

BACTERIAL KIDNEY DISEASE (BKD) IN INDIAN MAJOR CARP FISHES, LABEO ROHITA (HAM.) AND CIRRHINUS MRIGALA (HAM.) - NATURAL OCCURRENCE AND ARTIFICIAL CHALLENGE

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ABSTRACT

Objective: To isolate and identify the pathogens of diseased *Labeo rohita* and *Cirrhinus mrigala* and to check their pathogenicity. **Materials and Methods:** An outbreak of Bacterial Kidney Disease (BKD) was recorded during the months of March, April, May, June, 2011 from Upputeru, near Akividu, West Godavari district, Andhra Pradesh, India. A total of 1,680 fishes were screened. Only two species namely, *Labeo rohita* and *Cirrhinus mrigala* were found infected with disease. No other species of fishes could be found suffering with disease. Standard microbiological methods were used for isolation and characterization of bacteria associated with diseased fishes. **Results:** The infected fishes showed abnormal swimming behaviour, lethargic and swim lazily. The other clinical symptoms includes exophthalmia, skin becomes dark, open lesions with pus and hemorrhages on the body surface. In severe cases, lesions assumed the shape of ulcers. Internally, Kidney become swollen and showed discrete white areas which contained bacteria. In advance cases, kidney tissue was damaged severely. Bacteriological examinations of infected fishes indicate that the occurrence of *Renibacterium salmoninarum* in infected regions of skin and internal organs, particularly from kidneys. **Conclusion:** Experimental infection trails revealed that the isolated bacterium was pathogenic to both species of fishes.

Keywords: Bacterial Kidney Disease, (BKD), *Labeo rohita*, *Cirrhinus mrigala*,

INTRODUCTION

Bacterial Kidney Disease (BKD) was first described in early 1930's in Atlantic salmon fishes in Scotland [1], caused by *Renibacterium salmoninarum* [2]. This is considered as a serious chronic infection of cultured fishes [3, 4] apparently also feral Salmonids [5]. Initially, BKD was reported in freshwater fishes, now it is recognized as a serious problem of Salmonid farming [6,2,7]. Thereafter, it was reported from United States of America in trout fishes. In Canada, BKD was first observed in 1937 in mature trout Hatchery. In India also, outbreaks of BKD was also reported in different species of freshwater fishes [8,9]. In the present study attempts have been made to identify the causative agent of the BKD in two Indian Major Carp fishes, *Labeo rohita* and *Cirrhinus mrigala*.

MATERIALS AND METHODS

For the purpose of present study, a total of 1,680 fishes were screened. Only the infected specimens of *Labeo rohita* and *Cirrhinus mrigala* were brought to the laboratory in living condition and kept in large aquaria of the size 90x45x45 cm, filled with clean water. Diseased fishes were collected randomly every week at regular interval from Upputeru River of Kolleru Lake. Samples for bacteriological examinations were collected by inserting sterile platinum inoculating loop into the lesions. The inocula were sampled from the surface as well as deeper portion of lesions and internal organs particularly from kidneys. They were streaked on separate agar plates having Tryptone Soy Agar (TSA), Brain Heart Infusion Agar (BHIA) and Selective Kidney Disease Medium (SKDM) and incubated at 37°C for 2 to 4 days. Identification of bacterium was carried out on the basis of morphological, biochemical and serological tests. The biochemical tests were carried out following the methods of Austin and Austin [10], Bullock [11] Roberts [12] and Buchanan and Gibbons [13]. The isolated bacterial cultures were subjected for Gram's staining and tested for Oxidative/ Fermentative reaction, Oxidase activity, catalase activity, indol production, reduction of nitrate and nitrite, MR-VP test and gas production in

media contains different carbohydrates. The physico-chemical parameters of water were analyzed as per the methods described in APHA [14]. The experimental infection trials were conducted to test pathogenicity of isolated bacterium on the same species of fishes from which it was originally isolated.

RESULTS

In the present study, only two species of fishes namely *Labeo rohita* and *Cirrhinus mrigala* were found suffering with disease. No other species of fishes are found to be affected with disease. The incidences of disease reported were found only in the months of March, April May and June, 2011. The maximum percentage of infection was recorded to be 1.31 in May, 2011, while the minimum 0.71 in March, 2011 (Fig.1). In case of fish infection, the highest percentage of infection (2.68) was found to be observed in *Cirrhinus mrigala* while the lowest (1.37) in *Labeo rohita*. (Fig. 2)

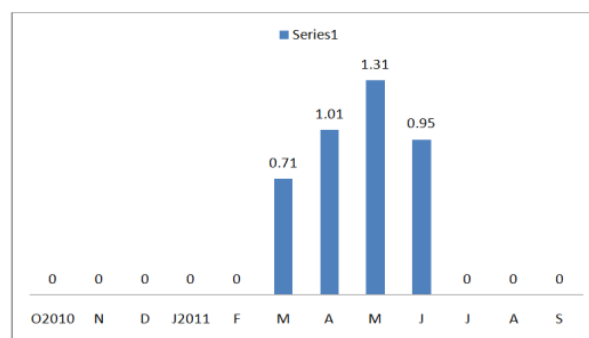


Figure 1: Monthly percentage of infection of fish during study period (Oct, 2010-Sept, 2011)

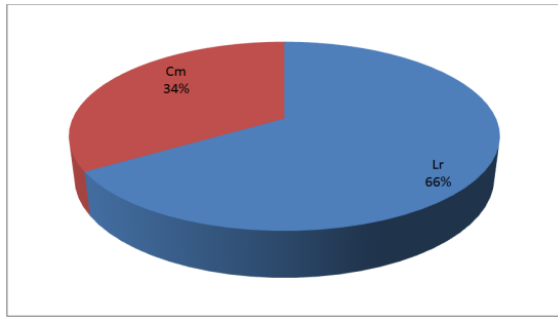


Figure 2: Species wise percentage of infection during study period.

