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FIELD INVESTIGATION ON THE EFFICACY OF *SALMONELLA* VACCINE PREPARED AT BANGLADESH AGRICULTURAL UNIVERSITY

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ABSTRACT

The research work was performed to investigate the immunogenicity of Salmonella vaccine produced at LPVRPC, BAU Mymensingh. The vaccination was performed at the Phenix Hatchery Ltd. Gazipur in Hy-sex brown and HY-sex white chicken designated as group A and group B respectively. Group A and B were subdivided into A1, A2, A3, B1, B2 and B3 groups containing eight birds each. Group C was maintained as unvaccinated control. Birds were immunized following schedule of the LPVRPC. Each bird was vaccinated SC at six weeks of age followed by a subsequent booster dose after 45 days. After four weeks of primary vaccination the mean PHA antibody titres were 96.00±34.21 in group A1 and 96.00±34.21 in B1 group. Prebooster vaccination the mean PHA antibody titres were 88.00±33.12 in group A2 and 88.00±33.12 in B2 group. At four weeks of booster vaccination the mean PHA antibody titres were 104.00±33.12 in group A3 and 104.00±33.12 in B3 group. The mean ± PHA antibody titre in chickens of group C was ≤ 4.0±0.00. Salmonella vaccine prepared at (LPVRPC) Department of Microbiology and Hygiene, BAU induces satisfactory level of antibody in chickens determined by PHA test conducted in an on-farm study of layer chickens.

Key words: Immunogenicity, salmonella, vaccine, antibody, PHA titre

INTRODUCTION

Poultry industries in Bangladesh have been facing many constraints. Outbreak of several devastating diseases constituting major constraints is causing economic loss and discouraging poultry rearing in this country (Das *et al.*, 2005). Surveys on both breeding flocks of commercial broiler and layer in major poultry raising belts in and around Dhaka and Gazipur districts in Bangladesh were conducted by Saleque *et al.* (2003) reported that the bacterial, viral mycoplasmal, protozoal, parasitic, fungal and non-infectious diseases cause 45%, 17% 12.4%, 6.6%, 4.5%, 1.5% and 12.4% of total death of poultry respectively. Among the infectious diseases, prevalence of Salmonellosis was recorded as 16.9% in breeding flock (Saleque *et al.*, 2003). Avian Salmonellosis may occur either as acute or chronic form by one or more member of genus *Salmonella*, under the family *Enterobacteriaceae* (Hofstad *et al.*, 1984). There are mainly two types of S*almonella* spp. namely *S. gallinarum* and *S. pullorum* that cause fowl typhoid and pullorum disease respectively. These two species of *Salmonella* are very important in poultry health because they are responsible for massive destruction. The major emphasis for preventing infections is to avoid the introduction of pathogens into the farms by enhancing bio-security (Gifford *et al*., 1987) along with vaccination. The vaccines available are both live (usually based on the Houghton 9R strain) and bacterins (killed/inactivated vaccine). *Salmonella* vaccines of both live and killed are imported and marketed in Bangladesh by different commercial companies. It is necessary to monitor purity, sterility, safety and protective efficacy of any biological or vaccines by respective controlling agency or an alternative agency prior to introducing it within the country for an extensive field use. Poultry Biologics unit (PBU) recently renamed as "Livestock and poultry Vaccine Research Development Centre (LPVRPC) incorporated with its parent organization the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU) Mymensingh produces vaccine against *S. pullorum* and *S. gallinarum* which are distributed for field use. The vaccine is tested for its purity, safety, potency and efficacy in the laboratory and field as well.With the expansion of volume of production of vaccine it has become imperative that the vaccine be tested while being used in the farm. The present piece of research was undertaken with the following objectives: (i) Isolation and identification of *Salmonella* spp from infected chicken reared in farm and (ii) On farm investigation of immunogenicity of *Salmonella* vaccine produced by LPVRPC.

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MATERIALS AND METHODS

Experimental design

The present research was conducted during the period of July 2010 to December 2010 *Salmonella* 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. Five 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), Chowdhury *et al.* (1987), Mondal *et al.* (1988), Sarker *et al.* (1992) Siddque *et al*. (1997), Supar *et al.* (2002), Akand *et al.* (2004) and Chowdhury (2008).

Principle of the test

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.

Collection and preparation of 2.5% horse red blood cells (HRBC)

Blood was collected from the right jugular vein of a normal adult horse with sterilized syringe and needle containing 5 ml of Alsever's solution for 10 ml of blood. The collected blood was centrifuged in graduated centrifuge tube at 1500 rpm for 10 minutes. The supernatant was then pipetted off and the blood cells were re-suspended with PBS and then centrifuged. The process was repeated for at least three times for washing the blood cells. During last washing the cells were maintained in PBS up to 15 minutes and then centrifuged at 2000 rpm for 10 minutes to obtain the packed cells. After final washing, the sedimentary blood cells were diluted with PBS to make 2.5% suspension of the blood cells and preserved at 4 to 8^0C in a refrigerator.

Tannic acid treatment of horse red blood cells

Five milliliters of 2.5% HRBC and 5 ml of 1: 20,000 dilution of tannic acid was taken in a test tube and mixed thoroughly. The mixture was then incubated at 37°C for 10 to 15 minutes in water bath according to the methods of Tripathy *et al.* (1970a and 1970b). The cells were centrifuged at 2000 rpm for 10 minutes; the sediment was then washed with PBS. Washed tanned HRBC was again diluted to make 2.5% suspension with PBS and used for the test.

