Modulation of systemic and mucosal immune responses of *Catla catla* (Hamilton, 1822) experimentally challenged with gill monogeneans

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**ABSTRACT**

The present study investigated the modulation of systemic and mucosal immunity of catla (*Catla catla*) experimentally challenged with gill monogeneans. Fingerlings of catla (24.2 ± 1.5 g) without any parasites were randomly distributed into two groups each comprising quadruplicate tanks (10 fish per tank). The fish in the infected group were challenged with gill monogeneans by cohabitation method. The control group fish were not exposed to parasites. Serum and mucus were collected from both control and infected fish and different immunological parameters were measured at weekly interval for four weeks. Monogeneans on the fish gills were also quantified from infected fish at weekly interval. Different systemic immunological parameters viz. myeloperoxidase content, alkaline phosphatase and hemolytic activity of serum showed a significant increase (*p < .05*) in infected group. However, respiratory burst activity, total serum protein and anti-protease activity did not show any significant enhancement (*p < .05*) in infected group compared to control. In case of mucosal parameters, myeloperoxidase content, alkaline phosphatase activity, total protein content and anti-protease activity of mucus showed significant enhancement (*p < .05*) in infected group compared to control. Parasite prevalence was 100% in the infected group in all the sampling weeks. The mean intensity of parasites was progressively increased and the highest number of monogeneans per gill (45 ± 4.56) was observed during 4th week post challenge. Control group had mean intensity and prevalence of zero. In conclusion, there was the participation of immune system components against monogenean infection in catla. However, the progressive increase in monogenean intensity indicates some immune evasion mechanism available with the monogenean to co-exist with the host in the face of host’s immune responses.

**1. Introduction**

Infectious diseases are considered as one of the major constraints for successful aquaculture. Among them, parasitic diseases are increasingly being recognized as emerging problem in aquaculture with serious economic implications [1]. Ectoparasite infections lead to damage of the fish integument facilitating entry of secondary pathogens [2]. Parasites interfere with nutrition, metabolism and several other physiological functioning of the host [3]. Ultimately, infections by parasites lead to either mortality or morbidity of fish with consequent economic losses.

Among the parasites, the gill monogeneans, primarily belonging to the Genus *Dactylogyrus*, are known to parasitize a large number of fish [4]. Species belong to this ectoparasitic helminth group is also known as gill fluke and commonly infect gills of cyprinid fish. The infected fish shows excessive mucus secretion, epithelial hyperplasia, edema, gill hemorrhages and asphyxia which often leads to mortality [5,6]. Indian major carps including catla (*Catla catla*), rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*) have been reported to be frequently infected by gill monogeneans with high prevalence and intensity [7,8]. Dactylogyrus infections, thus, can be accounted for production loss in many ways.

As far as the characterization of the immune mechanisms and their pathways against fish parasites are concerned, substantial progress has recently been made. In teleost, cellular and humoral factors of both innate and adaptive immunity are involved in immunity against monogeneans infecting gill tissue with superficial capillaries and several effector mechanisms has been suggested for that [9,10]. Apart from different systemic immunity components, several other cellular and humoral factors of the mucosa such as mucosal cells, leukocytes, complement, C-reactive protein, lysozyme, anti-microbial peptides, etc. are thought to play an important role in mediating immunity against monogeneans [10].

The majority of the progress in understanding the immune mechanisms against monogeneans has been reported in fish other than Indian major carps. Moreover, elucidation of immune responses has
been vastly reported in monogeneans other than gill flukes [10–13]. Also, responses of mucosal immune components have not been well documented.

Considering the fact that catla is highly susceptible to gill monogenean infections and the immune responses of this species against such pathogens have not been demonstrated, the present study aims to evaluate the modulation of systemic and mucosal immune responses of catla against experimental gill monogenean infection.

2. Material and methods

2.1. Experimental fish

Hundred fingerlings of catla (24.2 ± 1.5 g) with no signs of disease (as determined by gross and microscopic examination of representative tissues samples from skin and gill) were obtained from a local fish farm and acclimatized in 500 L outdoor fibre-reinforced plastic (FRP) tanks. The fish were acclimatized with aeration and were fed twice daily with a pelleted diet (2% of body weight). The physico-chemical parameters of tank water were maintained throughout the acclimatization period. Exchange of water (20–25%) was done daily to remove the waste materials.

Fifty fingerlings of naturally monogenean-infested catla (16.4 ± 1.03 g) were collected from another fish farm and acclimated in a separate set of FRP tanks. Whole gill tissues of representative samples (n = 10) were examined using a stereomicroscope (Olympus SZ51, Japan) for the presence of monogeneans. The mean intensity (MI) of the gill monogeneans-infested fish was 60.8 ± 8.6. Infected fish served as a source of infection in subsequent cohabitation challenge experiment.

