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Pathogenicity of *Aeromonas hydrophila* in Silver Carp *Hypophthalmichthys molitrix* and its Control Trial

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Abstract: *Pathogenicity of Aeromonas hydrophila, previously isolated from kidney of apparently healthy silver carp, was tested in silver carp Hypophthalmichthys molitrix. Trials were also made to control the pathogen in the experimentally infected fish. Before artificial infection the morphological, biochemical and physiological characters of A. hydrophila were studied. The infection was done by intramuscular (IM) injection method. Two different doses viz., 2.8 × 10^5 and 2.8 × 10^6 CFU/fish were used. In all the injected fish external pathology appeared. Reddish lesions on anal region and fin bases were observed. Injected A. hydrophila was re-isolated from liver, kidney and intestine of the challenged fish. It was understood that the isolate was highly virulent pathogen for silver carp. To control the infection by antibiotics, oxysentin 20% (oxytetracyline HCL BP), acimox (amoxicillin trihydrate BP) and oxy-D Vet (oxytetracycline 20% and + doxycycline 10%) were tested at lower, recommended and higher doses of the pharmaceutical companies, respectively. The antibiotic treatments were conducted for 10 days and the fish were observed for 15 days. Among the three antibiotics, acimox and oxy-D Vet at recommended dose showed the best result where 100% fish were recovered. But, oxysentin 20% at higher dose showed best results.*

Key words: *Aeromonas hydrophila, Hypophthalmichthys molitrix, pathogenicity, experimental infection and antibiotics.*

I. Introduction

Silver carp, *Hypophthalmichthys molitrix* is an important exotic fish species and it has been drawing the attention of more and more fish farmers in Bangladesh day by day due to its wide distribution, high growth rate, good market values, profitable culture and good taste. However, the fish has become susceptible to a disease condition in its intensive culture system by the bacteria *Aeromonas hydrophila*. The disease condition was characterized by swollen abdomen, red mouth, hemorrhage in external surface and surrounding the anus. *A. hydrophila* was frequently observed in various species of diseased farmed and wild freshwater fishes in different locations of Bangladesh [1] [2] [3]. The pathogenic bacteria *A. hydrophila* was isolated and identified in polyculture environment of five carp species namely *Labeo rohita, Cyprinas carpio, Cirrhinus cirrhosus, Catla catla* and *Hypophtahalmichthys molitrix* [4]. *A. hydrophila* was also found to be associated with EUS affected shing [5]. So, to confirm the etiology, experimental infection is important. A fish folk, already attacked by any bacterial pathogen, must be treated with a safe antibiotic. Considering the above facts, the present study was undertaken to experimentally infect silver carp with *A. hydrophila* bacteria and its control trial with some antibiotics to control the disease.

II. Materials and Methods

Apparently healthy silver carps were acclimatized for 7 days with aeration in 12 rectangular glass aquaria at wet laboratory. Previously isolated *Aeromonas hydrophila* from homologous fish were sub-cultured onto TSA plates for their morphological, biochemical and physiological characterization.

2.1 Pathogenicity Test

For the pathogenicity test intramuscular (IM) injection method was used to know the efficacy of the method in initiation of infection and pathogenicity of the pathogen. One ml insulin syringes (sterile and disposable) were used for the injection. For the IM injection, each 10 fish were injected intramuscularly with 0.1 ml of each of the two desired bacterial doses (2.8 × 10^5 and 2.8 × 10^6 CFU/fish, prepared by serial decimal dilution) just below the anterior part of the dorsal fin. Only PS was injected intramuscularly to 10 fish as negative control. The injected fish were released into 3 separate aquaria. The experimentally injected fish were observed for 15 days. Continuous aeration was maintained and no feed was given to the experimental fish. Infection was recorded by observation of lesion, clinical appearance and mortality. Moribund fish were

attended, observed and waited for their death. Immediately after death, they were transferred to the laboratory and were subjected to bacterial isolation from liver, kidney and intestine.

2.2 Isolation and Calculation of Bacteria from Liver, Kidney and Intestine

Liver, kidney and intestine of moribund fish were homogenized and two consecutive decimal dilutions, 10^{-1} and 10^{-2}, from the stock solution were made by sterile PS for each organ. The dilutions were used for spreading onto duplicate TSA plates. The plates were incubated at 25˚C for 48 hours. Appeared colonies were counted by digital colony counter and their numbers were used to interpret the pathogenesis of the pathogen in the organs of the experimentally infected fish. All the data of bacterial colony counts were recorded for calculating bacterial load in different organs. The bacterial load was counted by using the mentioned following formula [6].

