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***Aeromonas hydrophila* Infection in Fish of Swamps in Bangladesh**

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Abstract: For studying the load of the bacterium, *Aeromonas hydrophila* in swamps and artificial fish infection, four swamps were studied in Rajshahi University campus during November, 2001 to October, 2002. The load of *A. hydrophila* was found to vary from 1.16×10^7 to 4.90×10^7 CFU ml⁻¹. In the artificial infection to the snakehead, *Channa punctatus*, the mortality rates were recorded as 100, 75, 25, 12.5 and 0% at 3.42×10^9 , 3.42×10^8 , 3.42×10^7 , 3.42×10^6 and 3.42×10^5 CFU fish ml⁻¹, respectively. The average bacterial load in the swamps (3.42×10^7 CFU ml⁻¹) was slightly higher than that prevailed in the control pond (3.13×10^7 CFU ml⁻¹). Swamps can be utilized at the present bacterial load for the fish culture.

Key words: *Aeromonas hydrophila*, infection, swamp, fish, Bangladesh

INTRODUCTION

Bangladesh has vast inland water area. Swamps are potential water bodies for expanding fish culture. Rahman *et al.*^[1] reported that swamps are most important water bodies for various fish species of different size, which involve short time and small investment. Swamps are the sources of small fish which can grow easily without care and which provide the cheapest protein source for the people in Bangladesh. To meet the increasing demand for fish production in Bangladesh, swamps appeared to be a good candidate for fish culture. In Bangladesh, no specific research on bacterial diseases in fish has been carried out. It is recognized that bacteria are one of the important causative agents of fish diseases. Rahman *et al.*^[2] reported that *Aeromonads* are very important and destructive pathogens for warm water fishes. *Aeromonas hydrophila* is a ubiquitous free-living gram-negative bacterium prevalent in aqueous environments such as freshwater lakes and streams^[3], domestic tap water^[3] and Sewage. *A. hydrophila* is recognized as a scourge of freshwater fish farming worldwide and considered to be a major economic problem. It is the causative agent of haemorrhagic septicaemia^[4] and Epizootic Ulcerative Syndrome (EUS) of freshwater fishes of all Asian countries^[5]. The snakehead, *Channa punctatus* is an important fish prevalent in swamps and ponds in Bangladesh. The aim of the study was to investigate the load of *A. hydrophila* in swamps and its pathogenicity to *Channa punctatus*.

MATERIALS AND METHODS

Selection of swamp: A total of four swamps and a typical control pond were selected in Rajshahi University campus and its adjacent areas during November, 2001 to October, 2002. The swamps were mostly seasonal, derelict and sandy-muddy.

Load of *A. hydrophila* in swamp: Water samples were collected twice a week and counting of bacteria was performed according to Rahman *et al.*^[1] with slight modification. Water was inoculated into nutrient agar medium through 10 fold dilution in sterile physiological saline and then plates were incubated at 25°C for 48 h. After incubation the bacteria were counted directly by plate count method.

Collection of diseased fish: A total of four swamps and a control pond were selected for collection of diseased fish. Diseased fishes were collected twice a week from swamps.

Isolation of bacteria from diseased fish: Diseased fish were collected and bacteria were isolated from the kidney on to nutrient agar. After isolation, *A. hydrophila* was identified according to Bergey's manual. A pure colony of *A. hydrophila* was inoculated on nutrient agar and then cultured at 25°C for 24 h and used for further experiment.

Preparation of bacteria: Pure colony of *A. hydrophila* was identified and then bacteria were cultured in nutrient agar at 25°C for 24 h. Bacterial cells were harvested by

Table 1: Monthly variation in total number of *Aeromonas hydrophila* in different swamps and the control pond

Month	Load of <i>Aeromonas hydrophila</i> (CFU ml ⁻¹)				
	Swamp-1	Swamp-2	Swamp-3	Swamp-4	Control pond
Nov '01	3.30×10 ⁷	3.42×10 ⁷	3.24×10 ⁷	3.38×10 ⁷	3.08×10 ⁷
Dec	2.10×10 ⁷	1.90×10 ⁷	1.74×10 ⁷	1.52×10 ⁷	1.30×10 ⁷
Jan '02	1.70×10 ⁷	1.50×10 ⁷	1.96×10 ⁷	1.16×10 ⁷	1.04×10 ⁷
Feb	-	-	2.90×10 ⁷	2.92×10 ⁷	2.84×10 ⁷
Mar	-	-	3.26×10 ⁷	3.32×10 ⁷	3.12×10 ⁷
Apr	3.50×10 ⁷	-	3.42×10 ⁷	3.52×10 ⁷	3.28×10 ⁷
May	3.64×10 ⁷	3.72×10 ⁷	3.56×10 ⁷	3.68×10 ⁷	3.46×10 ⁷
Jun	3.84×10 ⁷	3.96×10 ⁷	3.74×10 ⁷	3.86×10 ⁷	3.64×10 ⁷
Jul	4.04×10 ⁷	4.12×10 ⁷	3.90×10 ⁷	3.96×10 ⁷	3.84×10 ⁷
Aug	4.16×10 ⁷	4.24×10 ⁷	4.16×10 ⁷	4.24×10 ⁷	3.96×10 ⁷
Sep	4.30×10 ⁷	4.90×10 ⁷	4.10×10 ⁷	4.84×10 ⁷	4.10×10 ⁷
Oct	4.10×10 ⁷	4.02×10 ⁷	3.96×10 ⁷	3.70×10 ⁷	3.90×10 ⁷

Yearly average in swamps was 3.42×10⁷ CFU ml⁻¹

Yearly average in control pond was 3.13×10⁷ CFU ml⁻¹

centrifugation at 3,000×g for 20 min. and washed twice in a physiological saline (0.85% NaCl ps). Experimental concentrations of bacteria for inoculation or injection were then prepared.

