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BACTERIAL INFESTATION IN DIFFERENT FISH AT RAJSHAHI

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ABSTRACT

The present study was carried out from July 2005 to June 2007 and diseased freshwater fishes were collected from different water bodies and fish landing centre of three study areas, namely City Corporation area, Rajshahi; Paba upazila and Charghat upazila, Rajshahi. In the present study the load of bacteria in lesion, liver and kidney of *H. molitrix* showed considerable variation in different months during the study period. Monthly variation of bacterial load in body lesion varied from 3.17×10^4 CFU/g (July'05) to 2.13×10^7 CFU/g (March'07), in liver it varied from 7.17×10^3 CFU/g (July'05) to 5.13×10^6 CFU/g (March'06) and in kidney it varied from 5.87×10^3 CFU/g (July'05) to 6.33×10^5 CFU/g (March'06). From two years observation, monthly variation of bacterial load in 3 sampling ponds were varied from 4.00×10^6 CFU/ml (June'07) to 3.93×10^7 CFU/ml (July'05) in case of pond-1. In pond-2 bacterial load varied from 1.27×10^6 CFU/ml (May'07) to 5.33×10^7 CFU/ml (September'06). In pond-3 the bacterial load varied from 2.10×10^6 CFU/ml (June'07) to 2.10×10^8 CFU/ml (July'06).

Keywords: Disease, Bacterial infestation, Bacterial load

INTRODUCTION

Bacteria are one of an important causative agent of fish diseases in both wild and cultured fish and are responsible for serious economic losses. Few pathogens infect both freshwater and marine fish. Many pathogens can present as only skin infections especially flexibacteria, aeromonads and vibrios. Fishes were present with ulcer, hemorrhage, scale loss, tail and fin rot, dropsy includes several bacterial diseases (Chowdhury, 1997). The bacteria of the genera *Aeromonas*, *Pseudomonas* are ubiquitous facultative parasites, where potential pathogenicity becomes threatening for fish, only under unfavourable conditions. They belong to the normal bacterial flora of aquaria, the water of hatcheries, fish farms and bodies of water for domestic use, in which the appearance of the colonization of these bacteria in the skin fins, gills and intestinal lumen of fish. Most fish pathogenic bacteria can reside in the environment or on/in apparently normal fish. Thus, infections are often precipitated by some stress that upsets the natural defenses against the agents (e.g. overcrowding, low DO, high ammonia).

A. hydrophila is the most common cause of bacterial haemorrhagic septicaemia. The disease occurs in three distinct form, (a) abdominal dropsy, characterized by distension of the visceral cavity with fluid, (b) ulcerative, characterized by skin and muscle lesion, and (c) generalized bacterial hemorrhagic septicaemia. It has also been given several other names, e. g. infections, dropsy, red disease. The

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disease is worldwide affecting cultured cyprinids and other pond fish. It is commonly associated with fish populations suffering from stress. Fish are abnormally dark, show large subcutaneous haemorrhages and have distended abdomen. *A. hydrophila* caused a severe disease outbreak. Cultured fish suffer from *Aeromonas* sp. and *Pseudomonas* sp. infections with similar signs like dermal lesion, scale loss, frayed fins, tail and fin rot and Dropsy. Red skin disease is caused by *Pseudomonas fluorescens* in China. Red spot disease was caused by *Aeromonas* sp.

In Bangladesh, there is no proper scientific research on bacterial disease in fish. Study of aquatic bacteria associated with fish is very limited in Bangladesh. Few attempts have been taken in order to assess the bacterial population in aquatic environment and their involvement in causing diseases in fish. Chowdhury *et al*. (1997) reported *Aeromonas* sp. and *Pseudomonas* sp. are very common in fish disease specially in carp and live fishes for these a successful investigation need to know, what kind of bacteria present associated with diseased fish, the quantity of bacteria present in different organs of diseased fish and pond water are harmful or not for fish culture.

The aim of the present study is to identify the common bacterial diseases in freshwater fishes. The loads in different organs and in the sampling ponds are to be assessed.

MATERIALS AND METHODS

Procedure: Petridishes, test tube, glass pipettes, conical flask, tips, cotton physiological saline, Agar media were sterilized in an autoclave at 121°C for 20 minutes. Nutrient Agar media was made by 8.4 g agar and distilled water up to 300 ml and TSA media was made by 19 g agar and distilled water up to 500 ml. The conical flask was shaken with stopper for dilution of Agar and distilled water. Conical flasks containing agar were kept in an autoclave at 121°C for 20 minutes. Then the sterilized agar solution was poured separately (20 ml) in the petridishes from conical flask. Petridishes were closed and kept at room temperature for one hour.

