycline, T-3 : Oral treatment with commercial cotrim, T-4 : Bath in NaCl and subsequent oral treatment with commercial oxytetracycline, T-5 : Bath in NaCl and subsequent oral treatment with commercial cotrim, T-6 : Dip in NaCl mixed with  $\text{Ca}(\text{OH})_2$ , T-7 : Control

From the lesions and kidney of affected fishes, *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Micrococcus* and *Staphylococcus* were isolated. Moreover, *Cytophaga* were also isolated from the lesions of affected fishes. However, the percentage composition of bacteria in kidney of both healthy and diseased fishes are shown in Table 1. In the present study, *Aeromonas* was detected as the dominant bacteria in the kidney of disease affected fishes which was 80% of the total bacterial content. In contrast, 35% *Aeromonas* was found in that of the healthy fishes. Abdullah (1989) isolated *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Edwardsiella* spp., *Moraxella* spp., *Flavobacterium* spp., *pasteurella* spp., *Bacillus* sp. and *Enterobacter* sp. from diseased catfish (*Clarias macrocephalus*) in Malaysia. Pal and Pradhan (1990) also isolated pseudomonads, aeromonads (*A. hydrophila*) and coccus (*Micrococcus varians*) from the lesions of ulcerative condition of air-breathing fish in India. However, Chowdhury *et al.* (1997) observed that the aeromonad content in the kidney of healthy *Puntius gonionotus* varied from 20-50% in different months but, Rahman and Chowdhury (1996) observed 72-82% aeromonad content in the kidney of farmed carp fishes suffering from an ulcer type of disease.

**Table 1.** Percentage composition of bacteria in the kidney of both healthy and diseased fish

Fish	Aero	Pseudo	Flavo	Micro	Staph
Healthy fish	35	15	7	31	12
Diseased fish	80	7	2	8	3

Aero : *Aeromonas*, Pseudo : *Pseudomonas*, Flavo : *Flavobacterium*, Micro : *Micrococcus*, Staph : *Staphylococcus*

As aeromonad was the dominant bacterial group recovered from the lesion and kidney of affected fish, they were further identified as *A. hydrophila*, *A. sobria* and *Aeromonas* spp. Rahman and Chowdhury (1996) also classified some aeromonad isolates as *A. hydrophila*, *A. sobria* and *Aeromonas* spp. recovered from some ulcer disease affected carp fishes. Species level identification of bacteria isolated from an exotic catfish, *C. gariepinus* is the first time report in Bangladesh.

The results of the bacterial challenge test are shown in Table 2. In the present study only the *A. hydrophila* isolate AGK 34 successfully produce disease both in *C. gariepinus* and in *P. gonionotus* with mortality. But, no pathogenic response were observed either in *A. hydrophila* isolate AGK 94 and or in *A. sobria* isolate AGK 25 and AGK 98. Esteve *et al.* (1993) observed that *A. hydrophila* causes ulcer disease in European eel, *Anguilla anguilla*. Rahman and Chowdhury (1996) also reported *A. hydrophila* as a pathogen causing ulcer disease in some carp fishes which supported the present study. However, in the present study two species of experimental fish were used. High mortality was observed in both of the fish species when they were challenged to the *A. hydrophila* isolate AGK 34. Thus it is suspected that the isolate might be highly virulent one. Moreover, among the two species of fish tested, *P. gonionotus* showed higher mortality. The reasons of such findings were not studied. But, it is suspected that *P. gonionotus* might be more susceptible to the pathogen.

