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PATHOGENICITY OF THE BACTERIAL ISOLATE *AEROMONAS HYDROPHILA* TO FISHES IN LABORATORY CONDITIONS

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ABSTRACT

The pathogenicity of a bacterial isolate *Aeromonas hydrophila* recovered from naturally diseased shing fish was investigated against catfishes (*Heteropneustes fossilis* and *Clarias batrachus*) and carp (*Channa punctatus*) of appropriate average body weights. Two different doses viz. 6.7 × 106 and 6.7 × 105 CFU/fish were injected intramuscularly. Pathogenicity of *A. hydrophila* was confirmed at water temperature of 30°C by mortality of 60% to 100% of all the tested fishes within 2-11 days. Injected *A. hydrophila* was re-isolated from liver, kidney and intestine of all the tested fishes. In all the cases of intramuscular injection, external pathology was found. Reddish anal region and fm bases were observed. It was understood that the isolate was a highly virulent pathogen for the challenged fishes.

Keywords: Pathogenicity test, *Aeromonas hydrophila*, Catfishes, Carps, Perch

INTRODUCTION

The bacteria *Aeromonas hydrophila* is a widely distributed pathogenic bacteria especially in warm water throughout the world. They are Gram negative, motile rods that are oxidase and catalase positive and are fermentative in nature (Sabur, 2006). *A. hydrophila* is the causative agent of MAS (motile Aeromonas septicemia). Both farmed and wild fishes have been found to be affected by this disease. Fishes become susceptible to the disease condition in their intensive culture system by *Aeromonas hydrophila*. The disease was characterized by swollen abdomen, red mouth,

hemorrhage in external surface and surrounding the anus (Alain, 2009).

A. hydrophila was frequently observed in various species of diseased farmed and wild freshwater fishes in different locations of India. It was recognized as a causative agent of ulcer type disease occurred in farmed fishes (Chowdhury, 1998). *A. hydrophila* were frequently isolated from various lesions of epizootic ulcerative syndrome (EUS) of different fishes (Roberts et al., 1990). Mamnur Rashid et al. (2008) identified *A. hydrophila* from EUS affected *Heteropneustes fossilis*. Hasan et al. (2008) found the histo-pathological changes in liver and kidney caused by this bacterium in the fish.

Experimental infection is done to know the pathogenicity of a pathogen in the body tissue of its susceptible host species. Present work was under taken to know the infectivity of the isolate from the kidney of catfishes (shing and magur) and carp (Garai).

MATERIALS AND METHODS

The pathogenicity test was conducted at the laboratory of the Zoology Department, ZA Islamia College, Siwan, Bihar in India. The experimental fishes of average body weight of 20.4 g for shing *Heteropneustes fossilis*, 25.6 g for magur *Clarias batrachus* and 30.5 g for *Channa punctatus* were used for the pathogenicity test of the isolate. Fishes were stocked in cemented cisterns for at least 15 days and then acclimatized in 04 aquaria for 7 days. Every day 50% of total water was changed and the aquaria were covered with synthetic net to prevent the fish from escaping.

Intramuscular injection method was used for the challenge test. One ml insulin syringe (sterile and disposable) was used for the injection. A total of 15 fishes (five fish from :each species) were injected intramuscularly with 0.1 ml of two pre-selected (Ahmed, 2009) bacterial doses (6.7×106 and 6.7×105 CFU/fish) just below the dorsal fin after disinfecting with 70% alcohol mixed cotton. Each group was then released in separate aquaria properly labeled to understand the dose and fish species. A negative control group of 05 fish of each species were injected with physiological saline as above. The injected fishes were observed up to 15 days. No feed was given to the experimental fishes and water temperature was recorded twice daily during the experimental period. The average temperature was recorded as 30°C.

Each fish was brought to the laboratory immediately after death, dissected out; kidney was touched with a sterilized loop and streaked onto AIM (Aeromonas isolation medium) plates. The plates were incubated at 25^0C for 48 hours for *A. hydrophila* colony appearance. Intestine, liver and kidney of each dead fish were dissected out aseptically and placed in sterilized separate plastic petridishes. After weighing, sample of each of the above organ was homogenized and suspended in sterile physiological saline (1 part of sample: 9 parts of PS) to obtain a stock solution. Two consecutive decimal dilutions, 10^{-1} and 10^{-2}, from the stock solution were made for each organ. At first the dilutions were used for spreading onto AIM plates to confirm *A. hydrophila*. Then the dilutions were used for spreading onto -duplicate TSA plates and incubated at 25°C for 48 hours for colony appearance.

Appeared colonies were counted by digital colony counter and all the data of bacterial colony counts were recorded for calculating bacterial load in different organs. The bacterial load was calculated by using the following formula after Mamnur Rashid et al. (1994).

Bacterial CFU/g of fish organ = No. of colonies counted in a plate × 10n × 100 Where, n was the dilution factor

RESULTS AND OBSERVATIONS

The abnormal movement and loss of balance were observed in all bacteria injected fishes. There lesions over experimental fishes were also evident. The posterior end of the body surface was found to develop grayish-white lesion that was extended up to caudal fin. Anal region and the fin bases developed red colour. After dissection of the freshly dead fish, the liver was observed to be swollen, unsmooth, and uneven turned blackish in colour.

