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HUMORAL AND PROTECTIVE RESPONSE OF INDIAN MAJOR CARPS TO IMMERSION VACCINATION WITH *AEROMONAS HYDROPHILA*

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ABSTRACT

Fry of the Indian major carps, *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) were immunized at 4 and 8 weeks post hatching (wph) by direct immersion in a suspension (10^8 cells ml^{-1}) of heat inactivated *Aeromonas hydrophila*. Following the same procedure, booster dose was administered 20 days after the first immersion. Antibodies as well as protective response produced in both the groups after the first and the booster immersion were different and significant ($P<0.05$). No significant difference was found between the species in the two age groups. The specimens immunized 8 wph showed higher antibody titres and protection than the 4 wph group. *C. catla* had higher relative percent survival followed by *L. rohita* and *C. mrigala*.

Keywords: Indian majors carps, immune response, *Aeromonas hydrophila*, immersion, challenge, relative percent survival

INTRODUCTION

The success of any vaccination depends largely on the structural and functional maturity of immune system of the fish at the time of vaccination (Ellis, 1989). Several reports are available on the ability of very young fish to produce humoral immune response. The onset of this ability depends more on the weight of fish than age (Johnson *et al.*, 1982a, b; Tatner and Horne, 1983). The number of lymphoid cells present in the fry is more closely related to its weight than age (Tatner and Manning, 1983). These authors suggested that immunocompetence is attained once a certain number of cells are present, possibly with the cells of differing functions dividing and maturing at different rates. Therefore, it is difficult to know exactly the appropriate age or size when the fish should be vaccinated. The present

investigation was carried out to test the ability of the Indian major carps of 4 and 8 weeks post hatching (wph) to elicit humoral and protective responses against *Aeromonas hydrophila*, delivered via immersion and ascertain a proper time to vaccinate.

MATERIAL AND METHODS

Four day-old hatchlings of the Indian major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, were procured from the State Fish Seed Farm, BR Project, Karnataka. The hatchlings were maintained in 25-m^3 cement cisterns at 3000 hatchlings per cistern and fed daily at 10% of the body weight till the start of the experiment. Two age groups (4 and 8 wph) of each species were used.

The experimental design for immersion vaccination is given in Table 1. A batch of 300 fry of each species was immunized by direct immersion in the heat-inactivated bacterial suspension of 10^8 cells ml^{-1} for one hour. The control group of fish was similarly treated by immersion in PBS of equivalent dilution. The vaccinated and the control groups of fish were maintained in 25-m^3 cement cisterns. A booster dose was given to the vaccinated fish 20 days after the first immersion following a similar protocol.

Table 1: Details of vaccination of Indian major carps of 4 and 8 wph using heat-killed *Aeromonas hydrophila*

Carps of 4 wph				Concentration of bacterin in immersion	Duration of immersion
Species	No. of fish vaccinated	Mean length (cm±S.E.)	Mean weight (g±S.E.)		
C. catla	300	4.30±0.60	0.95±0.03		
L. rohita	300	4.45±0.31	0.90±0.09		
C. mrigala	300	4.10±0.65	0.80±0.03	10^8 cells ml^{-1}	1 hour
Carps of 8 wph					
C. catla	300	6.00±0.96	4.50±0.18		
L. rohita	300	6.30±1.25	3.90±0.04		
C. mrigala	300	6.20±1.18	3.70±0.10		

Antibody titres against *A. hydrophila* were measured 20 days after the first immersion and 10 days after the second. Random samples of 10 to 15 fish from each treatment group were drawn and blood collected in capillary tubes by severing the caudal peduncle. Blood from individual fish was pooled and stored overnight at 4°C. Serum was separated by spinning the clotted blood at 6000 rev min^{-1} for ten minutes. The serum was then heat-inactivated at 55°C for 30 minutes on a thermostat water bath. Double dilutions of the serum were titrated against equal volumes of the heat-inactivated bacterial

suspension (10^9 cells ml^{-1}). The last dilution showing clear agglutination was taken as the titre and expressed as log 2 values (Sundick and Rose, 1980).

The challenge dose was determined to give less than 50% survival in the control fish. The vaccinated and control fish were challenged after 20 days of the first immersion and ten days of the second immersion to assess protection by direct immersion in a well-aerated suspension (10^6 cells ml^{-1}) of virulent *A. hydrophila* for one hour. A total of 50 fish in each treatment group were challenged. The challenged fish were maintained in fibreglass tanks (500 l) containing aerated fresh water for a period of seven days. Half of the water in each tank was exchanged daily. Post-challenge mortalities were confirmed by streaking the aseptic kidney samples of the moribund fish on Rimler Shotts' Aeromonas Selective medium (Hi-Media Laboratories Limited, Mumbai). Only specific mortalities were used for the computation of relative percent survival (RPS) as described by Amend (1981).

All the data were analyzed using ANOVA (Snedecor and Cochran, 1968) and least significant range ($P<0.05$) derived following Duncan (1955).

