J. Bangladesh Agril. Univ. 7(2): 295–299, 2009 **ISSN 1810-3030**

Efficacy study of bio-typhoid® vaccine against fowl typhoid in backyard layer chicken

M. Z. Uddin, M. S. R. Khan, F. Begum[1], M. A. Mannan
Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh and [1]Duck Plague Vaccine Section, Veterinary Training Institute, Mymensingh, Bangladesh

Abstract

The study was performed with a view to isolate and identify a virulent strain of *S. gallinarum* and determine the purity, safety and efficacy of BIO-TYPHOID® vaccine. A total of 40 backyard layer chicken were used for this study where Group A was used for experimental vaccination, Group B kept as control and Group C was used for calculating virulent *S. gallinarum* challenge dose. Primary and secondary vaccination was carried out at 40 days and 110 days of age, respectively. Blood samples were collected to obtain the sera after vaccination from both vaccinated and unvaccinated control group and antibody titres were determined by Microplate agglutination test. The antibody titre increased in primary vaccination up to days 56 days post vaccination (DPV) and then decreased gradually. The highest antibody titre (Mean ± SE) 384.00 ± 42.67 was obtained at 91 DPV (21 days later of secondary vaccination) and maintained up to 98 DPV. Safety test was done by inoculating mice and purity test of the vaccine was done by inoculating on to Blood Agar media. The efficacy of BIO-TYPHOID® vaccine was recorded as 90% which was determined by challenge infection with 0.1 ml of $5x10^5$ CFU virulent *S. gallinarum*. Results of this study revealed successful protections by BIO-TYPHOID® vaccine.

Keywords: BIO-TYPHOID® vaccine, Chicken, Efficacy, Fowl Typhoid

Introduction

Salmonellae in poultry have worldwide economic and public health significance. It may transmit from infected birds, their faeces and eggs, ingestion of contaminated food or water and contact transmission is most common. *S. gallinarum* can be detected serologically using macroscopic tube agglutination test, rapid serum test, stained antigen whole blood test or microplate agglutination test (Shah *et al.,* 2001). Control of Salmonella infections in poultry farms is by good farming and hygienic practices and the testing and removal of infected flocks from production (Purchase *et al.,* 2008). Vaccination against *S. gallinarum* is commonly used as preventive measure by both live (usually based on the Houghton 9R strain) and bacterins (killed/inactivated vaccine) are available (Lee *et al.,* 2007). In Bangladesh, both live and killed FT vaccines are commonly used for preventing Salmonellosis in chicken. Before introducing any biologics in a country, it must be evaluated for its purity, safety and efficacy by the respective controlling agency. Unfortunately, in developing countries like Bangladesh sometimes it would not be possible to perform such type of works. A preliminary study of BIO-TYPHOID® (FT vaccine imported and marketed by Advance Animal Science Company Limited) was conducted by Ferdous (2008) covering only the immunogenicity of the vaccine. Hence, a thorough study of FT vaccine covering purity, safety and efficacy in backyard layer chickens in order to examine the feasibility of using BIO-TYPHOID® vaccine.

Materials and Methods

Study place

The study was carried out in the experimental animal sheds and laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh-2202, from June 2008 to April 2009.

Culture media, chemicals and reagents

The reagents used during the study were physiological saline solution, phosphate buffered saline which were prepared as per the procedure suggested by Siddique *et al.,* (1997), 10% formalin, alcohol, sefranin, Salmonella Shigella (SS) agar media and selenite broth were also prepared as per the suggestion by Carter *et al.,* (1979).

Experimental chicken, mice and FT vaccine

A total of 40 backyard layer chicks were collected from the local market and healthy suckling day-old mice were used in this study. An inactivated FT vaccine BIO-TYPHOID® used in this experiment was imported and marketed by Advance Animal Science Company Limited. The vaccine was purchased from the local market and stored at 4-8°C until use.

The chickens (N=40) were divided into three groups. Group A (n=10) was used for experimental vaccination, group B (n=10) was maintained as unvaccinated control chicken and Group C consists of 20 chicken for calculating challenge dose.

