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Pathogenicity of the bacterial isolate *Aeromonas hydrophila* to catfishes, carps and perch

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Abstract

Pathogenicity of a bacterial isolate *Aeromonas hydrophila* recovered from naturally diseased shing fish was investigated against catfishes *(Heteropneustes fossilis* and *Clarias batrachus),* carps *(Labeo rohita, Catla catla* and *Cirrhinus cirrhosus)* and perch *(Anabas testudineus)* of average body weight of 20.4 g for *H. fossilis*, 25.6 g for *C. Batrachus*, 35.2 g for *L. rohita*, 25.7 g for *C. catla*, 30.5 g for *C. cirrhosus* and 20.3 g for *A. testudineus*. Two different doses *viz.* 6.7×10^6 and 6.7×10^5 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. The highest bacterial loads in catfishes were found to be 5.5×10^8 CFU/g in the liver of *H. fossilis* and 5.6×10^7 CFU/g in the intestine of *C. batrachus*. The lowest bacterial loads were found to be 2.2×10^2 CFU/g in the kidney of *H. fossilis* and 2.4×10^3 CFU/g in the liver of *C. batrachus*. The highest bacterial loads in carps were found to be 4.9×10^9 CFU/g in the liver of *C. catla*, 7.7×10^8 CFU/g in the intestine of *L. rohita* and 5.8×10^8 CFU/g in the intestine of *C. cirrhosus*. The lowest bacterial loads were found to be 2.7×10^4 CFU/g in the kidney of *C. catla*, 3.0×10^4 CFU/g in the kidney of *L. rohita* and 5.6×10^3 CFU/g in the kidney of *C. cirrhosus*. The highest and lowest bacterial load in perch was found to be 6.4 × 10' CFU/g and 1.6×10^2 CFU/g in the intestine and kidney of *A. testudineus* respectively. 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 Bangladesh (Sarker *et al.,* 2000). It was recognized as a causative agent of ulcer type disease occurred in farmed fishes (Chowdhury, 1998). Sabur (2006) isolated and identified five species of *Aeromonas* bacteria in polyculture environment of five carp species namely *Labeo rohita, Cyprinus carpio, Cirrhinus cirrhosus, Catla catla* and *Hypophthalmichthys molitrix. A. hydrophila* were frequently isolated from various lesions of epizootic ulcerative syndrome (EUS) of different fishes (Dooly *et al.,* 1986; Torres *et al.,* 1990; Roberts *et al.,* 1990). *A. hydrophila* were found to cause disease in fishes associated with fungus, *Aphanomyces invadans* to produce EUS (Hasan, 2007). lqbal *et al.* (1998) detected *A. hydrophila, A. veronii biover sobria* and *A. jandaei* as pathogenic bacteria recovered from EUS affected mrigal. Mamnur Rashid *et al.* (2008) identified *A. hydrophila* from EUS affected shing *Heteropneustes fossilis.* Hasan *et al.* (2008) found the histopathological changes in liver and kidney caused by this bacterium in the fish. Mostofa *et al.* (2008) studied experimental pathogenesis of *A. hydrophila* bacteria in the same fish. Islam *et al.* (2008) studied histopathological changes in experimentally infected shing with the same bacteria. Lately the bacteria *A. hydrophila* was isolated from Thai pangus *Pangasianodon hypophthalmus* (Siddik, 2009)) and from carps *Labeo rohita, Catla catla* and *Cirrhinus cirrhosus,* perch *Anabas testudineus,* catfishes *Heteropneustes fossilis* and *Clarias batrachus* and eel *Mastacembalus armatus* (Ahmed, 2009).

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 shing to catfishes (shing and magur), carps (rui, catla and mrigal) and the perch (koi).