Lr: *Labeo rohita*

Cm: *Cirrhinus mrigala*.

The infected fishes lose their appetite, become lethargic and swim lazily at surface of water. The other symptoms include dark pigmentation, exophthalmia, hemorrhages at base of the fins. Occasionally, superficial blisters and ulcerative abscesses occur on the body surface. In advance stages, fishes are anemic, pale gills and distended abdomen. Internally, the abdominal cavity filled with turbid fluid. The creamy whitish lesions were found in the kidneys. The intestine is gets filled with thin yellowish fluid.

Bacteriological examinations of infected fishes reveal that the presence of gram-positive diplobacillus, *R. salmoninarum* and it was

identified as the causative agent of BKD. It was a small, non-acid-fast, non-speculating, non-motile rod that grows best at the temperature of 18-24°C and not at all at 28°C. It was observed that a sufficiently long incubation period on Selective Kidney Disease Medium (SKDM), *R. salmoninarum* produced white or creamy, shiny, smooth, round, raised, entire which are measured about 2 mm in size. Microscopic observations of transverse sections through such colonies revealed the presence of gram-positive rods in a crystalline matrix. The crystalline material is thought to be cysteine precipitated from the medium. Growth does not occur on blood agar medium without cysteine supplement or on trypticase yeast agar. Other characteristics are shown in table-1

Artificial Challenging Studies

Artificial challenging studies were carried out by keeping the healthy fishes of *Labeo rohita* and *Cirrhinus mrigala* (an average weight of 80-100g and 18-22cm in length) in glass aquaria filled with fresh water at 18-25°C inoculated with bacterial suspension of *R. salmoninarum* at the dilution of 2.5×10^5 CFU ml⁻¹ and 2.0×10^5 CFU ml⁻¹ which got infected and died in seventeen to twenty days. All the experiments were conducted at 18-25°C in triplicate.

The marked variations have been observed in various physico-chemical parameters of water such as water temperature, pH, dissolved oxygen, alkalinity, hardness and chloride. Results of physico-chemical parameters of water are presented in table-2.

Table 1: Physical-Biochemical tests performed for characterization of *R. salmoninarum* isolated from *Labeo rohita* and *Cirrhinus mrigala* infected with BKD

S.No	Test Conducted	Response
1	Morphology of colony	Whitish yellow
2	Gram staining	+
3	Shape	Rods
4	Motility	-
5	Oxidation/Fermentation	F
6	Catalase test	-
7	Oxidase	+
8	MR-VP	-/+
9	Indole test	-
10	Citrate test	+
11	Nitrite	-
12	Nitrate	-
13	Arginine dihydrolase	+
14	Lysine	-
15	Triple sugar iron	-
16	H ₂ S production	-
17	Arabinose	-
18	Fructose	+
19	Detrose	+
20	Galactose	+
21	Mannitol	+
22	Xylose	-

-: Negative F: Fermentation

+: Positive O: Oxidative

Table 3: Physico-Chemical parameters of water during study period

S.NO	Parameter	Range
1	Water temperature	28-34°C
2	pH	6-6.8
3	Alkalinity	148-196 mg/l
4	Hardness	250-300 mg/l
5	Chloride	40-58 mg/l

DISCUSSION

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of micro-organisms may vary from a primary pathogen to that of an opportunistic organism which renders its host moribund by initiating a disease process. Bacterial flora of fish is a direct reflection of its environment. In the present study, marked variations in its physico-chemical parameters, such as water temperature, P^H, dissolved oxygen, alkalinity, hardness and

chloride have been observed. It seems that these variations in the water quality parameters favorable conditions for outbreak of the disease. The similar view has also expressed by Roberts¹⁵. He pointed out that BKD appear as a result of unfavorable environmental conditions.

During the one year of study period, only two species of fishes namely *L. rohita* and *C. mrigala* were affected with the disease. No other species of fishes could be found suffering with disease. The

affected fishes showed the symptoms like exophthalmia and bleb or open lesions on the surface of body of infected fishes. Ulcerations were also observed in some fishes when disease was in progress. Kidneys are the most often organ affected, became swollen and showed discrete white spots and which contains bacteria and host cell debris. More or less same symptoms have been observed by Austin and Austin [10], OIE [16] and Roberts [12]. The bacteriological studies of the fishes, infected with BKD, was carried out. It was observed that after sufficient incubation on Selective Kidney Disease Medium, *R. salmoninarum*, developed as white, shiny, round, entire with 2 mm in size. This finding is in agreement with the observations of Austin and Austin [10], Roberts, [12] and Perveez [9]. This observation is in agreement with the finding of Kimura et al, [17], Austin and Austin [10], Roberts [12], and Parveez [9]. Experimental infection trials revealed that this bacterium was able to produce the disease symptoms in the both species of *Labeo rohita* and *Cirrhinus mrigala*. More or less similar observation was reported by Parveez [9].

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