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Investigation on Infectious Dropsy of Indian Major Carps

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Abstract

Dropsy is a common disease often reported to cause mortality and morbidity in cultured Indian major carps. The present investigation was undertaken to find out the etiological agent of sporadic occurrences of infectious dropsy in Indian major carps cultured in different aquaculture regions of India. Among the three species of Indian major carps, *Catla catla* was most susceptible (41.66%) followed by *Labeo rohita* (31.49%) and *Cirrhinus mrigala* (26.66%). A survey of the three major aquaculture regions of the country such as Orissa, Andhra Pradesh and West Bengal over the last seven years revealed dropsy in 12.14 ±2.52, 9.4 ±1.36 and 12.14 ±2.52% cases, respectively. *Aeromonas hydrophila* was found to be the causative agent of the dropsy conditions in almost all cases. However, the present investigation ruled out the involvement of any viral agent in this condition of Indian major carp.

Introduction

Dropsy is a common disease responsible for the mortality and morbidity in a wide range of cultured fishes including Indian major carps. Different etiological agents for infectious dropsy have been reported, such as *Aeromonas hydrophila* (Shome et al. 1996), *A. salmonicida* (Bootsma et al. 1977; Kumar et al. 1986), *Rhabdovirus carpio* (Fijan et al. 1971; Fijan

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1972), *Saprolegnia* sp. (Havelka 1974) and *Myxosporidian* sp. (Kumar et al. 1986).

Indian major carps are the dominant freshwater cultured species in the Indian aquaculture system and suffer many diseases including infectious dropsy (Kumar 1984). *Aeromonas hydrophila* has been isolated from some cases of Indian major carps and the symptoms have been found to be similar to those described in other species (Gopalakrishnan 1961; Shome et al. 1996). However, these reports have neither ruled out the possibilities of any other etiology including virus infection associated with this nor reproduced this condition in these species.

Since this condition is an alarming problem and occurs widely in all the species of Indian major carps, the present investigation was undertaken to find out the involvement of *A. hydrophila* in addition to the possibility of involvement of any viral agent at the onset of dropsy syndrome in Indian major carps.

Materials and Methods

Sample collection

The study was conducted in three major aquaculture regions of India viz. coastal areas of Orissa, 925 km^2 area of Kolleru lake of Andhra Pradesh, freshwater and wastewater aquaculture areas of West Bengal from 1999-2005. During the study, all the three species of Indian major carps namely *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* were investigated. Samples were collected from fish showing typical symptoms of infectious dropsy. The samples included peritoneal fluid, bloods and tissue samples such as gills, liver, heart, kidney, spleen, muscle and blood sera. The data on occurrence of diseases were collected from progressive fish farmers and state department officers using specific questionnaires.

Isolation of bacteria

The samples were processed for bacteriological studies, i.e. bacterial isolation and subsequent identification by microbiological procedures (Cruickshank et al. 1975).

Virulence studies

The pathogenicity study for each *A. hydrophila* isolated from dropsy cases was conducted in duplicate groups of rohu juveniles (average

weight 50-70g). They were reared in 1000 l plastic pools with constant aeration and artificial diet, and the water temperature varied from 25 to 30°C. In each group, individual fish was injected intraperitoneally with 0.1 ml of bacteria suspension in phosphate buffer saline (PBS, pH 7.2) at a concentration ranging from 10^5 to 10^{10} CFU with one PBS injected group as control. Similarly, pathogenecity study for other isolated bacterial species such as *Aeromonas sorbia* and *Pseudomonas fluorescens* was conducted.

Sample preparation for isolation of viral agent

Tissue samples and peritoneal fluid collected from diseased fish were taken in a mortar and pestle, macerated thoroughly with PBS. The solution was transferred to eppendrof (1.5ml) tubes and given three cycles of freezing and thawing. It was then centrifuged at 10,000 x g for 10 min. at 4°C. After centrifugation, the supernatant was filtered through a 0.22 μ filter and the filtrate was used as an inoculum for infecting the cells.

Preparation of primary cell culture

Primary cell cultures of *Labeo rohita* ovary cells were done by using the standard protocol. Briefly, the ovary was aseptically collected from the matured female, minced into small pieces and trypsinised with 0.25% trypsin (Merck, India). The cells were seeded in 25 cm^2 flasks at 10^5 cells•ml^{-1} in Dulbecco's modified eagle's medium (DMEM) (Sigma, USA) containing 10% newborn calf serum (Hyclone, USA). The cells were incubated at 25°C in CO_2 incubator for 4 to 5 days till the completion of a monolayer.

