J Krishi Vigyan 2020 (Special Issue) : 304-308 DOI : 10.5958/2349-4433.2020.00123.3

Vaccination of *Catla catla* Employing Biofilm of *Aeromonas hydrophila* to Enhance its Immunity

Abhiman PB[1] and Garima[2]

[1]Department of Aquaculture, College of Fisheries, Mangalore-575002, Karnataka
[2]ICAR-Directorate of Coldwater Fisheries Research, Bhimtal-263136, Nainital, Uttarakhand

ABSTRACT

Vaccination is considered as a sustainable strategy for disease prevention in aquaculture and further mucosal vaccines are the need of the hour which brings about local immune response and hence enhanced immunity. Oral vaccination is the preferred method but its efficacy depends on its appropriate delivery. In this study the effect of biofilm oral vaccine on gut immunity (mucosal immunity) was evaluated in comparison with serum antibody titre and protection upon challenge. In catla vaccinated with biofilm the serum antibody (OD) increased gradually, compared to the control reaching highest on 30dpv. A similar trend was observed in mucus antibody of catla vaccinated with biofilm compared with that of control. However, antibody concentration (OD) in mucus was almost 50per cent compared to that of serum. In unvaccinated control group survival was only 20 per cent compared to 86.66 per cent with biofilm vaccinated group.

Key Words: Antibody, Biofilm, Catla, Vaccine.

INTRODUCTION

Catla (*Catla catla*) is the most important and popular farmed Indian major carp species. However, due to intensive culture, incidence of several diseases has been reported (Bondad *et al,* 2005). The control of these diseases using antibiotics is not desirable and therefore, development of appropriate prophylaxis is needed. Oral vaccination that is considered appropriate for aquaculture in general (Groove *et al*, 2006) is also important for managing enteric diseases. Preliminary studies in the laboratory, College of Fisheries, Mangalore, India on the effect of *Aeromonas hydrophila* biofilm oral vaccine on Indian major carp has given highly encouraging results (Azad *et al,* 1997, 1999). Increased serum antibody level and protection upon challenge was recorded in this study. Furthermore large quantities of biofilm antigens could be localized in lymphoid organs including gut; employing MAb based immunofluorescence (Azad *et al,* 2000). However, the information on the effect of oral vaccine on gut and skin mucus is lacking. The mucosal surfaces of skin, gills and gut are protected by both humoral and cellular mechanisms (Hart *et al*, 1988; Rowley *et al*, 1988). Leukocytes are present in all parts of the teleost digestive system, most extensively in the intestine, where lymphocytes, plasma cells, granulocytes and macrophages are present under the epithelium (Abelli *et al*, 1997). Mucosal immunization with lectins/adhesins of mucosal pathogens may induce antibody responses in secretions which inhibit the adhesion of the organisms to epithelial surfaces. This approach has been successfully demonstrated in eliciting immune protection against a number of bacterial infections in mammals (Danve *et al*, 1993; Toida *et al*, 1997). In fish, oral and anal delivery of soluble and particulate antigens can elicit systemic and mucosal immune responses - both humoral and cellular (McLean and Donaldson, 1990; Jenkins *et al*, 1994). This indicates the possible existence of a common mucosal immune system in fish similar to that in mammals (Joosten *et al*, 1995). The intestinal mucosa of common carp *Cyprinus carpio*

Corresponding Author's Email: abhifishco@gmail.com

L, has been studied with special consideration of mucin glycoproteins (Neuhaus *et al*, 2007) and after peroral application of *A. hydrophila* (Schroers *et al*, 2009). This study aims to see the effect of immunisation of Catla with a biofilm oral vaccine, focusing on gut mucosal immune responses.

MATERIALS AND METHODS

Vaccination of catla (*Catla catla*)

Catla (40±1 g) were maintained in circular fiberglass tanks (250 L) in the fish farm of College of Fisheries, Mangalore. The experimental set up consisted of a vaccination group (fed with biofilm vaccine containing 5.13×10^{10} CFUg^{-1} fish d^{-1}) and a control group (fed with normal feed) stocked with 20 fish. Every alternate day, half of the tank water was siphoned off to remove the waste material. During experiment the temperature of tank water ranged 27^{O} to 29^{O}C and pH 7.5-8.5; Fish were acclimatized to lab condition, 15 d prior to the experiment. Fish were vaccinated for a period of 10 d. The vaccinated feed was given at 2.5% of the body weight a day and the complete acceptance of feed was monitored. After 10 d of vaccination, the fish were fed with normal feed till the end of experiment.