Bacterial CFU/g of fish organ = No. of colonies counted in a plate × 10^n × 100

Where, n was the dilution factor

2. 3 Antibiotic Trial Against *Aeromonas hydrophila*

After confirmation of *A. hydrophila* from diseased silver carp, experimentally infected silver carp were treated with three different antibiotics as follows. The pharmaceutical company, Novertis produced oxysentin 20% (oxytetracycline HCl BP) and suggested a recommended dose of 28-40 g/100 Kg fish for 10 days. Advanced Chemical Industries (ACI) produced acimox (amoxicillin trihydrate BP) with a recommended dose of 5 g/15 Kg fish for 10 days, twice daily. EON had oxy-D Vet (oxytetracycline 20% and doxycycline 10%) with a 1 g/4 Kg fish for 10 days, twice daily.

2.3.1 Sensitivity test of the bacteria to the antibiotics

In-vitro: A 1 μl of the bacterial suspension from $10^{5\text{-}6}$ CFU/ml was spreaded onto duplicate TSA plates and the three antibiotics were put in each plate. The plates were incubated on 25˚C for 48 h and observed for the sensitivity of the bacteria to the antibiotics. Sensitivity was interpreted by no-growth zone.

In-vivo: For oxysentin 20%, lower than the recommended dose was selected as 25 g/100 Kg fish, recommended dose, 35 g/100 Kg fish and higher than the recommended dose, 45 g/100 Kg fish. In case of acimox lower than the recommended dose was 4 g/15 Kg fish, recommended dose, 5 g/15 Kg fish and higher than the recommended dose, 7.5 g/15 Kg fish. A lower than the recommended dose of oxy-D Vet was 0.80 g/Kg fish, recommended dose, 1 g/Kg fish and higher than the recommended dose, 1.5 g/Kg fish were selected. Total nine aquaria were used for three tretments, each was filled with 30 l ground water and then antibiotics were added to the aquaria. Total medicinal trial was conducted for 10 days and the experimental fish were observed for 15 days.

III. Results

3.1 Characters of *A. hydrophila* Bacteria

Results of morphological, biochemical and physiological characters of *A. hydrophila* compared with the characters shown by Popoff [7] are shown in Table 1.

3.2 Pathogenicity of *A. hydrophila*

Intramuscular method resulted in 100% mortality at a dose of 2.8×10^6 CFU/fish and 60% mortality at a dose of 2.8×10^5 CFU/fish of the experimental fish. 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 from the control group of the experimental fish. Results of pathogenicity tests are shown in Table 2. *Hypophthalmichthys molitrix* was proved to be sensitive to *A. hydrophila* as shown by their mortality to 100%, at a dose of 2.8×10^6 CFU/fish and 60%, at a dose of 2.8×10^5 CFU/fish. Post infection days of mortality were observed to be from 2 to 5 days and 4 to 9 days, respectively.

3.3 Gross Clinical and Pathological Symptoms

In moribund condition of each group of intramuscularly injected fish, clinical signs such as abnormal movement, loss of balance, grayish-white lesion extended up to caudal fin and reddish lesions on the fin bases and anal region were observed. After dissection of the freshly dead fish, the liver was observed to be swollen, unsmooth, and uneven and turned blackish in colour.

Table 1. Characters of *A. hydrophila* isolates in comparison to those shown by Popoff (1984)

Characters		Characterization by Popoff (1984)	Present result
Gram stain		-[1]	-
Shape		Rod	Rod
Motility		+[2]	+
Sensitivity to 0/129		ND[3]	-
Oxidase		+	+
Catalase		+	+
OF test		F[4]	F
Acid and gas production from Glucose		+	+
Acid production	Lactose	+	+
	Sucrose	+	+
	Maltose	+	+
	Manitol	+	+
Esculin hydrolysis		ND	+
Methyl-red test		-	-
Voges-Proskaur		+	+
H_2S production		+	+
Growth at	4˚C	-	-
	5˚C	+	+
	37˚C	+	+
	40˚C	-	-
Salt tolerances	≤ 3%	+	+
	4%	-	-