**Table 2.** Experimental infection with the selected aeromonad isolates

Aeromonad isolates	Experimental fish	Nos. of fish		Mortality (%)
		Tested	Died	
AGK 25	<i>Clarias gariepinus</i>	20	0	0
AGK 34	"	20	16	80
AGK 94	"	20	0	0
AGK 98	"	20	0	0
Control (no bacterium)	"	20	0	0
AGK 25	<i>Puntius gonionotus</i>	20	0	0
AGK 34	"	20	18	90
AGK 94	"	20	0	0
AGK 98	"	20	0	0
Control (no bacterium)	"	20	0	0

#### **Data summarizes two repeated trials**

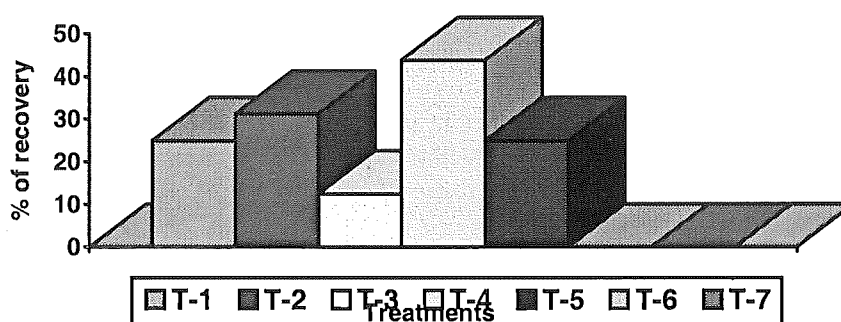
In the drug sensitivity test, all of the selected aeromonads including the pathogenic isolate were found to be sensitive to oxytetracycline (OT), oxolinic acid (OA) and chloramphenicol (C). Most of these isolates were found to be resistant to erythromycin (E), streptomycin (S) and sulphamethoxazole (SXT) (Table 3). The results were correlated with the findings of Sunthonnann *et al.* (1981). However, Banu (1996) observed that 26% aeromonad isolates recovered from different fish farms of Mymensingh were resistant to oxytetracycline. But in the present study, all of the isolates were found to be sensitive to the antibacterial agent. The reasons of such findings were not studied. It is believed that such findings were due to the serological difference of these isolate from other isolates.

**Table 3.** Sensitivity patterns of the selected Aeromonad isolates recovered from diseased fishes

Aeromonad Isolate	Response to different antibiotic agents with their zone of inhibition (mm)					
	OT	OA	C	SXT	S	E
AGK 34	+27	+23	+28	±15	R	R
AGK 94	+30	+30	+36	+20	R	R
AGK 98	+27	+21	+25	R	±20	R
AGK 25	+23	+17	+18	± 18	R	15

OT : Oxytetracycline (30 µg/disc), OA: Oxolinic acid (2 µg/disc), C: Chloramphenicol (30 µg/disc), S XT : Sulphamethoxazole (25 µg/disc), S: Streptomycin (10 µg/disc), E: Erythromycin (10 µg/disc)

In the chemotherapy trials, the best result was observed in T-IV, successive bath in NaCl and subsequent oral treatment with commercial oxytetracycline. In this treatment, the percentage of recovery was 43.75% (Fig. 2). In the oral treatment with commercial oxytetracycline (T-II ) 31.25% fish were recovered. No fish were recovered from the disease in T-VI, dip in NaCl mixed with Ca(OH)<sub>2</sub>. The prevalence of recovery of diseased catfish (*C. gariepinus*) in T- I, T-III and T-V were recorded 25%, 12.5% and 25% respectively. Roberts *et al.* (1989) found tetracycline to be effective in oral treatment of *Puntius gonionotus* at 500 mg/500 g feed in an ulcer type of disease. Jhingran (1990) also found oxytetracycline to be effective for the treatment of EUS affected fishes. In the present study all the treated fish were not cured in none of the treatment. It was suspected that unrecovered fish were severely infected before the treatment started. However, the results of the chemotherapy trials might be applicable to other ulcer disease affected farmed fish. The present study will help fish farmers in choosing suitable treatments in order to save there fish from ulcer disease.

**Fig. 2.** The recovery patterns of diseased catfish (*C. gariepinus*) with different treatments.

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(Manuscript received 30 May 1998 )