Serum antibody collection

After vaccine feeding for 10 days, 5 fish per sample time were bled from their caudal veins on 0, 10, 20 and 30 days post vaccination (dpv) with non-heparinised disposable syringes. Blood samples were also collected from the control group. Blood was transferred into micro centrifuge tubes and allowed to clot at room temperature for 1 h. Supernatant clot-free serum was collected following centrifugation at 10,000 rpm for 10 min.

Gut mucus antibody collection

The gut was cut open and the mucus was scrapped out using blunt end of a scalpel and transferred into the tube containing PBS. At each sampling point, ten fish (five from each treatment and control) were sampled.

Evaluation of immune response by MAb based ELISA

Antibody (IgM) in serum and mucus from the control and vaccine fed fish (Catla) corresponding to the different sampling points were quantified by MAb based ELISA (OD at 405 nm). Briefly microplates (Polystyrene F-bottom, Nunc, Germany) were coated with 10^{9} cells (100 µl well $^{-1}$) of heat inactivated *Aeromonas hydrophila*. The plate was blocked with 300 µl of PBS containing 0.05% of Tween (Merck, Darmstadt, Germany) and 1% milk powder (Himedia) and incubated in a box with wet tissues at 37^{O}C. The blocking solution was poured off and the plate was rinsed with distilled water and then washed with PBS-tween (0.05%). Serum and mucus samples 200 µl were added in the first row (starting dilution1:4) and 2 log dilutions were made by transferring 100 µl from one well to another containing 100 µl of PBS and incubated for 1.5 h at 37°C. The plates were washed again and, 100 µl of 1:250 MAb C5H3 (reacting to IgG of catla) for was added to the respective wells except negative control well (blank) and incubated for 1.5 h. The MAb was poured off and the plate was washed as mentioned before. Rabbit-antimouse-HRP (Sigma, USA 1:4000, 100 µl) was added and incubated for 1 h at 37^{O}C. The conjugated antibodies were poured off and the plate was washed again as mentioned above. Substrate (100 µl) of tetramethylbenzidine (TMB/H_2O_2) in distilled water (1:20) was added and incubated for 10 min. The reaction was stopped by adding 50 µl of 1 N H_2SO_4 and OD of the resultant blue colour developed was measured at 450 nm using ELISA reader (Bio-Tek instruments, inc. USA).

Protection with vaccine upon challenge

Fifteen, orally immunized catla (40±1 g) were used for protection studies. Catla was challenged on 30th day post vaccination, each fish was injected (IM) with 0.5ml of 18 h old culture of *A. hydrophila* at 10^{10} cells /g fish. Appearance of gross clinical lesions and mortality pattern if any, were observed for 15 d. Post challenge mortalities were recorded

for a period of 7 d. Only specific mortalities with *A. hydrophila* as determined by PCR were used in evaluating the potency of vaccine. Relative percent survival (RPS) was calculated according to Amend *et al.,* 1981.

RPS = [1-(% mortality of vaccinated group/ % mortality in control] × 100

Statistical analysis

Chi-square test was applied to test the dependence of survivability on vaccines; ANOVA followed by Dunnett's multiple comparison tests was employed for the vaccination group to compare between control and the other time points. Unpaired t-test was used for the comparison between the control and vaccination group at 30dpv.

RESULTS AND DISCUSSION

Oral vaccination of catla with biofilm of *A. hydrophila* and evaluation of immune response by MAb based ELISA

Catla maintained in circular fibre glass tanks were also fed with biofilm incorporated feed and the immune response was evaluated employing MAb based ELISA; Catla F2D9. Specific IgM in serum and mucus from all the treatments at different DPV were quantified (OD) at 450nm in an ELISA. Quantity of *A. hydrophila* cells coated in ELISA was uniform (10^9 cells /100µl) for both the vaccinated and non vaccinated fish groups. Compared to the control serum and mucus, biofilm vaccinated fish gave the highest OD for all the two species Catla (Fig.1 & 2). In catla, fed with *A. hydrophila* biofilm vaccine serum antibody increased with time reaching highest on 30dpv, Similar to serum antibody, mucus antibody increased with time in biofilm vaccinated catla, but impact was comparatively low OD. Similarly, impact of biofilm vaccine on mucus antibody was low compared to serum it might be due to no effective antibody present to detect mucosal antibodies. However biofilm oral vaccine gave a good trend of antibody production in the body.