Collection of diseased fish

18 types of fishes were collected from different fish landing centre, different water body and sampling ponds from July 2005 to June 2007. Bacterial isolates were collected from ulcer type lesion on body surface such as caudal, dorsal, ventral, lateral, head lesion and fin rot of different fish species viz. *C. catla, L. rohita, C. mrigala, H. molitrix, C. idellus, C. carpio* var. *specularis, C. carpio* var. *communis, P. gonionotus, L. calbasu, P. ticto, C. punctatus, C. striatus, C. batrachus, H. fossilis, M. tengara, M. armatus, M. pancalus, A. testudineus*. The samples were brought to the laboratory immediately after collection for bacteriological study.

Bacteriological investigation

Tryptic Soy Agar (TSA) was used for culture of *Aeromonas* sp. Nutrient Agar was used for culture of *Pseudomonas* sp. And finally the identification of *Aeromonas* sp. and *Pseudomonas* sp. was made by Cowan and Steel's Manual, for the identification of Medical Bacteria edited by Barrow and Felthem (1993) and confirmed with the help of Bergey's Manual (Krieg and Holt, 1984).

Determination of bacterial load in fish and pond water

Bacterial load in diseased *H. molitrix*

Sampling: Three specimens of *H. molitrix* were collected monthly from the sampling pond. Experiment was carried out by the method of Banu *et al*. (2001). Samples were collected from body lesion, liver and kidney of fish.

Body lesion: Liver samples were collected monthly for investigation. Fishes were killed by a light hurt on the neck region and then the lesion was collected by sterile scalpel and kept in a pre-weighed sterile weighing boat. After weighed (0.1g) the lesion was homogenized for the preparation of suspension in sterile 1ml physiological saline (0.85% NaCl). These samples were diluted in sterile 9ml physiological saline through 10 fold dilution. Pteridishes were set out, 3 plates for dilution were tested and each dilution was micropipetted (100 µl) into the centre of the petridishes using sterile tips for each dilution. With a sterilized glass rod the content was spread as quickly as possible. Each dilution had mixed with the help of cyclomixture before spread in petridish. Then the petridishes were inverted and kept in incubator at 25°C for 24-48 hours. Colony was counted by directed counting method and then the number of bacteria was calculated.

Liver: The body surface of fish samples were disinfected with 70% ethyl alcohol. The abdomen of diseased fish was opened by aseptic dissection and then the liver was taken out carefully with the help of sterilized forceps. The liver samples after being weighed (0.1 g) individually in a sterile weighing boat was homogenized for preparation of the suspension in physiological saline. Thereafter, the suspension was diluted in physiological saline through 10 fold dilution for incubation on the culture media.

Kidney: The kidney samples were prepared following the same procedure as done for the preparation of liver. Nutrient Agar was used for culture.

Bacterial load in pond water: Water samples were collected from 3 sampling ponds, once a month during the study period. Water samples were collected in sterilized reagent bottles from the depth of 25-35 cm below the surface at the time from 10:00 am to 12:00 pm. These samples were 1ml diluted in sterile 9 ml physiological saline through 10 fold dilution for inoculation on the culture media (Nutrient Agar).

RESULTS AND DISCUSSION

Identification of bacteria

The investigations demonstrated that freshwater fishes were affected by infection with different types of bacteria. During the investigated period July 2005 – June 2007 dermal lesion, ulcer, EUS, tail and fin rot, scale loss type of bacterial diseases were very common. Bacterial investigation were collected from ulcer type of lesion and tail and fin rot type of diseases. The recovered bacterial genera were identified as *Aeromonas* sp. and *Pseudomonas* sp (Table-1). *Aeromonas* sp. are gram negative, acid

fast, non sporing rod with a single polar flagellum, measuring about 1.0-1.5×6.5 μm. Bacteria of the *Pseudomonas* sp. are gram negative, non acid fast, non sporing rod with a single polar flagellum, measuring about 2×6.4 μm.

Table 1: Name of fish investigated and positive isolation of *Aeromonas* sp. and *Pseudomonas* sp.

