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Development of Washed Cell Fowl Cholera Vaccine in Bangladesh

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Abstract: An experiment was conducted to develop washed cell fowl cholera (WCFC) vaccine with virulent avian *Pasteurella multocida* (PM 38) serotype 1 (X-73). A total of 20 Fayoumi birds of either sex of 10 weeks aged were divided into two groups as group A (immunized with washed cell fowl cholera vaccine) and group B (unvaccinated control). Primary vaccination was given through IM route in each birds of group A and booster dose was given through SC route after 15 days of primary vaccination. The presence of antibody against *P. multocida* was determined by slide agglutination test (SAT) and growth inhibition test (GIT). The degree of antibody levels of prevaccination and post vaccination sera were determined by passive haemagglutination assay (PHA). Sera mean PHA titres at 15, 21, 28 and 42 days post-vaccination in group A were 30.4±4.43, 46.4±6.06, 67.2±11.14 and 134.4±22.28 respectively. The present results revealed that WCFC vaccine worked satisfactory in terms of protection rate against Avian Pasteurellosis. It was also demonstrated that experimental WCFC vaccine conferred 80% protection against challenge infection when all chickens of control group failed to survive against challenge infection.

Key words: Washed cell fowl cholera vaccine, slide agglutination test, growth inhibition test

Introduction

Fowl cholera (FC) is a commonly occurring avian disease that infects all types of birds in Bangladesh. It usually occurs as an acute septicemia associated with high morbidity and mortality. Fowl cholera causes mortality about 25 to 35% in chickens of Bangladesh (Choudhury *et al*., 1985). Fowl cholera, caused by *Pasteurella multocida* which is mainly prevented by vaccination in Bangladesh. Alum precipitated formalin killed fowl cholera vaccine is produced in the Poultry Biologic Unit of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh and Livestock Research Institute (LRI) and oil adjuvant fowl cholera vaccine is also produced in LRI, Mohakhali, Dhaka. In Bangladesh two types of adjuvanted vaccines are used for the prevention of fowl cholera in chickens and ducks. Some reports on the immune response and efficacy of locally prepared fowl cholera vaccines in chickens have been made from Bangladesh (Choudhury *et al*., 1988; Mondal *et al*., 1988 a, b; Khan *et al*., 1994). Therefore the present study was under taken for the development of new washed cell fowl cholera (WCFC) vaccine in Bangladesh.

Materials and Methods

***Pasteurella multocida* vaccine strain:** *Pasteurella multocida* organisms were obtained from laboratory stock culture of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Birds: Twenty Fayoumi birds were selected for this study. Healthy 10 weeks older birds were purchased from the Bangladesh Agricultural University Poultry Farm, Mymensingh, Bangladesh.

Cultural examination of *P. multocida*: Cultural examination was conducted according to the standard method described by Cowan (1985). The collected organisms were inoculated in blood agar media, nutrient agar media, and MacConkey agar media to observe the growth characteristics of the organisms. Biochemical tests were also performed for confirmation of the organism according to the methods described by Cheesbrough and Cowan (1985).

Preparation of WCFC vaccines: Formaldehyde was added at the rate of 0.8% bacterial culture (5.6×10^7 CFU/ml) for WCFC vaccine preparation. Then, the cultural broth kept for WCFC vaccine preparation was centrifuged at 3500 rpm for 30 minute to settle down the cells. The sediment was washed with PBS for 3 times. Finally washed cell sediment was diluted with physiological saline as 100 ml volume for the production of WCFC vaccine. Later, 1 ml of treated bacterial culture was inoculated on blood agar media and incubated at 37^0C for overnight. The plate with no bacterial growth indicated complete inactivation and negative for bacterial contamination.

Experimental immunization: The experimental immunization was made according to procedure

described by Siddique *et al.* (1997). Fayoumi birds of layer breed were divided into two groups consisting of 10 birds in each A and B groups. The birds of group A vaccinated with experimentally prepared WCFC vaccine and the remaining group B served as control. The initial dose of vaccine was given to the birds of group A through IM route at the age of 10 weeks. These birds were revaccinated with same dose of vaccine through SC route after two weeks of primary vaccination as booster.

Collection of serum from the immunized birds: Five ml of blood was collected from the wing vein of all vaccinated chickens of group A without anticoagulant and was poured gently in the sterile glass test tubes. Blood samples were collected from the chickens at prevaccination and 15, 21, 28, 42 days post vaccination. Sera were separated from the blood and stored at -20°C in the sealed-capped vial until tested.

Determination of the humoral immune response: Humoral immune response was determined by the application of slide agglutination test (SAT), growth inhibition test (GIT) and passive hemagglutination assay (PHA). Antibody titres of the chickens that immunized with WCFC vaccine was determined by modified passive hemagglutination assay according to the method described by Tripathy *et al.* (1970); Siddique *et al.* (1997), with slight modification.

Protection test: For the protection test, 10 birds from vaccinated group A and 10 birds from control group were challenged after 15 days of boostering intramuscularly with 1 ml of virulent *P. multocida* (5.6 x 10^7 CFU/ml).