The chicken of group A were vaccinated with BIO-TYPHOID® FT vaccine, where 1st dose of vaccine was administered at 40 days of age and 2nd dose was administered at 110 days of age. To determine the antibody response against the vaccines, blood sample of the chicken were collected at every 7 days interval up to day 98 of 1st vaccination. Sera were prepared from blood samples following the method of Shah *et al.,* (2001) to determine the antibody titres by microplate agglutination test. Efficacy of BIO-TYPHOID® vaccine on backyard layer chicken was determined by virulent challenge exposure of *S. gallinarum.*

Estimation of Colony Forming Unit (CFU) and challenge dose was prepared by the method of Heddleston and Reisinger (1960) using various combination of CFU/ml of *S. gallinarum* bacteria. Purity test was performed by the inoculation of FT vaccine on to Blood Agar (BA) media and the safety test was carried out through mice inoculation.

Serological analysis

The serological analysis was conducted through slide agglutination and microplate agglutination test by the method of Wambura *et al.,* (2006) and Schlink *et al.,* (1979) respectively to determine the presence of specific antibody against *S*. *gallinarum.*

Challenge and post-challenge observation of chicken

Chicken of both vaccinated and unvaccinated control groups were subjected to challenge exposure by intravenous inoculation in the jugular vein and were observed frequently up to one to four weeks for any clinical signs and symptoms. The clinical findings of both vaccinated and unvaccinated chicken were observed and recorded every 6 hours intervals following the procedure of Collins and Carter (1972). The procedure of Matsumoto and Helfer (1977) was followed for the isolation of the bacteria after 15 days of challenge exposure

Results and Discussion

The present study was undertaken to determine the purity, safety and efficacy of BIO-TYPHOID® FT vaccine in backyard layer chicken. Immunogenicity of the vaccine was studied by the determination of the serum antibody titre with Microplate Agglutination test.

Purity test was done by inoculating of FT vaccine on to BA media and incubated for 48 hours at 37^0C in incubator. It was observed that the vaccines did not exhibit the growth of any aerobic or anaerobic organisms, which indicated the vaccine as pure and similar report was found from Heddleston and Reisinger (1960).

The safety test was carried out following the method of Dorsey (1963). Five mice were inoculated subcutaneously (S/C) with 0.2 ml of vaccine. The inoculated mice remained alive and healthy during observation which indicated the vaccine as safe for further use.

In slide agglutination test, the sera of all vaccinated chicken of group A showed agglutination with *S. gallinarum* that indicated the positive slide agglutination test. Whereas, the serum of all unvaccinated control chicken of the group B did not agglutinate, which indicated negative result that is similar to Haider *et al.*, (2007).

Plate-1. Mice without showing any clinical signs after vaccination with experimental FT vaccine BIO-TYPHOID®

Plate-2. The chickens of group B showed the typical signs of FT in the post challenge observation

The prevaccinated plate agglutination titre of sera samples of all the vaccinated and unvaccinated chicken were found with a mean value of ≤4±00 which is closely related to the findings of Mandal *et al.,* (1988).

Chicken of group A, 0.5 ml of BIO-TYPHOID® vaccine was administered through subcutaneous route where the first dose was on day 40 and second dose on day 110 of age. Blood samples were collected from chicken up to 138 days of age (at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 DPV).

In group A, the lowest post primary vaccination titres which ranged from 16 to 32 was obtained at day 7 and the highest post primary vaccination titres which ranged from 64 to 128 was obtained at 21 DPV and maintained up to day 56 DPV, then started to decline before secondary vaccination and the highest post secondary vaccination titre ranged from 256 to 512 was obtained at 91 DPV (21 days after secondary vaccination) and maintained up to day 98 DPV (28 days after secondary vaccination) when the age of chicken was 138 days (Table 1).

Table 1. Results of Microplate Agglutination antibody titre of sera of chicken in group A vaccinated with BIOTYPHOID® FT vaccine

TagNo.	Antibody titre at days post vaccination (DPV)													
	First dose at 40 and second dose at110 days of age													
	Primary vaccination									Secondary Vaccination				
	7	14	21	28	35	42	49	56	63	70	77	84	91	98
A_1	32	64	64	128	64	128	128	64	64	64	128	256	512	256
A_2	16	32	64	64	128	64	64	128	64	32	64	128	256	256
A_3	32	64	128	128	128	64	64	64	64	64	128	256	512	512
A_4	16	32	128	128	64	64	64	64	64	64	128	256	256	256
A_5	16	16	64	64	64	128	128	64	64	32	64	128	256	256
A_6	32	32	64	128	128	64	64	64	64	64	128	512	512	512
A_7	32	32	64	64	64	64	128	128	128	64	128	256	512	256
A_8	16	32	128	64	64	64	128	64	64	32	64	128	256	256
A_9	16	64	64	128	128	64	64	64	64	64	128	256	256	256
A_{10}	16	32	64	64	64	128	64	64	64	32	64	128	512	512