Sl. No.	**Species**	**Site of isolation**	***Aeromonas* sp. positive isolation**	***Pseudomonas* sp. positive isolation**
1	*C. catla*	Lesion, Fin	+	+
2	*L. rohita*	Lesion, Fin	+	+
3	*C. mrigala*	Lesion, Fin	+	+
4	*H. molitrix*	Lesion, Fin	+	+
5	*C. idellus*	Lesion	+	+
6	*C. carpio* var *communis*	Lesion	-	+
7	*C. carpio* var *specularis*	Lesion	-	+
8	*P. gonionotus*	Lesion	+	+
9	*L. calbasu*	Lesion	+	+
10	*P. ticto*	Lesion, Fin	-	+
11	*C. punctatus*	Lesion, Fin	+	+
12	*C. striatus*	Lesion, Fin	+	+
13	*C. batrachus*	Lesion, Fin	+	+
14	*H. fossilis*	Lesion	+	+
15	*M. tengara*	Lesion	-	+
16	*M. armatus*	Lesion	+	+
17	*M. pancalus*	Lesion	+	+
18	*A. testudineus*	Lesion, Fin	+	+

+ = present, - = not present

Circular, smooth raised colonies were produce on both Agar (TSA and nutrient agar). Colonies of *Aeromonas* sp. were yellowish and *Pseudomonas* sp. creamy whitish. Glucose fermentation is a critical reaction that differentiates the *Aeromonas* sp. from *Pseudomonas* sp. Bacteria were inoculated into two tubes of oxidation fermentation (OF) basal medium supplemented with 1% glucose. The medium in one tube is overlaid with a plug of sterile petrolatum and both tubes are incubated at 25°C for 24 to 48 h. Results were interpreted as follows: yellow coloration in both tubes indicates acidic fermentation of glucose typical of *Aeromonas* sp., whereas yellow coloration only in the tube without petrolatum indicates oxidation of glucose characteristic of *Pseudomonas* sp. Production of gas is evidenced in either test by the formation of bubbles in the medium. Although most strains of *Aeromonas* sp. produce gas during the fermentation of glucose. Bacterial strains were identified by conventional biochemical test (Table 2).

Table 2: Identifying characteristics of fish pathogenic strain *Aeromonas* sp. and *Pseudomonas* sp.

Identifying character	*Aeromonas* sp.	*Pseudomonas* sp.
Colony	Yellowish	Creamy whitish
Morphology	Small rods	Small rods
Gram stain	-	-
Catalase	+	+
Oxidase	+	+
Gelatin liquefaction	+	+
Indole production	+	-
OF test	F	O
Arabinose	+	-
Manitol	+	-
Sucrose	+	-
Inositol	+	-
Esculin hydrolysis	+	-
Voges-Proskauer reaction	+	-
Ammonium production (nutrient growth)	-	+
Glucose	G	S

Note:

+ = positive reaction, - = negative reaction

F = fermentation, O = oxidation, G = gas, S = acid

The present findings support the previous works done by Sarker *et al*. (1999). Chowdhury (1998) reported the involvement of *Aeromonas* sp. and *Pseudomonas* sp. in the ulcer type diseases in freshwater fishes. Only *Aeromonas* sp. and *Pseudomonas* sp. were isolated from EUS affected freshwater fishes in the endemic area of Punjab, Pakistan (Rab *et al*., 2001). These results agree with present study and *Aeromonas* sp. and *Pseudomonas* sp. were isolated from diseased fish. Only *Pseudomonas* sp. were identified from *C. carpio* var. *communis*, *C. carpio* var. *specularis*, *M. tengara* and *P. ticto* and *Aeromonas* sp. and *Pseudomonas* sp. both were identified from all the rest. In India a broad spectrum of bacterial forms belonging to *Pseudomonas* sp. *Bacillus* sp., *Anthrobacter* sp. *Staphylococcus* sp., *Micrococcus* sp., *Actinomycete* sp. and *Aeromonas hydrophila* were isolated from disease fish *C. mrigala*, *L. rohita* and *C. catla* (Kumar and Day, 1992). *A. hydrophila*, *A. veronii* biover *sobria*, *A. veronii* biover *veronii*, *A. schubertii* and *A. jandaei* were isolated from EUS affected fishes in Bangladesh (Khan, 2001). *Aeromonas, Pseudomonas, Flavobacterium*, *Micrococcus* and *Staphylococcus* were isolated from ulcer type of disease in wild fishes of Bangladesh (Majumder *et al*., 2001). *Aeromonas* and *Pseudomonas* bacteria were isolated from infected fin and viscera of Asian catfish, *Clarias batrachus* (Saha *et al*., 1998).

Load of bacteria in lesion, liver and kidney of *H. molitrix*

Bacterial load in body lesion, liver and kidney of fish sample during the study period July 2005 – June 2006 were found to considerable variation. The bacterial load in body lesion varied from 3.17×10^4 CFU/g (July 2005) to 2.13×10^7 CFU/g (March 2006), in liver varied from 7.17×10^3 CFU/g (July 2005) to 5.13×10^6 CFU/g (March 2006) and in kidney varied from 5.87×10^3 CFU/g (July 2005) to 6.33×10^5 CFU/g (March 2006), respectively The lowest and the highest bacterial load in all the three organs were found in the month of July 2005 and March 2006. There were no diseased fish found in the month of May and June 2006.