Materials and Methods

The pathogenicity test was conducted at the wet laboratory and fish disease laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. The experimental fishes of average body weight of 20.4 g for shing *Heteropneustes fossilis,* 25.6 g for magur *Clarias batrachus,* 35.2 g for rui *Labeo rohita,* 25.7 g for catla *Catla catla,* 30.5 g for mrigal *Cirrhinus cirrhosus* and 20.3 g for koi *Anabas testudineus* were used for the pathogenicity test of the isolate. Fishes were stocked in cemented cisterns for at least 15 days and then acclimatized in 12 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 60 fishes (ten fish from :each species) were injected intramuscularly with 0.1 ml of two pre-selected (after Ahmed, 2009) bacterial doses (6.7×10^6 and 6.7×10^5 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 10 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 petri dishes. 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 (stock, 10^{-1} and 10^{-2}) 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 $\times 10^n \times 100$
Where, n was the dilution factor

Results and Discussion

Clinical and Gross Pathology

In moribund condition of each group of intramuscularly injected fish, abnormal movement and loss of balance were observed. Clinical external pathologies 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, uneven and turned blackish in colour.

Pathogenicity

Intramuscular injection method resulted in 100% mortality at a dose of 6.7×10^6 CFU/fish (6.7×10^7 CFU/ml) and 60 to 80% mortality at a dose of 6.7×10^5 CFU/fish (6.7×10^6 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.

Table 1. Results of pathogenicity test of *Aeromonas hydrophila* in experimental fishes by intramuscular injection method (five fish of each species were challenged with each dose of the bacterial suspension)

Species of fishes	Dose (CFU/fish)	Average weight of fish (g)	No. of fish died	Mortality (%)	Post infection days of mortality
C. catla	6.7×10^6	25.7 ± 0.32	5	100	2-3
	6.7×10^5		4	80	4-10
L. rohita	6.7×10^6	35.2 ± 0.41	5	100	1-4
	6.7×10^5		4	80	3-11
C. cirrhoses	6.7×10^6	30.5 ± 0.56	5	100	2-5
	6.7×10^5		3	60	4-12
H. fossilis	6.7×10^6	20.4 ± 0.27	5	100	2-8
	6.7×10^5		4	80	3-13
C. *batrachus*	6.7×10^6	25.6 ± 0.18	5	100	3-8
	6.7×10^5		3	60	4-11
A. testudineus	6.7×10^6	20.3 ± 0.34	5	100	3-9
	6.7×10^5		3	60	5-14
Control (PS)	0.1 ml	30.2 ± 0.66	0	0	0

Pathogenicity of *A. hydrophila* to *Heteropneustes fossilis* by IM was measured through their mortality as 100% at a dose of 6.7×10^6 CFU/fish and 80%, at a dose of 6.7×10^5 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 6.7×10^6 CFU/fish and 60%, at a dose of 6.7×10^5 CFU/fish. Post infection days of mortality were from 3-8 days and 4-11 days respectively.

By intramuscular injection method 100% of *Labeo rohita* died at a dose of 6.7×10^6 CFU/fish and 80%, at a dose of 6.7×10^5 CFU/fish, post infection days of mortality being from 1-4 days and 3-11 days respectively.

A. hydrophila caused 100% mortality in *Catla catla* at a dose of 6.7×10^6 CFU/fish and 80%, at a dose of 6.7×10^5 CFU/fish taking post infection days of mortality from 2-3 days and 410 days respectively. *Cirrhinus cirrhoses* showed their mortality as 100% at a dose of 6.7×10^6 CFU/fish and 60%, at a dose of 6.7×10^5 CFU/fish with post infection days of mortality from 2-5 days and 4-12 days respectively.

Anabas testudineus was proved to be sensitive to *A. hydrophila* as shown by their mortality to 100%, at a dose of 6.7×10^6 CFU/fish and 60%, at a dose of 6.7×10^5 CFU/fish. Post infection days of mortality were observed to be from 3-9 days and 5-14 days respectively.

Bacterial load in experimentally infected fishes

In case of intramuscular injection, the highest bacterial load in catfishes was found to be 5.5×10^8 CFU/g in the liver of shing and 5.6×10^7 CFU/g in the intestine of magur. The lowest bacterial load was found to be 2.2×10^2 CFU/g in the kidney of shing and 2.4×10^3 CFU/g in the liver of magur. The highest bacterial load in carps was found to be 7.7×10^8 CFU/g in the intestine of rui, 4.9×10^9 CFU/g in the liver of catla and 5.8×10^8 CFU/g in the intestine of mrigal. The lowest bacterial load was found to be 3.0×10^4 CFU/g in the kidney of rui, 2.7×10^4 CFU/g in the kidney of catla and 5.6×10^3 CFU/g in the kidney of mrigal. The highest and lowest bacterial load in perch (koi) was found to be 6.4×10^7 CFU/g in the intestine and 1.6×10^2 CFU/g in the kidney.

