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IMMUNE RESPONSE OF FOWL CHOLERA VACCINE PRODUCED AT BANGLADESH AGRICULTURAL UNIVERSITY IN FARM LEVEL

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ABSTRACT

The work was carried out to determine the immunogenicity of fowl cholera vaccine (FCV) produced by Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) at Bangladesh Agricultural University (BAU). A total five hundred of seven-week old Hy-sex chickens (both white and brown) were vaccinated @ 0.5 ml of 2.93×10^8 CFU through subcutaneous route in each selected groups such as A1, A2 and A3; and B1, B2 and B3. Booster dose was provided at 13 weeks of age in group A3 and B3. Group C was kept as unvaccinated/control. Postvaccination sera were collected at different time schedule from all the groups of birds and antibody against fowl cholera were determined by Passive haemagglutination (PHA) test. At 4 weeks of primary vaccination (11 weeks aged birds) the mean PHA titres of sera were 96.00±34.21 and 96.00±34.21in group A1 and B1 respectively. On the other hand mean PHA titres at 5-weeks following vaccination (12 weeks aged birds) were 88.00±33.12 and 96.00±34.21in group A2 and B2, respectively. After 4 weeks of booster vaccination the mean PHA titres were 104.00±33.12in A3 and 104.00±33.12 in B3 group. The mean PHA titres in chickens of unvaccinated control group C was <4±0.00.Fowl cholera vaccine prepared at LPVRPC induced a good level of immunity at the farm level and it was also demonstrated that booster (secondary) vaccination is essential to develop protective level of immunity.

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INTRUDUCTION

Poultry industry is an excellent agribusiness with its tremendous development during the last decades (1996-2006) in Bangladesh (Rahman, 2003). However, a number of infectious diseases of different etiologies such as bacteria, virus, fungi, mycoplasma etc. are found to be the most leading causes of economic loss often discouraging poultry rearing in this country (Das *et al.,* 2005). Among the bacterial diseases, fowl cholera (FC) is a major threat to the poultry industry. It is a contagious acute fatal septicemic disease of various domestic and wild bird species (OIE, 2004).Vaccination is practiced as preventive measures in Bangladesh like other countries of the world to reduce the incidence of the disease. Michael *et al.,* (1979) suggested that a local strain of higher immunogenic value should be selected as vaccine strain for preparation of a prophylacticbacterin. Fowl cholera vaccines (FCV) are made available in Bangladesh by a number of Pharmaceutical companies, Livestock Research Institute (LRI) of Department of Livestock Services (DLS) and Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) (erstwhile known as poultry biologics unit) of Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU). The volume of FCV, an adjuvantedkilledbacterin produced at LPVRPC is on increasing demand. For this, it was thought plausible to investigate the on farm immunogenicity that is produced by this vaccine. The present study was undertaken with following specific object: To isolate and identify *p.multocida* from naturally infected chicken and to determine on farm immunogenicity of adjuvant fowl cholera vaccine produced by LPVRPC.

MATERIALS AND METHODS

Experimental design

The present research was conducted during the period of July 2010 to December 2010.Fowl cholera vaccine as prepared by LPVRPC at BAU was investigated immunogenicity for measured in term of production of antibody in vaccinated chicken determined by PHA test. Seven weeks aged Hy-sex chicken (white and brown) were selected for these experiment. These chicken were divided into two groups- (Vaccinated group and Unvaccinated group) under this vaccinated group collection of blood prior to vaccination from Hy-sex brown A and Hy-sex white B. Primary vaccination were done at 6 weeks of age in Hy- sex brown A and Hy-sex white B. Post primary vaccination bleeding occurred at 10 weeks of age (A_1 and B_1) or 30 days after primary vaccination and Prebooster bleeding at 11weeks of age (A_2 and B_2) or 37 days after primary vaccination. Booster vaccination of Hy-sex brown A and Hy-sex white B were done at 12 weeks of age. Post booster bleeding at 16 weeks of age (A_3 and B_3) or 74 days after primary vaccination. Then collection of sera and PHA test was performed.

Passive haemagglutination (PHA) test

The test was used to determine the antibody titers in chickens and was performed according to the methods described by Tripathy*et al.* (1970a), the sensitivity of PHA test depends upon the use of soluble antigens. In this case, capsular antigens (soluble antigen) of *P. Multocida* were coupled to chemically modified erythrocytes (sheep erythrocytes) and then antigen-coated erythrocytes readily react with specific antibodies and results in haemagglutination.