Cell culture infection

After the completion of a monolayer, the cells were infected with the inoculums for virus isolation described previously for 1h followed by incubation at 24°C with maintenance medium (DMEM containing 2% calf serum). The cells were checked every day for 5 to 6 days for the appearance of any cytopathic effect. Three passages were given for each sample, before they were declared negative.

Serology

Preparation of ELISA antigen

A standard strain of *A. hydrophila* (MTCC Strain No- 646) was obtained from the Institute of Microbial Technology, Chandigarh, India and used for the preparation of ELISA antigen (Swain and Nayak 2003). For

detection of viruses, triturated tissue samples were heated at 56°C for 30 min. for inactivation of endogenous peroxidase activity and coated directly to the nitrocellulose paper strips for dot-ELISA.

Monoclonal antibodies and anti – mouse- HRPO conjugate

Monoclonal antibodies against known freshwater fish viruses *viz.* infectious haematopoietic necrosis (IHN), viral haemorrhagic septicemia (VHS), spring viremia of carp (SVC) and infectious pancreatic necrosis viruses (IPN) and anti-mouse IgG- HRPO conjugate were procured from Biox Diagnostics, Belgium for use in ELISA.

Indirect ELISA

The antibodies against *A. hydrophila* were detected using indirect ELISA (Swain et al. 2001). Briefly, microtitre plates (Nunc, Denmark) were coated overnight at 4°C with 50 µl of ELISA antigen (2-4 µg per well, diluted in PBS, pH 7.2). The plates were then washed in PBS containing Tween-20 (PBS-T) and blocked with 100 µl of 3% skim milk powder for 2 h at 37°C. The wells were further washed with PBS-T and then 50 µl of serially diluted fish sera was added followed by incubation at 37°C for 45 min. The plates were then washed in PBS-T and added with 50 µl of (1:20) anti-rohu globulin-HRPO conjugate obtained from the Fish Health Management Division Central Institute of Freshwater Aquaculture, Bhubaneswar. They were incubated further for 45 min at 37°C, washed thoroughly and added with 50 µl of substrate solution (5mg of O-phenylene diamine dihydrochloride and 10 µl of H_2O_2, 38% V/V in 5ml of acetate buffer, pH 5.0). The plates were incubated at 37°C for 5 to 10 min. in a dark chamber and the optical density (OD) was recorded at 450/655 nm in a microplate reader (Bio-Rad, USA). The reciprocal of the highest dilution of the infected serum sample showing OD value double that of the control (negative sera) was considered to be positive.

Dot ELISA

Nitrocellulose paper (NCP) strips were coated with minced infected tissue supernatants prepared as previously described. The antibody used was monoclonal antibodies against different viruses listed as above with conjugate (anti-mouse Ig G – HRPO conjugate) (1:200) and substrate Tetramethyl Benzidine (TMB) / H_2O_2 (Bangalore Genei, India) for localization of antigens (Swain et al 2001).

Results

During the present investigation, a total of 400 cases of dropsy of different species of Indian major carps showing typical signs of infectious dropsy were collected. Among which 31.49, 41.66 and 26.66% cases were of *L. rohita*, *C. catla* and *C. mrigala*, respectively (Fig. 1). Figure 2 shows regional composition of the cases for three major aquaculture regions, i.e. Orissa, West Bengal and Andhra Pradesh of India.

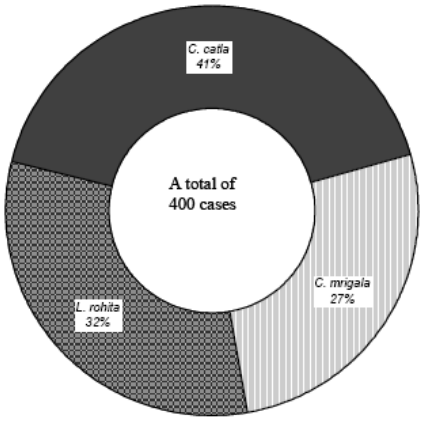

Figure 1. Composition of the cases of dropsy for Indian major carp species during the survey period.

Bacteria

For the bacteriological study, *A. hydrophila* was isolated and identified from almost all infected fish. Other isolates such as *Bacillus* (7.23%), *Escherichia coli* (9.90%), *A. sorbia* (6.25%) and *P. fluorescens* (3.2%) were isolated from the dropsy affected fishes. In all the three fish species, *A. hydrophila* was the most prominent isolate whereas *P. fluorescens* was the least isolated species.

Pathogenicity study

For the pathogenicity study, majority of the isolates of *A. hydrophila* were found to be pathogenic with LD_{50} which varied from 10^5 to 10^6 CFU•ml^{-1}. However, the LD_{50} value of *Pseudomonas aeruginosa* and *A. sorbia* were above 10^9 CFU•ml^{-1} and found to be non-pathogenic.