In the second year (July 2006-June 2007) minimum and maximum length varied from 24.2 cm (September 2006) to 36.6 cm (March 2007) and weight varied from 176.00g (September 2006) to 345.00g (April 2007). The bacterial load in body lesion varied from 1.27×10^6 CFU/g (September 2006) to 2.23×10^7 CFU/g (March 2007), in liver varied from 1.10×10^5 CFU/g (December 2006) to 3.03×10^6 CFU/g (March 2007) and in kidney varied from 6.17×10^3 CFU/g (January 2007) to 5.67×10^5 CFU/g (March 2007), respectively (Appendix Table 4.1). The highest bacterial load in all the three organs were found in the month of March 2007. There were no diseased fish found in the month of July, August 2006 and May, June 2007.

From two years observation, monthly variation of bacterial load in body lesion varied from 3.17×10^4 CFU/g (July'05) to 2.13×10^7 CFU/g (March'07), in liver it varied from 7.17×10^3 CFU/g (July'05) to 5.13×10^6 CFU/g (March'06) and in kidney it varied from 5.87×10^3 CFU/g (July'05) to 6.33×10^5 CFU/g (March'06).

Bacterial load in pond water

In the First year bacterial load of sampling pond-1 was varied from 4.37×10^6 CFU/ml (June 2006) to 3.93×10^7 CFU/ml (July 2005). Bacterial load of pond water varied from 4.00×10^6 CFU/ml (June 2007) to 3.73×10^7 CFU/ml (September 2006) for the second year observation.

In the sampling pond-2 the minimum and maximum bacterial load 1.33×10^6 CFU/ml (June 2006) to 5.33×10^7 CFU/ml (September 2005) were observed in the first year. In the second year minimum and maximum bacterial load 1.27×10^6 CFU/ml (May 2007) to 5.43×10^7 CFU/ml (September 2006) was found.

In case of sampling pond-3 the minimum and maximum bacterial load 3.23×10^6 CFU/ml (May 2006) to 2.10×10^8 CFU/ml (July 2005) were observed in the first year. In the second year minimum and maximum bacterial load 2.10×10^6 CFU/ml (June 2007) to 2.13×10^8 CFU/ml (July 2006) were observed.

The highest bacterial loads in all three organs were found in the month of March. Horseley (1973) investigated relationship between the bacterial flora of Salmon and its environment. He recorded 10^2 to 10^3 bacteria per cm^2 at skin and similar number of bacteria found/ ml of water. Charganowski (1985) reported that the total bacterial population was 1.1×10^3 cells/m^3 of water in lake Arlington and

cell volume was substantially larger in winter than summer and were negatively correlated with the temperature. Iqbal (1995) reported that the total bacterial loads in pond water, body slime and kidney of fish at Trisal Fish Seed Multiplication Farm, varied from 1.3×10^2 to 5.9×10^5 CFU/ml, 5.4×10^3 to 8.5×10^7 CFU/g and 0.0 to 2.4×10^4 CFU/g, respectively, while those of the Jhalak Fish Farm were 2.0×10^2 to 3.0×10^5 CFU/ml, 3.8×10^2 to 2.3×10^8 CFU/g and 0.0 to 5.3×10^4 CFU/g. Banu *et al.* (2001a) reported that the total bacterial load in pond water varied from 1.39×10^5 to 3.11×10^7 CFU/ml in surface water and 5.90×10^7 CFU/ml in bottom water. They also reported that the total bacterial loads in body slime, liver and kidney of *C. mrigala* varied from 0.58×10^3 to 2.37×10^7 CFU/g, 0.22×10^3 to 9.64×10^6 CFU/g and 0.15×10^3 to 9.36×10^6 CFU/g, respectively. These results agree with the findings of the present study.

The present study revealed that the bacterial load in body lesion in *H. molitrix* varied from 3.17×10^4 CFU/g (July 2005) to 2.23×10^7 CFU/g (March 2007), in liver varied from 7.17×10^3 CFU/g (July 2005) to 5.13×10^6 CFU/g (March 2006) and in kidney varied from 5.87×10^3 CFU/g (July 2005) to 6.3×10^5 CFU/g (March 2006).

In the present study the highest bacterial load in three sampling pond water was in the month of July 2006 and lowest was in the month of May, 2007. Bacterial load varied from 4.00×10^6 CFU/ml (June 2007) to 3.93×10^7 CFU/ml (July 2005) in sampling pond-1. In the sampling pond-2, bacterial load varied from 1.27×10^6 CFU/ml (May 2007) to 5.43×10^7 (September 2006). In the sampling pond-3 it was observed that bacterial load was varied from 2.10×10^6 CFU/ml (June 2007) to 2.13×10^8 CFU/ml (July 2006).

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