2.2. Cohabitation challenge

Healthy fish were given a bath treatment with formalin (250 μl L⁻¹) and salt (3%) to kill parasites, if any, consecutively for three days before introducing any monogenean infested fish. The treatments were performed in a separate set of FRP tanks. The treated healthy fish were used for the subsequent challenge experiment after seven days of acclimatization. The experiment was conducted with a completely randomized design with quadruplicates for each treatment. The first treatment comprised of uninfected control where 40 fish were distributed equally in 4 tanks without introducing any monogenean infested fish. The second treatment was the cohabitation challenge group where the same numbers of fish were distributed in another four tanks and each tank was introduced with five monogenean-infested fish. Infected fish were marked by cutting the tip of the left pectoral fin to differentiate them from the healthy individuals.

2.3. Sample collection

Eight fish per treatment (two fish from each tank) were sampled and anesthetized with clove oil (50 μl L⁻¹) for gross and microscopic observation of gills, collection of mucus and blood. Sampling was carried out at weekly interval for four weeks.

2.3.1. Collection of blood and serum

Blood was collected directly from the caudal vein, allowed to clot at room temperature for 2 h and then centrifuged at 1500 g for 10 min at 4 °C. Collect sera were then stored at −20 °C in the sterilized vial until further use. An aliquot of blood was also kept with anticoagulant (EDTA) for nitroblue tetrazolium (NBT) assay.

2.3.2. Parasite counting

Monogeneans on the fish gills were quantified for the infected group at weekly interval [14]. Parasites were quantified only from the right gill of the infected fish (the number of parasites was not multiplied by two for further calculations). The four right side gill arches of each fish were placed into Petri dishes containing water and parasites were counted using a stereomicroscope (Olympus SZ51, Japan). The prevalence and MI of parasites were estimated following the formula proposed by Margolis et al. [15].

Prevalence (%) = (Total number of infected fish/Total number of fish hosts examined) × 100

Mean Intensity (MI) = Total number of parasites/Total number of infected fish

2.3.3. Mucus collection

Mucus was collected from the infected fish as well as from the uninfected control from the posterior to the operculum region by gentle scraping [16]. Briefly, an equal volume of PBS containing 0.02% azide was added to the collected mucus, and samples were vortexed for 1 min and stored overnight at 4 °C. Mucus was then centrifuged at 1500 × g for 10 min and the supernatant stored at −20 °C until needed for analysis.

2.4. Assessment of immune responses

Different immunological responses were measured from the collected serum and mucus samples. Oxygen radical production (respiratory burst) by catla phagocytes in the blood was measured using NBT reduction assay [17]. The total serum and mucosal myeloperoxidase content were determined as described by Mohanty and Sahoo [18] with minor modification. Briefly, serum (10 μl) was added with PBS (90 μl) in a microtitre plate to which 35 μl of 20 mM 3,3′,5,5′-tetramethyl benzidine hydrochloride (TMB) (HiMedia) and 5 mM H₂O₂ were added. After 2 min of incubation, the reaction was stopped by adding 35 μl of 4 M sulphuric acid and the optical density (OD) was read at 450 nm. Total protein content of serum and mucus was estimated by the biuret method [19]. The alkaline phosphatase activity of serum and mucus was assayed based on the method by Kind and King [20]. Total serum and mucosal anti-protease in fish were determined according to Zuo and Woo [21] with minor modification. In short, serum (10 μl) was mixed with 100 μl of trypsin (HiMedia; 200 μg/ml of PBS) in tubes. One positive control without serum was taken containing only trypsin and PBS. After incubation at 25 °C for 30 min, 1 ml of casein dissolved in PBS (2.5 mg ml⁻¹) was added and all the tubes were further incubated for 15 min at 25 °C. The reaction was stopped following addition of 500 μl of 10% trichloroacetic acid. Finally, the sample was centrifuged at 10,000 g for 5 min, the OD of the supernatant was read at 280 nm and the percentage of trypsin inhibition was calculated. The hemolytic activity of serum was determined as described by Dash et al. [1] with slight modification. Briefly, 2 ml of chicken blood was collected in Alsever's solution and separated RBC was further diluted with PBS to make 1% v/v. Two-fold serial dilution of the sera (unheated) in PBS was added with 50 μl of 1% chicken RBC in each well of a microtitre plate. The microtitre plate was incubated for 1 h at 37 °C. Haemolysin titer was defined as the last dilution of serum showing complete lysis of RBC.

2.5. Statistical analysis

Statistical analysis of data was performed using SPSS-16.0 for windows software (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± standard error of mean (SEM). Data were analyzed by the student’s T-test. Probability levels of 0.05 were used to find out the significance in all cases.
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