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Investigation of Bacterial Flora in Catla (*Catla Catla*) Associated with EUS (Epizootic Ulcerative Syndrome) in a Commercial Fish Farm

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Abstract

An investigation was carried out to find the status of bacterial flora in the farmed fish, Catla (*Catla catla*) cultured in a pond of a commercial fish farm, "Dhaka Fisheries Ltd." in Gazipur district, Bangladesh. The total bacterial load in the fish kidney varied from $4.8x10^3$ to $8.1x10^4$ CFU/g whereas in fish body surface (slime), it varied from $3.0x10^5$ to $3.8x10^8$ CFU/g. The isolated bacterial genera and groups were *Aeromonas, Flavobacterium, Micrococcus, Pseudomonas, Staphylococcus* and *Coryneforms, Acinetobacter*. In case of slime *Micrococcus* was the dominant genus whereas *Aeromonas* was dominant in the case of kidney.

Keywords: Fish, *Catla Catla*, Bacteria, EUS.

Introduction

Fish is the major source of animal protein to replenish the protein deficiency of Bangladeshi people. Fisheries resources are vast in the country having large riverine system, canals, estuaries, lagoons, rain water depressions (hoars, baors, beels, ponds) and other water bodies. But fish demand for human consumption is increasing rapidly with increasing population. As a result, the tendency of the people of the Bangladesh has turned towards fish farming. Simultaneously, microbial studies have become important to know the ecological condition of fish farms as well as disease condition of the farmed fishes. In a previous report (Chowdhury *et al*, 1994) a number of bacterial genera were identified from various wild and cultured fish species presumably affected by epizootic ulcerative syndrome. The research work on fish disease especially on microbial pathogens in Bangladesh is still in a preliminary stage due to various reasons such as lack of laboratory facilities, skilled manpower, and adequate funds.

Earlier some workers Ahsan *et al.* (1992) isolated some common pathogenic bacteria from diseased fish. Chowdhury, (1993) published information on bacterial diseases of indigenous carps, and found that *Flavobacterium columnaris, Aeromonas hydrophila* and *Pseudomonas flurescens* are pathogenic to fish. Chowdhury *et al.* (1994) isolated pathogenic bacteria from farmed fishes. Rahman and Chowdhury (1996) isolated bacterial pathogens from ulcer disease of farmed carps in Bangladesh. Alam *et al.* (1999) studied the pathogenicity of *Edwardsiella tarda* in *Pangasius sutchi*.

Catla (*Catla catla*) is one of the most popular culturable species in Bangladesh, because of its taste and market value. However, disease has gradually become a great problem for fish production. The fish especially in farmed condition has been reported with

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lesions including EUS or disease of unknown causes. Bacterial study on Catla (*Catla catla*) has not yet been done in the Bangladesh, though trials were made on Rui, Mrigel and other fishes. Considering the importance, attention was paid to investigate bacterial flora of Catla (*Catla catla*) in the present study with following objectives:

1. Isolation of bacteria available in the slime and of lesions fish.

2. Determination of total bacterial load in the kidney of the fish.

3. Characterization of the isolated bacteria for their identification

Materials and Methods

Fish samples:

A polyculture fish pond was selected from Dhaka Fisheries Ltd. in Gazipur district of Bangladesh for regular sampling of the fish Catla (*Catla catla*). The fish (50 to 100 g in weight.) were collected twice a month regularly from September 1999 to June 2000. Fish samples were taken in polythene bags containing water of the same pond and carried immediately to the Fish Disease laboratory of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Culture and isolation of bacteria:

Tryptone Soya Agar (TSA, Oxoid) was exclusively used for culture and isolation of bacteria. Firstly, the fish were killed by a blow on the head. Initial visual inspections of the external body surface were carried out to identify any gross external lesions. The slime from external body surface (including affected surface) was aseptically scrapped with a sterile scalpel and weighed in a sterile test tube and suspended in physiological saline solution (0.85% NaCl) to have the stock suspension. The kidney (either partly or wholly) was taken out carefully by a sterilized scalpel and weighed. It was then homogenized aseptically and suspended in sterile physiological saline (0.85%) to have a stock solution. In every case desired dilutions were made in physiological saline by a ten-fold dilution method. From the dilution sample an amount of 0.1 ml was aseptically pipetted and spreaded over the culture plates and incubated at 25°C for 36-48 hours.

Determination of total number of bacteria:

Total number of bacteria was determined by counting the developed colonies on the triplicate plates of the same dilution after incubation. Average number was obtained from the mean of five replications in each sampling. For determination of percentage composition, 45 colonies were separated randomly from the total colonies grown in each of the duplicate plates having the number within 50 to 80 to obtain the pure culture and to characterize the isolates.