RESULTS AND DISCUSSION

The humoral response of carps measured as antibody titres (log 2) is

Table 2: Antibody titres in vaccinated and control Indian major carps of 4 and 8 wph

Age at (wph)	Species	Test group	No. sampled	Mean antibody titre (log 2)	
				20 dppi	10 dpbi
4	*C. catla*	V	15	5	6
		C	15	1	1
	L. rohita	V	15	5	6
		C	15	2	1
	C. mrigala	V	15	5	5
		C	15	2	2
8	*C. catla*	V	10	6	7
		C	10	1	1
	L. rohita	V	10	6	7
		C	10	2	1
	C. mrigala	V	10	6	7
		C	10	2	2

Booster immersion given 20 days after priming. V: Vaccinated; C: Control; dppi: Days post priming immersion; dpbi: Days post booster immersion

presented in Table 2. The direct immersion of carp fry of 4 and 8 wph irrespective of their age groups resulted in the production of humoral immunity as evidenced by the antibody titres. Carps elicited higher antibody titres in the 8 wph group compared to that of the 4 wph after the first and second immersions, and was significant ($P<0.05$). Tatner (1986) demonstrated that the immersion-immunization of rainbow trout fry can elicit humoral immune response against *Aeromonas salmonicida* and showed that 1 wph did not produce antibodies while the fish of 4 wph did. Botham and Manning (1981) reported that the effective allograft rejection of carp lymphocytes occurred at 16 days post hatching, which coincided with the detection of immunoglobulin-positive cells (Koumans-van Diepen *et al.*, 1994). The entry of antigens during immersion take place predominantly through gills and lateral line, and a small percentage via skin and vent (Alexander *et al.*, 1982; Smith, 1982). This process appeared to be an active mechanism in Indian major carps also to activate systemic immune response. The lower antibody response in 4 wph *C. mrigala* after the booster immersion, compared to the other two carps *C. catla* and *L. rohita,* possibly reflects the late onset of immune memory as the fish was vaccinated at an early stage of life, whereas the positive response was achieved when vaccinated at an advanced stage. It was clear from the results that *C. mrigala* of 8 wph responds equally well to the booster immersion. Antibody titres following the first immersion were significantly lower than those of the booster immersion. However, between the species, no difference in response between the two age-groups was observed. A single route for both priming and boosting the immune response was followed. Lamers *et al.* (1985) also confirmed that a single route of priming and booster help in eliciting better immune response than when the route of priming is different from the route of booster. Karunasagar *et al.* (1991) recorded very high titres of antibodies in the fingerlings of Indian major carps to *A. hydrophila* vaccine after three boosters but no significant difference was observed between the immunized batches.

Protective response of the immersion-vaccinated Indian major carp fry (Table 3) supported the findings on the humoral response. Unidentical routes of vaccination and challenge can greatly affect the protective response. Orally-vaccinated carp, *Cyprinus carpio* registered better protective response when challenged via immersion than challenged via injection (Azad *et al.*, 2000). In the present study, carps of 4 wph elicited lower protective response compared to those of 8 wph after priming as well as boosting, and there was no difference in their protective response between the species in each group. Booster-treated carps performed better than the primed ones and the protective responses corresponded to the antibody titres elicited. Post (1966), Plumb (1984) and Karunasagar *et al.* (1997) found that the

Table 3: Protective response of 4 and 8 wph groups of Indian major carps to immersion vaccination with *A. hydrophila*

Age (wph)	Species	Test group	Number challenged	20 days post immersion		10 days post booster immersion	
				Mortality (%)	RPS	Mortality (%)	RPS
4	*C. catla*	V	100	40	50.62	35	58.82
		C	100	81		85	
	L. rohita	V	100	46	48.31	36	57.65
		C	100	89		85	
	C. mrigala	V	100	48	46.67	36	56.63
		C	100	90		83	
8	*C. catla*	V	100	33	58.23	29	63.29
		C	100	79		79	
	L. rohita	V	100	37	57.95	31	61.25
		C	100	88		80	
	C. mrigala	V	100	36	55.56	33	60.71
		C	100	81		84	

V: Vaccinated; C: Control; RPS: Relative percent survival

extent of protection showed good correlation with the titres of the agglutinating antibody when fish were immunized with antigens of motile aeromonads. Chandran *et al.* (2002) observed that a high degree of protection against *A. hydrophila* is associated with a good agglutinating antibody response in the three Indian major carps. Kamilya *et al.* (2006) also reported a good degree of protection against *A. hydrophila* in all the vaccinated groups of *C. catla* and indicated that antibody titres correlate with survival. However, working on the different fish sizes at vaccination in rainbow trout, Johnson *et al.* (1982a) suggested that the rainbow trout of less than 0.5 g could not produce the protective response against either *Vibrio anguillarum* or *Yersinia ruckeri* after immersion-vaccination and opined that enhanced bacterin concentration for immunizing the fish in the size range of 0.5 to 1.0 g could help in elevating the levels of the protective response. Tatner and Horne (1984) reported in rainbow trout that some degree of protection is achieved by immersion (16-100%) and intraperitoneal vaccination (25-100%) against *V. anguillarum*, once the fry had reached 0.5 g in size. In this study, age-dependant protective response in Indian major carps is in conformity with the findings of the above investigations. Higher protective response of *C. mrigala* in 4 wph was noticed after the booster dose despite the fact that the fish did not produce enhanced antibody titres. The role of cell-mediated immunity and the natural agglutinins could be the reasons for the enhanced protective response which had been

recognized in fish. Hazen *et al.* (1981) showed the presence of natural agglutinins in many fish species, and Lamers and Muiswinkel (1986) reported the presence of a variety of other non-antibody molecules that could provide a natural resistance in fish.

The present study clearly revealed that the age/size of fry showed prominent effect in producing antibody titres and protection. The fry of Indian major carps could, therefore, effectively be vaccinated as early as by 4 wph.

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