Fig. 1: Serum antibody titre (OD at 405nm; data are shown as mean±SEM) in catla from control (a), Days post vaccination (b, c, d, e)

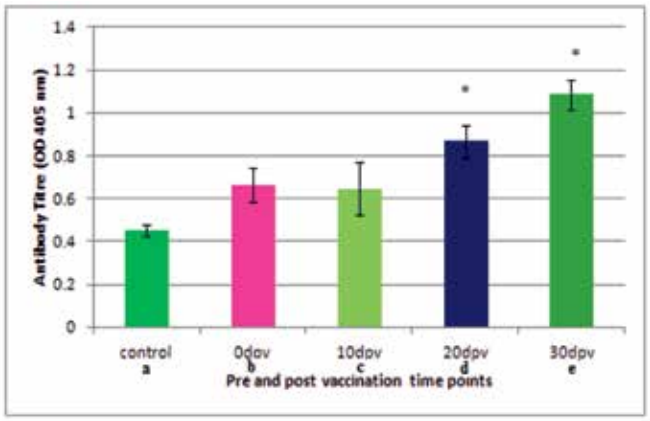
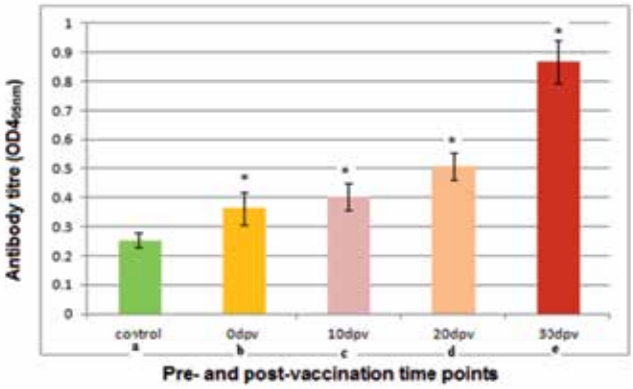


Fig 2: Gut mucus antibody titre (OD at 405nm; data are shown as mean±SEM) in Catla from control (a), Days post vaccination (b, c, d, and e)



Protection with biofilm vaccine upon challenge

Vaccinated catla showed an elevated protection to the homologous injection challenge. In unvaccinated control group survival was only 20 per cent compared to 86.66 per cent with biofilm vaccinated group. The protective immunity calculated as relative percent survival (RPS) was 83.32 for biofilm. Biofilm of bacteria are known for their resistance properties and are believed to protect oral vaccines in gut and they can act as very good vaccine carrier in oral immunization (Azad *et al,* 1997). Higher (86.66%) protection in BF vaccinated catla compared to control observed were in consistence with findings of Azad *et al,* 1999

also fed catla with biofilm vaccine (10^{10} CFU/g fish/ day for a period of 20 d to record RPS of 68.29 compared to 53.66 with free cell vaccine. A similar study involving a carnivore (*Clarias batrachus*) model by Nayak *et al.,* 2004 gave convincingly good results, i.e., 100 and 33.33 RPS for BF and control respectively followed by 20 d vaccination.

CONCLUSION

One of the challenges in the development of effective oral delivery is to protect the antigen from degradation due to acid and enzymes in the stomach, thereby enhancing the uptake, processing and presentation of the antigen to gut-associated lymphoid tissue at the mucosal level. Therefore, to protect fish from enter pathogenic agents, induction of mucosal immunity and especially an intestinal immune response, is very important. The present study showed that biofilm oral vaccine elicits antibody response at the mucosal level, although the titre may be low compared to that in serum there was a gradual increase in antibody indicating the presence of local immune response in the gut and hence an effective vaccination.

REFERENCES

Abelli L. Picchietti S, Romano N, Mastrolia L and Scapigliati G (1997). Immuno histochemistry of gut-associated lymphoid tissue of sea bass *Dicentrarchus labrax* (L.). *Fish Shellfish Immunol* **7**: 235–245.