Characterization of bacteria:

Morphological characteristics of the bacterial colonies such as shape, size and color were recorded by visual observation. Shape of the individual isolate was determined by Gram staining method with the young culture. To know the Gram's staining response sometimes 3% KOH was used. The motility test

was performed by hanging drop method. Biochemical tests such as catalse activity test, oxidase test, Hugh and Leif son's test, indole production test, gelatine liquefaction test, proteinase test were performed with the fresh culture of bacterial isolates according to the methods described by the Cowan and Steel's Manual for the identification of Medical Bacteria (Barrow and Felthem, 1993). However, the identification of bacterial isolates was confirmed with the help of Bergey's Manual of Systematic Bacteriology (Kireg and Holt, 1984; Sneath *et al.* 1986).

Results and Discussion

A total of 45 isolates were identified during the study period (September 1999 to June 2000) and were categorized into 7 genera according to their morphological and biochemical characteristics (Table I). These were *Micrococcus, Aeromonas, Pseudomonas, Flavobacterium, Corynebacterium, Acinetobacter, Staphylococcus.* The isolates were recovered from slime and kidney (Table 2). In the slime, the bacterial genera / groups were *Micrococcus, Aeromonas,Pseudomonas, Flavobacterium,Coryneformes, Acinetobacter, Staphylococcus* and in the kidney *Micrococcus,Aeromonas, Pseudomonas, Staphylococcus* were identified.

The total bacterial load in kidney of the sampled fish was found to vary between different months. This variation was $8.1x10^4$ - $4.8x10^3$ CFU/g. The highest load was found in January and the lowest was in May as shown in Fig 1. Among the percentage composition of different genera *Aeromonas* was found to be dominant in the case of kidney and in case of slime *Micrococcus* was the dominant genera (Table 3).

Bacterial flora found on the fish body surface (slime) varied from those in the kidney. Total number of bacteria in the fish slime varied during various months. However, qualitative composition of bacterial flora did not vary significantly both in the case of slime and kidney. Percentage composition of individual bacterial genera/group in the fish slime and kidney varied in different months (Table-4).

Table1: Primary characterization of different isolates from various organs of *Catla catla*.

Characteristic	A	B	C	D	E	F	G
Gram staining	+	-	+	+	-	-	-
Shape	S	R	R	S	R	R	R
Motility	-	-	-	-	+	+	-
Growth in air	+	+	+	+	+	+	+
Growth anaerobically	-	-	+	+	-	+	-
Catalse	+	+	+	+	+	+	+
Oxidase	+	+	-	-	+	+	+
Glucose (acid)	+/-	+	+	+	+/-	+	-
Carbohydrates (o/f/-)	O/-	O	F	F	O/-	f	-

A : *Micrococcus* B : *Flavobacterium* C : *Coryneformes* D : *Staphylococcus*
E : *Pseudomonas* F : *Aeromonas* G : *Alcaligenage* S : Spherical
R: Rod f : Fermantative o : Oxidative + : Positive
- : Negative

Table 2: Isolates of bacterial genera from slime and kidney of *Catla catla*.

Sl. No	Bacterial isolates from fish slime/lesion	Bacterial isolates from fish kidney
1.	*Flavobacterium*	*Flavobacterium*
2.	*Aeromonas*	*Aeromonas*
3.	*Micrococcus*	*Micrococcus*
4.	*Pseudomonas*	*Pseudomonas*
5.	*Staphylococcus*	----
6.	*Coryneformes*	----
7.	*Alcaligenes*	----

Table 3: Monthly variation in the total load of bacteria in fish slime (including lesion) and kidney of *Catla catla*.

Name of months	Total load (CFU/g) of slime/lesion	Total load (CFU/g) of kidney
September	$1.8x10^7$	$2.4x\ 10^4$
October	$7.8\ x10^6$	$3.8\ x\ 10^4$
November	$3.8\ x10^7$	$5.8\ x\ 10^3$
December	$3.2\ x\ 10^7$	$5.6\ x\ 10^3$
January	$3.8\ x\ 10^8$	$8.1\ x10^4$
February	$3.1\ x\ 10^6$	$7.5\ x\ 10^3$
March	$6.1\ x\ 10^7$	$1.2\ x\ 10^4$
April	$5.9\ x\ 10^5$	$4.8\ x\ 10^3$
May	$3.0\ x10^5$	$3.7\ x\ 10^3$
June	$3.2\ x\ 10^6$	$3.1\ x\ 10^4$

Table 4: Monthly prevalence of bacterial genera/groups in slime (including lesion) and kidney of *Catla catla*.