Sensitization of somatic antigen with tannic acid treated horse red blood cells

Three ml of sensitized HRBC (2.5%), 1 ml of somatic antigen (1:10 dilution) and 8 m1 PBS were mixed together. This mixture was incubated at 37°C for 20 to 30 minutes. After sensitization, the cells were centrifuged at 1500 rpm for 10 minutes, then the supernatant fluid was discarded and the sedimentary HRBC was collected and diluted with 1% normal rabbit serum diluents (NRSD) at the ratio of 1: 4. This was then mixed thoroughly and kept at room temperature for an hour and centrifuged for 10 minutes. The cells were re-suspended in 1% NRSD to make 0.5% sensitized cells for use in microtitre plate method and stored at 4° C until used (Tripathy *et al.,* 1970a and 1970b).

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 1st well 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

Microplate PHA test was conducted to determine the antibody titres of test sera collected from the vaccinated and unvaccinated control chickens at different stages of this experiment and the titres are calculated and presented as mean ± standard error. The prevaccination mean PHA antibody titre was $\leq 4.0\pm0.00$ in chickens of all groups. After four (4) weeks of primary vaccination t he mean PHA antibody titres were 96.00±34.21 in group A_1 and 96.00±34.21 in group B_1. Prebooster vaccination mean PHA antibody titres were 88.00±33.12 in group A_2 and 88.00±33.12 in group B_2. After four (4) weeks of booster vaccination the mean PHA antibody titres were 104.00±33.12 in group A_3 and 104.00±33.12 in group B_3. The mean ± SE PHA titre of viccnated birds are presented in the following table.

Table 1. Mean PHA titre with standard error of sera of chickens vaccinated with *Salmonella* vaccine prepared at BAU

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Group	Pre- vaccinated PHA titre of all vaccinated and control birds (Mean±SE)	Post Vaccination PHA titre of vaccinate group (Mean ±SE)	P value
A_1	<4±0.00	96.00±34.21	
A_2	<4±0.00	88.00±33.12	0.869
A_3	<4±0.00	104.00±33.12	
B_1	<4±0.00	96.00±34.21	
B_2	<4±0.00	88.00±33.12	NS
B_3	<4±0.00	104.00±33.12	

NS= Statistically Not Significant, **means p≥ 0.05

DISCUSSION

Vaccination is one of the most important methods of prevention of Salmonellosis. In Bangladesh only a good number of commercial companies import *Salmonella* vaccine for marketing. Such imported vaccines are used without any field trial, which should have been mandatory for testing the efficacy. It is necessary to study the immunogenicity of vaccines and to suggest the more effective schedule of vaccination. The immunogenicity was studied by the determination of the serum antibody titre by PHA test. PHA test was conducted for determination of antibody titre of the sera of vaccinated and unvaccinated chickens as per the method described by Schlink *et al.* (1979). The pre-vaccinated PHA titre of sera samples of all vaccinated chickens were recorded as $\leq 4\pm00$ (Table 7) which was closely related to the findings of Ferdous (2008). The antibody titres in this study ranges from 64 to 128 after 30 days of boostering. The lowest antibody titre was 64 and the highest antibody titre was 128. This finding is similar to Bhattacharya *et al.* (2004), who observed that the antibody titre induced by primary vaccination first reached to a peak and declined gradually thereafter. Rahman *et al.* (2005) also used rapid serum plate agglutination test and tube agglutination test to determine the antibody titre. The authors stated that antibody titres of the vaccinated birds increased quickly and reached peak four (4) weeks after vaccination.

The mean value of antibody titres of group A_1, A_2, A_3, B_1, B_2 and B_3were 96.00±34.21, 88.00±33.12, 104.00±33.12, 96.00±34.21, 88.00±33.12, 104.00±33.12 respectively. The highest Mean ± SE titres of was 104.00±33.12 in group A_3at 30 days after booster vaccination and the highest Mean ± SE titres of was 104.00±33.12 in group B_3 at 30 days after booster vaccination.

These results were in agreement with the statement of Rahman *et. al.*, (2005), who found that the antibody titre reached peak at 4 weeks vaccination in chickens. In this present study, it was observed that in group A, the sub group A_3 produced comparatively better immune response than A_1 and A_2. In group B, the subgroup B_3 produced comparatively better immune response than B_1 and B_2. In group A_3 sera sample showed higher PHA titer when booster dose was given at 45^{th} days after primary vaccination. In group B_3 sera sample showed higher PHA titer when booster dose was given at 45^{th} days after primary vaccination. There were several limitations of this study such as antibody titres could not determined by ELISA. Due to short study period immune response of vaccine could not be studied through various routes. Moreover, the identified *Salmonella* spp (cultural, staining, biochemical test) could not be confirmed further by molecular study. Limitations which we have to consider for the different routes of vaccination could be used and different age of birds could be considered for determination of protective efficacy. As the continuation of the present study, the following research works may be undertaken in future that confirmation of *Salmonella* isolates at species level by molecular assays and determination of protective efficacy by using different routes of vaccination.

In conclusion, The suspected disease of chicken at Phenix Hatchery Ltd was caused by *Salmonella* spp. *Salmonella* vaccine prepared at (LPVRDC) Department of Microbiology and Hygiene, BAU, Mymensingh induces good level of antibody in chickens determine by PHA test conducted in an on farm study of layer chickens.

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