The result showed that antibody level was higher when induced by BIO-TYPHOID® administered in the chicken of group A. Antibody level decreased gradually up to second vaccination. BIO-TYPHOID® produced lower level of antibody but induced a longer lasting immune response. Similar findings were also reported by Lee *et al.,* (2007).

The sera from chicken were collected at 7, 14, 21, 28, 35, 42, 49, 56, 70, 77, 84, 91 and 98 DPV. All those samples were subjected to Microplate Agglutination test. The mean values of agglutination titres of the sera collected from the chicken of group A (Mean±SE) were 22.40±2.61, 40.00±5.47, 83.20±9.78, 96.00±10.67, 89.60±10.45, 83.20±9.78, 89.60±10.45, 76.80±8.53, 70.40±6.40, 51.20±5.23, 102.40±10.45, 230.40±37.20, 384.00±42.67 and 332.80±39.10 respectively (Table 2).

Table 2. Results of post vaccination antibody titres in Mean±SE of chicken in group A vaccinated with BIO-TYPHOID® Fowl typhoid vaccine

Age of vaccination		Prevaccination antibody titre (Mean±SE)	Days post vaccination (DPV)	Antibody titres (Mean±SE) Group A (Tag no.A_1 to A_{10})
First dose	Booster dose			
40 days	110 days	≤4±0	07	22.40±02.61
		≤4±0	14	40.00±05.47
		≤4±0	21	83.20±09.78
		≤4±0	28	96.00±10.67
		≤4±0	35	89.60±10.45
		≤4±0	42	83.20±09.78
		≤4±0	49	89.60±10.45
		≤4±0	56	76.80±08.53
		≤4±0	63	70.40±06.40
		≤4±0	70	51.20±05.23
		≤4±0	77	102.40±10.45
		≤4±0	84	230.40±37.20
		≤4±0	91	384.00±42.67
		≤4±0	98	332.80±39.10

The highest antibody titres of group A (Mean ± SE) 384.00±42.67 was obtained on 91 DPV or 21 days after secondary vaccination, when the age of the chicken reached 131 days. Here, in chicken of group A, vaccinated with BIO-TYPHOID® FT vaccine with the first dose at 40 days and second dose at 110 days of age, the antibody titre reached peak at 91 DPV which is closely similar to the findings of Rahman *et al.,* (2005).

The variation in the antibody titres might be due to the quality of the vaccines used, vaccination method, age of vaccination etc. Agglutination formation began more quickly and the immune response was greater with increasing age and persisted longer in the blood (Vodas 1978). Agglutination formation against *S. gallinarum* in chicken was directly correlated with age.

A total of 20 chicken at group C used for calculating the challenge dose. Group C was divided into four sub-groups such as C_1, C_2, C_3 and C_4 where each sub-group possessed 5 chicken. $5x10^5$/0.1 ml CFU, $5x10^4$/0.1 ml CFU and $5x10^3$/0.1 ml CFU of virulent *S. gallinarum* challenge dose were given to the sub-group C_1, C_2, and C_3, respectively. Sub-group C_4 was kept as control. The infectivity or death of chicken was observed after challenge exposure in sub-group C_1, C_2, C_3; five, two and one chicken were dead, respectively out of 5 chicken. Survivility after challenge exposure in sub-group C_1 was zero which indicates the dose of *S. gallinarum* was highly virulent. Sub-group C_2 was moderately and C_3 was less virulent. Highly virulent S*. gallinarum* challenge dose ($5x10^5$/0.1 ml CFU) was used for this study to determine the efficacy of BIO-TYPHOID® vaccine.