Indirect ELISA

Antibodies against virulent *A. hydrophila* were detected in the test sera of dropsy affected fishes by indirect ELISA. In indirect ELISA, 83 % of samples were found to be seropositive for *A. hydrophila.*

Virological study

The virological study showed negative reaction with monoclonal antibodies against IHN, VHS, SVC and IPNV with the tested fish tissues samples. Similarly, no cytopathic effect and other abnormal changes were observed in the cell lines even after three consecutive passages.

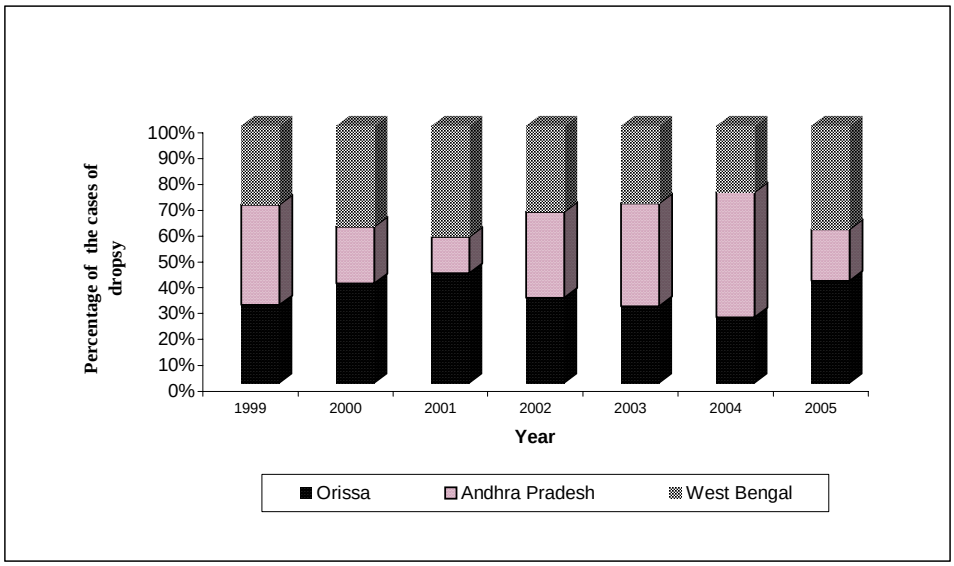

Figure 2. Composition of the cases of dropsy in Indian major carps for three aquaculture regions, i.e Orissa, West Bengal, Andhra Pradesh from 1999-2005.

Discussion

Dropsy is a chronic problem of fishes throughout the world. In India, several workers have already reported the incidences of dropsy in Indian major carps (Gopalakrishnan 1961; Shome et al. 1996; Shome et al. 1999). During the present investigation, the three major aquaculture regions of India viz. Orissa, Andhra Pradesh and West Bengal were covered and the incidence of disease was recorded to be 12.14 ±2.52, 9.4 ±1.36 and 12.14 ±2.52%, respectively. Among the three species of Indian major carps, *C. catla* was found to be the most susceptible (41.66%), followed by *L. rohita* (31.49%) and *C. mrigala* (26.66%). Similar observations had been made by Gopalakrishnan (1961) who reported the susceptibility of Indian major carps to this disease condition with *C. catla*, *L. rohita*, *C. mrigala in* descending order.

The present finding also supports the view of earlier workers like Lakshmanan et al. (1986), Karunasagar et al. (1989) and Shome et al. (1996) that *A. hydrophila* is the causative agent of dropsy. The bacteriological, pathogenicity and serological studies also confirmed the involvement of *A. hydrophila* in dropsy cases. Highly virulent strains of *A. hydrophila* with LD_{50} values of 10^5 to 10^6 CFU was predominantly isolated from

almost all the dropsy affected fish in these localities. Though, in a few infected fishes, *P. aeruginosa*, *P. fluorescens*, *A. sorbia*, or *E. coli* along with *A. hydrophila* were isolated, but the non pathogenic nature of these isolates indicated them as secondary invaders after primary infection with *A. hydrophila*. In indirect ELISA, 83% of sera were positive for *A. hydrophila* antibodies.

The present investigation also ruled out the possible involvement of Myxosporodian parasite in dropsy as recorded earlier by Mishra et al. (1987). Moreover, negative reaction of the antiviral monoclonal antibodies with the infected samples and absence of cytopathic effect in cell culture ruled out the possibilities of any viral infection associated with this condition of Indian major carps.

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