Pathogenicity test: Different concentrations of bacteria was made in physiological saline by 10 fold dilution. They were injected intraperitoneally into 5 groups of *C. punctatus*, each consisting of eight fishes and kept in a 100 L. tank. Injected fish groups were reared for 15 days in water at 20-25°C and then mortality was recorded. Infection was confirmed by reisolating the bacteria from the kidney of dead fishes using agar medium.

RESULTS

Load of *A. hydrophila*: The maximum number of *A. hydrophila* was recorded as 4.90×10⁷ CFU ml⁻¹ in September, 2002 and the minimum number was 1.16×10⁷ CFU ml⁻¹ in January, 2002. In the control pond, the highest number was 4.10×10⁷ CFU ml⁻¹ in September, 2002 and the lowest was 1.04×10⁷ CFU ml⁻¹ in January, 2002. The yearly average numbers of the bacterium were 3.42×10⁷ CFU ml⁻¹ and 3.13×10⁷ CFU ml⁻¹ in swamps and in control pond, respectively (Table 1).

Diseased fish: Diseased fishes were recorded from November, 2001 to October, 2002 from the swamps. It was found that the highest number of diseased fishes was found during May to June, 2002 and the minimum number was recorded during March, September and October, 2002. A total of 7 genera of diseased fishes were collected.

Pathogenicity: *C. punctatus* were injected by five different doses of *A. hydrophila* 3.42×10⁹, 3.42×10⁸, 3.42×10⁷, 3.42×10⁶ and 3.42×10⁵ CFU/fish/ml. The mortality

Table 2: Artificial infection of *Channa punctatus* with *Aeromonas hydrophila*

Injected dose (CFU/fish/ml)	No. of injected fishes	No. of dead fishes	Mortality (%)
3.42×10 ⁹	8	8	100.0
3.42×10 ⁸	8	6	75.0
3.42×10 ⁷	8	2	25.0
3.42×10 ⁶	8	1	12.5
3.42×10 ⁵	8	0	0.0

were recorded as 100, 75, 25, 12.5 and 0%, respectively at the stated doses (Table 2).

DISCUSSION

Effective water management is one of the most important factors contributing to the success of fish culture. Environment plays a crucial role in disrupting the balance between the host and pathogen. Disease associated with microorganisms was first recognized in the 19th century when Sonarelli^[6] reported an outbreak of a disease in eels.

Disease has become a major problem in fish production both in culture system and wild condition in Bangladesh^[7]. In Bangladesh, fishes have been seen suffering from ulcer type of diseases of different expressions including Epizootic Ulcerative Syndrome (EUS), bacterial haemorrhagic septicaemia, tail and fin rot, bacterial gill rot, dropsy, columnaries disease, fungal disease and parasitic disease^[8]. Bacterial pathogens to vertebrates have developed many strategies to overcome structural, humoral and cellular defense systems of host animals. A number of biological features known as virulence factors are common among many pathogenic bacterial species, although some characteristics are only in certain bacteria^[9]. As *A. hydrophila* is one of the most important pathogens of freshwater fishes^[10-11], rich data are available regarding the pathogenic mechanism and virulence of *A. hydrophila*^[12-16]. Unfortunately enough information on the pathogenicity of *A. hydrophila* to swamp water fishes is not available.

Different types of diseased fishes (*Channa punctatus*, *C. Striatus*, *Mastacembelus armatus*, *M. pancalus* and *Puntius ticto*) were collected from different areas by Chowdhury^[17]. During the experimental period, commonly diseased fishes were *Channa punctatus*, *C. gachua*, *C. striatus*, *Puntius ticto*, *P. sarana*, *P. gonionotus*, *Mastacembelus pancalus*, *Anabas testudineus*, *Tilapia nilotica*, *T. mossambica*, *Labeo rohita*, *Hypophthalmichthys molitrix* and *Cirrhinus mrigala*, which were found in swamps and the control pond. Study of aquatic bacteria associated with fish is very limited in Bangladesh^[17]. Romanenko^[18] reported that total bacterial number in reservoir water was

1.43-0.18×10⁶ CFU ml⁻¹ of water. Chowdhury^[17] reported that the range of bacterial load was 1.01×10⁶ to 5.06×10⁸ CFU ml⁻¹ of water and the highest number was recorded in August and September. In the present investigation the range of bacteria (*A. hydrophila*) load was recorded from 1.16×10⁷ to 4.90×10⁷ CFU ml⁻¹.

The average load of *A. hydrophila* was recorded as 3.42×10⁷ CFU ml⁻¹ in swamps. The same concentration when injected produced 25% mortality in *C. punctatus*. An average load of 3.13×10⁷ CFU ml⁻¹ was recorded in control pond where fish culture was well developed. This indicates that swamps can be profitably utilized for fish culture.

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