Months	Prevalence of bacterial genera/groups in slime/lesion (%)							Prevalence of bacterial genera in kidney (%)			
	Fla.	Aer.	Mic.	Cor.	Ale.	Sta.	Pse.	Mic.	Pse.	Aer.	Fla.
September	24	06	32	08	15	10	05	40	Nd	50	10
October	30	05	20	15	Nd	10	10	35	05	60	Nd
November	25	12	25	15	12	Nd	06	40	05	50	05
December	25	10	30	05	05	05	20	55	Nd	45	Nd
January	25	20	30	05	05	Nd	15	35	15	40	10
February	30	08	35	12	10	Nd	05	30	15	50	05
March	20	15	35	10	Nd	05	Nd	35	20	45	Nd
April	30	15	30	20	Nd	05	Nd	25	10	60	05
May	25	15	30	10	15	05	Nd	40	15	35	10
June	25	12	35	15	13	Nd	Nd	30	15	45	10

Fla: *Flavobacterium* Sta : *Staphylococcus* Aer : *Aeromonas* Cor : Coryneformes
Mic: *Micrococcus* Alc : Alcaligenes Pse : *Pseudomonas* Nd : Not detected

The causes of such variation of the bacterial composition were not studied but water quality parameters including nutrients, temperature, and pH were suspected to be the possible factors. Nutritional composition plays an important role and influences decisively the composition of the micro flora in the aquatic environment (Rhienheimer, 1985). Among the detected bacterial genera/groups in the present study *Micrococcus* and *Aeromonas* were found to be dominant bacterial flora in the cases of slime and kidney, respectively. The total bacterial load was high in January and low in the May.

The result of the present study agrees with the reports on the EUS affected fish by Lloborera and Gaculan (1987). The EUS affected fishes correspond toisolation of *Pseudomonas, Micrococcus, Flavobacterium,* and Enterobacterium described by Lilley *et al.* (1992). These authors believed that all of these bacteria were involved in the secondary infection. Subasinghe *et al.* (1990) isolated *Aeromonas hydrophila* .*Pseudomonas fluorsecens* and some gliding bacteria from the lesions and ulcers of EUS affected fish. Roberts et al. (1989) carried out a survey of ulcerative disease in Bangladesh, where *Aeromonas hydrophila* and *A sobria* were dominant bacteria in the EUS lesions and ulcers.

The study gives an idea about the variation in the bacterial composition in kidney of Catla (*Catla catla*) under polyculture fish farming. Although, the pathogen was not clearly detected even from the affected fish, but *Aeromonas* was suspected as one of the causative agent due to its high numbers found in the kidney of the affected fish. Further studies are necessary to explain and ascertain the reasons and to test the pathogenicity of the isolates and their drug sensitivity to control the EUS problem.

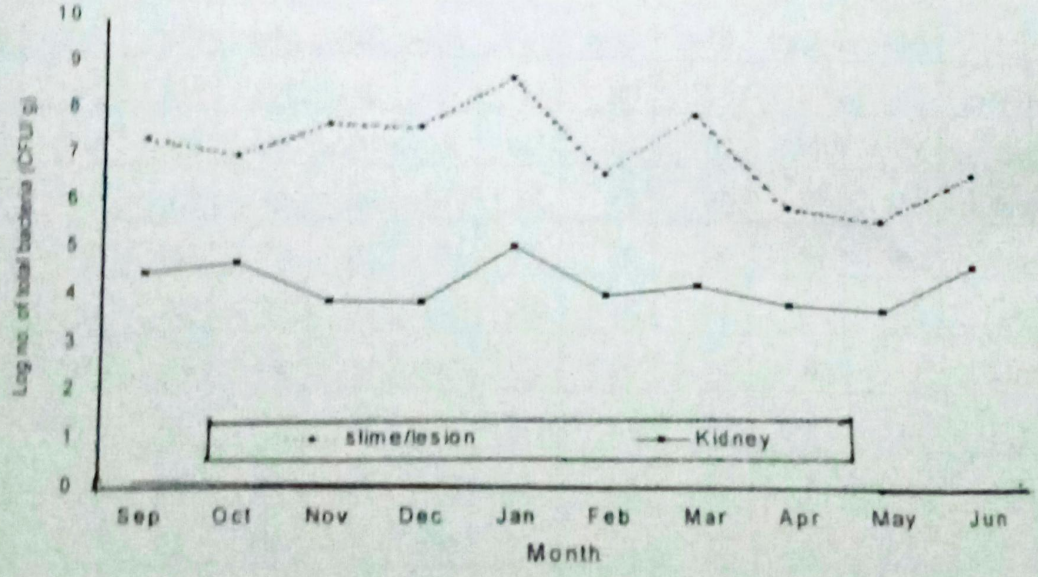

Fig. 1 Monthly variation in the total load of bacteria in the slime (including lesion) and kidney of *Catla catla*.