Intramuscular injection method resulted in

Table 1. Results of pathogenicity in experimental fishes by intramuscular injection method.

Fish species	Dose	Average weight (g)	No. of fish died	Mortality percentage	Post- infection Days of mortality
C. batrachus	6.7×1056.7×106	25.6±0.18	0503	10060	3-84-11
H. fossilis	6.7×1056.7×106	20.4±0.27	0504	10080	2-83-13
C. punctatus	6.7×1056.7×106	25.7±0.32	0504	10080	2-34-10
Control (PS)	0.1 ml	30.2±0.66	00	00	00

100% mortality at a dose of 6.7 × 106 CFU/fish (6.7 × 107 CFU/ml) and 60 to 80% mortality at a dose of 6.7 × 105 CFU/fish (6.7 × 106 CFU/ml) of the experimental fishes. Kidney streaking from all dead fish gave rise to the growth of *A. hydrophila* and thus the isolates were proved to be pathogenic. No fish died in the control group. Results of pathogenicity tests are shown in Table 1.

Pathogenicity of *A. hydrophila* to *Heteropneustes fossilis* by IM was measured through their mortality as 100% at a dose of 6.7 × 106 CFU/fish and 80%, at a dose of 6.7 × 105 CFU/fish having post infection days of mortality from 2-8 days and 3-13 days respectively. *Clarias batrachus* was found to be susceptible to *A. hydrophila* expressed by their mortality to 100%, at a dose of 6.7 × 106 CFU/fish and 60%, at a dose of 6.7 × 105 CFU/fish. Post infection days of mortality were from 3-8 days and 4-11 days respectively. *A. hydrophila* caused 100% mortality in *Channa punctatus* at a dose of 6.7 × 106 CFU/fish and 80%, at a dose of 6.7 × 105 CFU/fish taking post infection days of mortality from 2-3 days and 410 days respectively.

In case of intramuscular injection, the highest bacterial load in catfishes was found to be 5.5 × 108 CFU/g in the liver of shing and 5.6 × 107 CFU/g in the intestine of magur. The lowest bacterial load was found to be 2.2 × 102 CFU/g in the kidney of shing and 2.4 × 103 CFU/g in the liver of magur. The highest bacterial load in carp was found to be 5.8 × 108 CFU/g in the liver of Garai. The lowest bacterial load was found to be 2.7 ×104 CFU/g in the kidney of Garai.

DISCUSSIONS

Pathogenicity of *A. hydrophila* was measured intramuscularly at 30°C with two different doses of 6.7 × 106 CFU/fish and 6.7 × 105 CFU/fish and showed mortality of up to 100% and 80% of the experimental fish within 2-8 days and 3-13 days in *Heteropneustes fossilis* of 20.4 g, 100% and 60% within 3-8 days and 4- 11 days in *Clarus batrachus* of 25.6 g, 100% and 80% within 2-3 days and 4-10 days in *Channa punctatus* of 25.7 g, 100% and 60% within 2-5 days, respectively. Islam (2007) conducted an experimental infection of *Heteropneustes fossilis* with *A. hydrophila by two* different methods *viz*. intra-peritoneal and intramuscular injection. A standard dose of infection (6.4 × 107 CFU/fish) was selected based on predetermined LD_{50}. Mortality gave rise to 85%. Mostafa *et al* (2008) conducted an experimental infection of *Heteropneustes fossilis* with *A. hydrophila* by *two* different methods viz. intra-peritoneal and intramuscular injection at a dose of 9.6 × 107 CFU/fish that resulted in 100% mortality of the tested fish within 1-9 days. Experimental infection by *A. hydrophila* of the fishes (catfishes, carps and perch) showed that the fishes were seriously affected which caused mortality. Thus it was proved that *A. hydrophila* was pathogenic to all experimental fishes. Angka (1990) conducted same type of experiment with *A. hydrophila*, injected intraperitonealy and found that the bacteria was pathogenic to *Clarias batrachus* fingerlings, causing 93% mortality in fish infected with 107 CFU/ml, with peak mortalities occurring on days 14 and 15. At lower dosage mortalities were significantly lower.

Mamnur Rashid *et al.* (2008) observed the highest and the lowest loads of *A. hydrophila* in liver, intestine and kidney to be 6.46 x 108 CFU/g, 1.18 × 109 CFU/g and 3.70 × 108 CFU/g and 1.67 × 104 CFU/g, 1.71 × 103 CFU/g and 1.47 × 104 CFU/

g in the natural EUS affected shing *Heteropneustes fossilis* respectively. Mostafa *et al.* (2008) conducted infection experiment of shing *Heteropneustes fossilis* with 105 and 108 CFU/fish of *A. hydrophila* and found the highest bacterial load in the kidney, intestine and liver of the experimentally infected fish to be 1.3 × 107 CFU/g, 3.5 × 106 CFU/g and 2.42 × 107 CFU/g and the lowest bacterial load to be 2.1 × 102 CFU/g, 9.0 × 103 CFU/g and 2.0 × 104 CFU/g respectively.

From the above discussion it is clear that the pathogen *Aeromonas hydrophila* is an opportunistic and serious pathogen for catfishes and carps. These pathogenicity test results will be helpful for further study to observe the fate of the pathogen in the organs of these fishes as well as to study the experimental histopathology of these fishes with the bacteria.

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