Results and Discussion

Fowl cholera is one of the important bacterial disease of chickens. It causes severe economic loss and hampers the development of poultry industry in the developing countries like Bangladesh. Vaccination of chickens is one of the most important methods of prevention of this disease. The protective efficacy of WCFC vaccine was thoroughly investigated in this experiment using passive haemagglutination (PHA) test and challenge experiment. Chung *et al.* (2001) and Boyce *et al.* (2000) suggested that capsulated strain of *P. multocida* should be selected as vaccine strain. They also reported that capsular strain was more antigenic and produced better immune response in chickens against fowl cholera. For this, a capsulated strain was selected for the preparation of WCFC vaccine in this experiment.

Slide agglutination test (SAT): In slide agglutination test, the serum of all immunized chickens of group A agglutinated with *P. multocida* and that indicated the positive slide agglutination test. Where as, the serum of control chickens did not agglutinate which indicated the negative slide agglutination test (Table 1). The results correlated with the study of Choudhury *et al.* (1985) that determined humoral immune response in vaccinated chickens by slide agglutination test. But the agglutination test did not clearly indicate the immune status of birds that were vaccinated and tested after challenge with homologous cultures (Heddleston and Watko, 1965).

Growth inhibition test (GIT): *Pasteurella multocida* strain treated with test serum was inoculated on blood agar and nutrient agar plates. After overnight incubation, no bacterial growth was observed on media which indicated positive GIT for vaccinated chickens. But there were growth on media incase of control samples which indicated negative GIT (Table 1).

Passive haemagglutination assay (PHA): Microplate PHA was conducted to determine the PHA titres of test sera samples collected from WCFC vaccinated and control birds used in this study (Table 2). Prevaccination PHA titres of sera samples of all vaccinated and control birds were found at a mean of $\leq$ 4.0±0.00 which was closely related with Mondal *et al.* (1988a,b). The post vaccination sera mean antibody titres after 15 days of primary vaccination in group A were 30.4±4.43. At 21 days postvaccination sera mean antibody titres in group A were 46.4±6.06 and at 28 days postvaccination sera mean antibody titres were 67.2±11.14. Two weeks after challenge infection the mean antibody titres of the immunized birds of group A which survived challenge exposure were recorded 134.4±22.28. Therefore, the mean antibody titres recorded in this experiment indicated that IM route of vaccination induced better results in respect of protection against experimental challenge infection and higher antibody titre than SC route of vaccination. The findings of this experiment partly correlated with the results of Leonchuk and Tsimokh (1977) who reported that the immunogenicity of the vaccine depended on the route of vaccination and IM route gave stronger and more lasting immunity than SC route. The chickens received booster dose of vaccination which induced significantly higher antibody titres than the chickens of primary vaccinated groups. Wu *et al.* (1986) observed that two inoculations provided better immunity than a single inoculation. Onet *et al.* (1994) reported that the level of specific antibodies increased at least 3 times by twice vaccinations.

Survivability of chickens at challenge experiment: In post-challenge observation, two chickens of group A and all chickens of group B were died. So survivability

Table 1: Results of the slide agglutination test (SAT) and growth inhibition test (GIT)

Parameters	Name of vaccine	Group	Route of vaccination		Results		
			Primary vaccination	Booster vaccination	Primary vaccine	Booster vaccine	Post challenge
Slide agglutination	WCFC vaccine	A	IM	SC	+	+++	+++
test (SAT)	Control		B		-	-	-
Growth inhibition	WCFC vaccine	A	IM	SC	+	+	+
test (GIT)	Control		B		-	-	-

Legend: IM = Intramuscular route, SC = Subcutaneous route.
Slide agglutination test (SAT): + = Agglutination, = No agglutination; Growth inhibition test (GIT): + = Unable to grow in culture media, - = Able to grow in culture media

Table 2: PHA titers of sera of vaccinated Fayoumi chickens and survivability of vaccinated and control chickens of challenge experiment

	WCFC vaccine	
Route of vaccination		
Primary vaccination	IM	Control
Booster vaccination	SC	
Group	A	B
No. of chickens	10	10
Prevaccination PHA titre	≤ 4	≤ 4
Post vaccination sera PHA titres (mean PHA titres with SE)		
15 DPV sera	30.4±4.43	-
21 DPV sera	46.4±6.06	-
28 DPV sera	67.2±11.14	-
42 DPV sera	134.4±22.28	-
Survivability (%)	8 (80%)	0 (0%)

WCFC vaccine = Washed cell fowl cholera vaccine, IM = Intramuscular route, SE = Standard error
DPV = Days post vaccination, SC = Subcutaneous route

rate of WCFC vaccinated chickens was 80% (Table 2). Supar *et al.* (2002) observed protection rate was 50-75% and Avakian *et al.* (1989) showed a survival rate of 86% and Lin *et al.* (1986) observed the rate of protection ranged from 70 to 100%.

From the present study, it was found that the protective efficacy of the WCFC vaccine was 80% and the IM route of vaccination was more effective than SC route of vaccination. So, we developed washed cell fowl cholera vaccine first time in Bangladesh.

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