Microtitre plate method

The procedure of the PHA test was followed according to the method described by Tripathy *et al*., (1970).An amount of 50 µl of PBS was first poured in each well up to 8th well of horizontal row of microtitre plate. 50 µl of test serum was added in the 1st well. Two fold dilutions of serum ranging from 1: 2 to 1: 256 were made by transferring 50 µl of the mixture from the 1stwell to 2nd well and thus continuing successively up to the 8th well from where an excess amount of 50 µl of the mixture was poured off. A volume of 50 µl 0.5% somatic antigen sensitized hRBC was taken in each of the eight wells. The Control system, horizontal row of microtitre plate (9th well: equal volume of 50 µl of normal serum and PBS and10th well: equal volume of 50 µl of sensitized tanned RBC and PBS). The content of the wells of the test system and control were mixed by gentle agitation of the microtitre plate and kept at room temperature for 4 to 5 hours.

The PHA titre was the highest dilution of test sera were complete haemagglutination occur due to the reaction of specific antibody and antigen sensitized tanned HRBC. The results were recorded by deposition of a diffuse thin layer of clumping of RBC on the bottom of the wells, which indicated HA positive, and a compact buttoning with clear zone indicated HA negative. The reciprocal of the highest dilution of sensitized tanned HRBC was considered as titre of the serum.

RESULTS

PHA antibody titer

The PHA antibody titres of the serum obtained from the chicken's belonging to group A1, A2, A3, B1, B2 and B3 are presented in Table-1. The pre-vaccination mean PHA titer were <4±0.00 in sera of chickens of all groups. After 4 weeks of primary vaccination the mean PHA titres were 96.00±34.21 in A1 and 96.00±34.21 in B1 group. Prebooster vaccination PHA titres were 88.00±33.12 in A2 and 96.00±34.21in B2 group. After 4 weeks of booster vaccination the mean PHA titres were 104.00±33.12 in A3 and 104.00±33.12 in B3 group. The mean PHA titres in chickens of unvaccinated control group C were <4±0.00.

Table 1. Mean PHA titres of sera of chickens vaccinated and revaccinated with fowl cholera vaccine through SC route as determined by *t*-test

Groups	Schedule	PHA titer (Mean ±SE)	P value
A1	Primary vaccination	96.00±34.21	
A2	Prebooster vaccination	88.00±33.12	
A3	Postbooster vaccination	104.00±33.12	
B1	Bleeding at post Primary vaccination	96.00±34.21	0.934
B2	Bleeding at Prebooster vaccination	96.00±34.21	NS
B3	Bleeding after booster vaccination	104.00±33.12	

Level of significance: NS (P>0.05); Legends: PHA=Passive hemagglutination
Mean= Geometric mean of 8 birds; SE = Standard error. NS=Not significant

DISCUSSION

Vaccination is one of the most important methods of prevention of Fowl cholera. This study was undertaken proximately with a view to evaluate the immune responses following usual schedule of vaccination at farm. The immunogenicity was studied by the determination of the serum antibody titre by passive haemagglutination (PHA) test suggested by Carter (1955). PHA test was conducted to determine the humoral immune response of the serum of chickens having been inoculated at 7 weeks aged birds as per the method described by Carter (1955), and Chang (1987) but slight modification was done as suggested by Mondal*et al.,* (1988), Sarker*et al.,* (1992), Siddque*et al.,* (1997), Supar *et al.,* (2002), Akand *et al.,* (2004) and Chowdhury (2008).The prevaccination PHA titres of sera samples of all vaccinates and control birds was found with a mean of <4.00±0.00 that was closely related with Mondal*et al.,* (1988). After 4 weeks of primary vaccination the mean PHA titres were 96.00±34.21 in A1 and 96.00±34.21 in B1 group. Prebooster vaccination PHA titres were 88.00±33.12 in A2 and 96.00±34.21in B2 group. After 4 weeks of booster vaccination the mean PHA titres were 104.00±33.12 in A3 and 104.00±33.12 in B3 group. The mean PHA titres in chickens of unvaccinated control group C were <4±0.00.In this present study, it was observed that group A3 and B3 produced comparatively slightly better immune response than group A1, A2, B1 and B2 and group A1 and A3 produced comparatively better immune response than group A2. There were several limitations of this study such as antibody titres could not determine and compared by ELISA.

Due to short study period immune response of vaccine could not studied elucidated through various routes. *P. multocida* used as antigen in case of microplate agglutination test were identified tentatively by cultural, staining and biochemical test. That could have been identified by molecular characterization such as polymerase chain reaction (PCR).

In conclusion, Fowl cholera vaccine produced by Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) at BAU induced a good level of antibody in layer chicken at farm level. The vaccine produced higher level of antibody when booster dose was given after primary vaccination.

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