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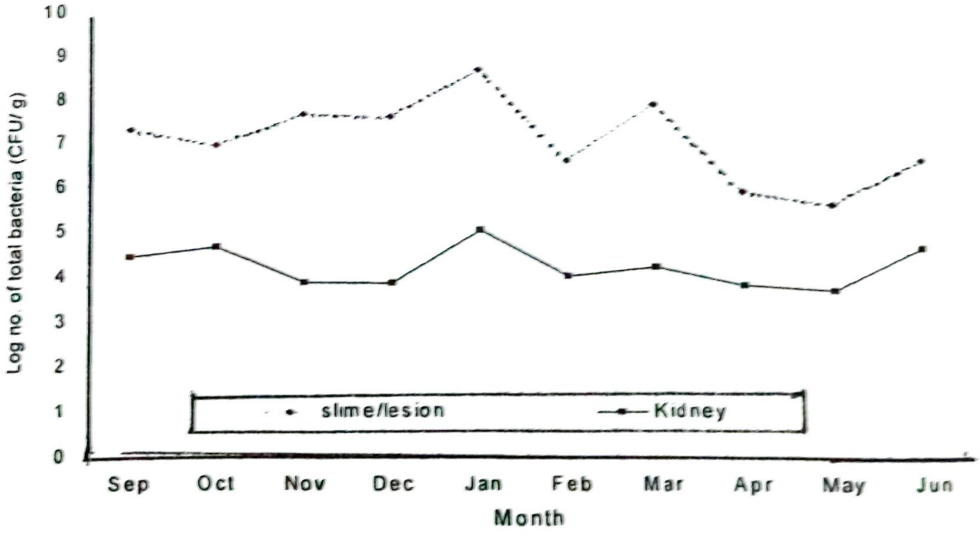

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References

Ahsan, C. R., M. M. Rasul and M. M. Hoq. (1992) Enterotoxicity of *Vibrio furnissi* isolated from eel. *World J. Microbial. Biotech.* **8**: 187-189.

Alam, M., M. Sardar and M. Mamnur Rashid, (1999) Pathogenicity of *Edwardsiella tarda* isolated from apparently healthy *Pangasius sutchi* by experimental method in the homologous fish. Bangladesh J. Fish., **22** (2): 63-66.

Barrow, G. I., Feltham, R. K. A., (1993) Cowan and Steel's Manual for the identification of Medical Bacteria. 3rd edi. Cambridge University Press. 331 p.

Chowdhury, M. B. R. (1993) Research priorities for microbial fish disease and its control in Bangladesh. In: Proceedings of the workshop on research priorities in Bangladesh for fish health disease prevention and pathology, 17th May, (ed. A. G. Tollervey) pp. 8-11.

Chowdhury, M. B. R., Islam, M. A., Islam, M. N. (1994). Studies on the bacterial involvement in epizootic ulcerative syndrome in fresh water fishes of Bangladesh. BAU Research Progress No. **8** B; 579-583.

Kireg, N. R., Holt, J. G., (1984) Bergeu's Manual of Systematic Bacteriology Volume 1, Williams and Wilkings. 964 pp.

Lilley, J., H. Phillips, K. Tonguthai, (1992) A review of epizootic ulcerative syndrome (EUS) in Asia. Pub. Aquatic Animal Health Research Institute and Network of Aquacultural centers in Asia Pacific Bangkok. Thailand 19 pp.

Lloborera, A.T., R. Q. Gaculan, (1987) *Aeromonas hydrophila* associated with Ulcerative Disease Epizootic in Laguna de bay Philippines. Aquaculture **67**: 273-278.

Rahman, M. M and M. B. R. Chowdhury (1996) Isolations of bacterial pathogen causing an ulcer disease in farmed carp fishes of Mymensingh. Bangladesh J. Fish., **19** (1-2): 103-110.

Rhienheimer, G. (1985) Aquatic Microbiology. University of Kien, West Germany. John. Wiley and Sons Chichester, NewYork, Brisbane Toronto. 3rd edi. 257 pp.

Roberts, R. J., Wootten, R., Macrae, I., Millar, S., Struthers, W. (1989) Ulcerative Disease Survey Bangladesh final report to the Government of Bangladesh and the Overseas Development Administration, Institute of Aquaculture, Stirling University. Scotland 40 pp.

Sneath, P. H. A., Mair, N. S., Shape, M. E., Volt, J. G., (1986). Bergey's Manual of Systematic Bacteriology. Volume **2**. Williams and Wilkins. 15-99 p.

Subasinghe, R, Lakshitha, P., Kamal, P. J., Balasuriya, S. W., Kulathilake, M. (1990). Preliminary Investigations into the Bacterial and Fungal Pathogens Associated with the Ulcerative Fish Disease Syndrome in Srilanka. The Second Asian Fisheries Forum 655-657 pp.