During the *experimental* period of pathogenicity test the *average* water temperature was 30°C. Kluyver and Niel (1936) reported that the optimum growth temperature of *A. hydrophila was* 28°C. Mostafa (2007) calculated $_{LD50}$ of *A. hydrophila* in *Heteropneustes fossilis* at 28°C.

Pathogenicity of *A. hydrophila* was measured intramuscularly at 30°C with two different doses of 6.7×10^6 CFU/fish and 6.7×10^5 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 *Glarus batrachus* of 25.6 g, 100% and 80% within 14 days and 3-11 days in *Labeo rohita* of 35.2 g, 100% and 80% within 2-3 days and 4-10 days in *Catla catla* of 25.7 g, 100% and 60% within 2-5 days and 4-12 days in *Cirrhinus cirrhosus* of 30.5 g, and 100% and 60% within 3-9 days and 5-14 days in *Anabas testudineus* of 20.3 g, respectively. Islam (2007) conducted an experimental infection of *Heteropneustes fossilis* with *A. hydrophila by two* different methods *viz.* intraperitoneal and intramuscular injection. - A standard dose of infection (6.4×10^7 CFU/fish) was selected based on predetermined $_{LD50.}$ Mortality gave rise to 85%. Mostafa *et al* (2008) conducted an experimental infection of *Heteropneustes fossilis* with *A. hydrophila* by *two* different methods viz. intraperitoneal and intramuscular injection at a dose of 9.6×10^7 CFU/fish that resulted in 100% mortality of the tested fish within 1-9 days. Sabur (2006) observed that *A. hydrophila* was found to be pathogenic for both indigenous (rui *Labeo rohita,* catla *Catla catla* and mrigal *Cirrhinus cirrhosus)* and exotic (silver carp *Hypophthalmichthys molitrix* and common carp *Cyprinus carpio)* carps. He observed that intramuscular method was found to be the most effective method that resulted 80 to 100% mortality at a dose of 2×10^6 CFU/fish and 60 to 80% mortality at a dose of 2×10^5 CFU/fish for three indigenous and two exotic carp species within 2-12 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 10^7 CFU/ml, with peak mortalities occurring on days 14 and 15. At lower dosage mortalities were significantly lower.

Iqbal *et al.* (1996) investigated bacterial flora in slime and kidney of mrigal *Cirrhinus mrigala* from two fish farms. In first farm, the total bacterial load varied from 5.4×10^3 CFU/g to 4.7×10^7 CFU/g in slime and undetectable to 1.7×10^4 CFU/g in kidney and in the second farm, the total bacterial load varied from 4.8×10^3 CFU/g to 1.4×10^8 CFU/g in slime and undetectable to 3.0×10^4 CFU/g in kidney. Mamnur Rashid *et al.* (2008) observed the highest and the lowest loads of *A. hydrophila* in liver, intestine and kidney to be 6.46×10^8 CFU/g, 1.18×10^9 CFU/g and 3.70×10^8 CFU/g and 1.67×10^4 CFU/g, 1.71×10^3 CFU/g and 1.47×10^4 CFU/g in the natural EUS affected shing *Heteropneustes fossilis* respectively. Mostofa *et al.* (2008) conducted infection experiment of shing *Heteropneustes fossilis* with 10^5 and 10^8 CFU/fish of *A. hydrophila* and found the highest bacterial load in the kidney, intestine and liver of the experimentally infected fish to be 1.3×10^7 CFU/g, 3.5×10^6 CFU/g and 2.42×10^7 CFU/g and the lowest bacterial load to be 2.1×10^2 CFU/g, 9.0×10^3 CFU/g and 2.0×10^4 CFU/g respectively.

From the above discussion it is clear that the pathogen *Aeromonas hydrophila* is an opportunistic and serious pathogen for catfishes, carps and perch. 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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