Amend D F (1981). Potency testing of fish vaccines. In: Fish biologics: serodiagnostics and vaccines (ed. by D. P. Anderson and W. Hennessen). Karger, Geneva, pp. 447-454.

Azad I S, Shankar K M, Mohan C V and Kalita B (2000). Uptake and processing of biofilm and free-cell vaccines of Aeromonas hydrophila in Indian major carps and common carp following oral vaccination e antigen localization by a monoclonal antibody. *Diseases of Aquatic Organisms* **43**:103-8.

Azad I S, Shankar K M and Mohan C V (1997). Evaluation of an *Aeromonas hydrophila* biofilm for oral vaccination of carps. In: Flegel TW,McRae IH (eds) Diseases in Asian aquaculture III. Fish health section, Asian Fisheries Society, Manila, pp. 519-528.

Azad I S, Shankar K M and Mohan C V (1999). Evaluation of biofilm of *A. hydrophila* for oral vaccination of carps. *Dis Asian Aquaculture*. III: 181-185.

Azad I S, Rajendran K V, Rajan J J S, Vijayan K K and Santiago T C (2001). Virulence and histopathology of *Aeromonas hydrophila* (Sah 93) in experimentally infected tilapia, *Oreochromis mossambicus* (L.). *J Aquaculture in the Trop* **16**: 265-275.

Bondad-Reantaso M G, Subasinghie R P, Richard Arthur,and Shariff M (2005). Disease and health management in Asian Aquaculture. *Vety Parasitology* 1-24.

Danve B, Lissolo L, Mignon M, and Quentin-Millet M J (1993). Transferrin-binding proteins isolated from *Neisseria meningitidis* elicit protective and bactericidal antibodies in laboratory animals. *Vaccine*, **11**: 1214–1220.

Groove S, Johansen R, Reitan L J and Press C M (2006). Immune- and enzyme histochemical characterisation of leukocyte populations within lymphoid and mucosal tissues of Atlantic halibut (Hippoglossus hippoglossus). *Fish Shellfish Immunol* **20**: 693–708.

Hart S, Wrathmell A B, Harris J E and Grayson T H (1988). Gut immunology in fish: A review. *Dev and Comparative Immunology* **12**: 453–480.

Jenkins P G, Wrathmell A B, Harris J E and Pulsford A L (1994). Systemic and mucosal immune responses to enterically delivered antigen in *Oreochromis mossambicus*. *Fish Shellfish Immunol* **4**: 255–271.

Joosten P M, Avilés-Trigueros M, Sorgeloos P and Rombout J (1995). Oral vaccination of juvenile carp (Cyprinus carpio) and gilthead seabram (*Sparus aurata*) with bioencapsulated Vibrio anguillarum bacterin. *Fish Shellfish Immunol* **5**: 167–177.

Mclean E and Donaldson E M (1990). The absorption of biactive protein by the fish gastrointestinal tract: a review. *J Aquatic Anim Health* **2**: 1–11.

Nayak D K, Asha A, Shankar K M And Mohan C (2004). Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of *Clarias batrachus* – a carnivore model. *Fish Shellfish Immunol* **16**: 613–19.

Neuhaus H, Van Der Marel M, Caspari N, Meyer W, Enss M and Steinhagen D (2007). Biochemical and histochemical effects of perorally applied endotoxin on intestinal mucin glycoproteins of the common carp *Cyprinus carpio*. *Dis Aquat Org* **77**: 17-27.

Rowley A F, Hunt T C, Page M and Mainwaring G (1988). *Fish*. In: *Vertebrate Blood Cells* (eds A. F. Powley & N. A. Ratclie) Cambridge, UK, Cambridge University Press, pp. 19–27.

Schroers V, Van Der Marel M, Neuhaus H and Steinhagen D (2009). Changes of intestinal mucus glycoproteins after peroral application of *Aeromonas hydrophila* to common carp (*Cyprinus carpio*), *Aquaculture* **288**: 184-189.

Toida N, Hajishengallis G, Wu H Y and Russell M W (1997). Oral immunization with the saliva-binding region of *Streptococcus mutans* AgI/II genetically coupled to the cholera toxin B subunit elicits T-helper-cell response in gut-associated lymphoid tissues. *Infection & Immunity* **65**: 909–915.

Received on 15/06/2020 *Accepted on 20/08/2020*