[1]: Negative [2]: Positive [3]: Not Done [4]: Fermentative

Table 2. Results of pathogenicity test of *Aeromonas hydrophila* in experimental fish by intramuscular injection method

Injected with	Dose (CFU/fish)	No. of fish challenged	No. of fish died	Mortality (%)
Aeromonas hydrophila	2.8×10^6	10	10	100
	2.8×10^5	10	6	60
PS (control)	0.1	10	0	0

3.4 Sensitivity Test Results of the Antibiotics: *In-vitro* and *in-vivo*

The antibiotics oxysentin, acimox and oxy-D Vet showed minimum, medium and highest inhibitory zone, respectively. It was proved that the bacteria, *A. hydrophila* was less, medium and highly sensitive to oxysentin, acimox and oxy-D Vet, respectively. Results found after treatment with antibiotics are shown in Table 3. After oxysentin 20% treatment at higher dose, haemorrhagic lesions were recovered. Recommended dose of acimox and oxy-D Vet could cure erosion in anal region. Hemorrhagic lesions, caudal fin ray loss and ulcerative lesions were quite recovered. Feeding affinity was increased and there was no more dark skin colouration.

Table 3. Effects of antibiotics on *Hypophthalmichthys molitrix* infected with bacteria

Antibiotics	Selected dose	No. of fish treated	No. of fish cured	No. of fish not recovered	Percentage of recovery
Oxysentin 20%	Lower	3	0	3	0
	Recommended	3	2	1	67
	Higher	3	3	0	100
Acimox	Lower	3	0	3	0
	Recommended	3	3	0	100
	Higher	3	3	0	100
Oxy-D Vet	Lower	3	1	2	33
	Recommended	3	3	0	100
	Higher	3	3	0	100

IV. Discussion

Experimental infection by *A. hydrophila* of the fish silver carp, *H. molitrix* showed that the fish were seriously affected which caused mortality. Thus it was proved that *A. hydrophila* was pathogenic to silver carp, causing 100% mortality by a suspension of the bacterial cells of 2.8×10^6 CFU/fish, with peak mortalities occurring on days 2 to 5 and 60% mortality by a suspension of the bacterial cells of 2.8×10^5 CFU/fish, with peak mortalities occurring on days 4 to 9. Through experimental infections in carps (rui, catla, and mrigal), perch, and catfishes (shing and magur), 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 to 4 days and 3 to 11 days, respectively [8]. *Cirrhinus cirrhosus* 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 to 5 days and 4 to 12 days, respectively [8]. In another 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 to 9 days [9]. The *A. hydrophila* was found to be pathogenic for both indigenous (rui *Labeo rohita*, catla *Catla catla* and mrigal *Cirrhinus cirrhosus*) and exotic (Silver carp *Hypopthtalmichthys molitrix* and common carp *Cyprinus carpio*) carps; it was observed that intramuscular methods 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 to 12 days [4]. In the case of intramuscular injection, the highest bacterial load were found to be 9.4×10^8 CFU/g in the intestine and the lowest bacterial load was found to be 2.8×10^3 CFU/g in the kidney. The highest and the lowest loads of *A. hydrophila* was found in liver, intestine and kidney to be 1.18×10^9 CFU/g and 6.46×10^8 CFU/g, 3.70×10^8 CFU/g and 1.67×10^4 CFU/g, 1.47×10^4 CFU/g and 1.71×10^3 CFU/g in the natural EUS affected shing *Heteropneustes fossilis*, respectively [5]. It was carried out to control *A. hydrophila* and justify the recommended dose and method of application of particular antibiotics. Among the three antibiotics, effect of acimox and oxy- D Vet at recommended dose treatment showed the best result where 100% fish were recovered. However, oxysentin 20% at higher dose showed best result. The acimox and oxysentin 20% at higher dose showed good result but oxy-D Vet at recommended dose showed the best result [10].

V. Conclusion

The present work generated some information on the pathogenicity and the control of *A. hydrophila* against silver carp *H. molitrix*. This study would help the fish farmer, technical personnel and pharmaceutical companies which may have contribution in further disease diagnosis and control of fish diseases. The knowledge generated from this study could be suggested at the common fish farmer level through an action research. So, the disease of fish could be controlled with appropriate dose and dosages by using the desired medicine.

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