Challenge infection was given to the chicken of both vaccinated and unvaccinated control group. Among the 10 chicken of group A one was found to be dead while nine chicken resisted with the virulent challenge exposure against *S. gallinarum* organism. From the 10 chicken of unvaccinated control group B all chicken were recorded as dead which was similar to Purchase *et al.,* (2008). This results revealed that the commercially available BIO-TYPHOID® (FT vaccine imported by Advance Animal Science Company Ltd) vaccine provided 90% protection against virulent *S. gallinarum* organism and closely similar (95-100% with 9R vaccine) findings was reported by Lee *et al.,* (2005). The results obtained are also furnished in Table 3.

Table 3. Efficacy of the BIO-TYPHOID® FT vaccine in experimental chicken

Group of chicken	Name of vaccine used for vaccination	Number of chicken challenged	Number of chicken survived to challenge	Number of chicken infected/ died	Percent of survival
Chicken of vaccinated group	BIO-TYPHOID®	10	09	01	90
Chicken of control group	Not applicable	10	10	10	00

Therefore, from the above study, it was observed that chicken of group A vaccinated with BIO-TYPHOID® FT vaccine exhibited higher antibody titre after secondary vaccination. The stimulation of higher degree of immunity induced by BIO-TYPHOID® FT vaccine might be due to higher immunogenic character of the vaccine strain. Thus, the vaccine is more effective for further use.

References

Calnek, B.W., Barnes, H.J., McDougald, L.R. and Saif, Y.M. 1997. Diseases of Poultry. 10th edition. *Iowa State University Press*. America, Iowa. pp. 143.

Cheesbrough M. 1985. Medical laboratory Mannual for Tropical Countries. First edition. Vol-II Microbiology, Chapter 35, *London English Language Book Society*. pp. 40-57.

Collins, C.M. and Carter, S.B. 1972. Immunogenicity of living and heat killed *Salmonella pullorum* vaccines. 7 (5): 735-742.

Dorsey, T.A. 1963. Studies on Fowl cholera. II. The correlation between biochemic classification and the serologic and immunologic nature of avian *Pasteurella multocida*. *Avian Disease*, (7): 393-402.

Haider, M.G., Rahman, M.M., Hossain, M.M., Rashid, M., Sufian, M.A., Islam, M.M. and Haque, A.F.M.H. 2007. Production of formalin killed fowl typhoid vaccine using local isolates of *Salmonella gallinarum* in Bangladesh. *Bangladesh Journal of Veterinary Medicine.* 5 (2): 33-38.

Headleston, K.L. and Reisinger, R.C. 1960. Studies on Pasteurellosis. IV. killed fowl cholera vaccine absorbed on aluminium hydroxide. *Avian Disease*, 4: 429-435.

Lee, Y.J., Mo, I.P. and Kang, M.S. 2005. Safety and efficacy of *Salmonella gallinarum* 9R vaccine in young laying chickens. *Avian Pathology.* 34 (4): 362-366.

Lee, Y.J., Mo, I.P. and Kang, M.S. 2007. Protective efficacy of live *Salmonella gallinarum* 9R vaccine in commercial layer flocks. *Avian Pathology.* 36 (6): 495-498.

Mandal, S.k., Choudhyury, K.A., Amin, M.M., Rahman, A. and Sarker, A.J. 1988. Immune response in chickens induced by Alum precipitated fowl cholera vaccine. I. Humoral immune response. *Bangladesh Veterinary Journal.* 22: 966-976.

Matsumoto, M. and Helfer, D.H. 1977. A bacterin against fowl cholera in turkeys: Protective quality of various preparations originated from broth culture. *Avian Disease*. 21: 382-393.

Purchase C., Picard J., McDonald R. and Bisschop S.P. 2008. A comparison of the oral application and injection routes using the onderstepoort biological products fowl typhoid vaccine, its safety, efficacy and duration of protection in commercial laying hens. *Journal of South African Veterinary Association*, 79 (1): 39-43.

Rahman, M.M., Khan, Z.U.M., Rahman, A.N.M. and Islam, M.S. 2005. Recovery of Salmonella from incubated eggs newly hatched chicks and contaminated environments. *Journal Animal and Veterinary Advances.* 5 (7): 598-601.

Schlink, G.T. and Olson, L.D. 1979. A Microtiter Agglutination Test for Anti *Pasteurella multocida* Antibodies in Turkeys. *Avian Diseases*. 23: 1066-1071.

Shah, D.H., Roy, A. and Purohit, J.H. 2001. Characterization of *Salmonella gallinarum* avian strains isolated from Gujarat State. *Indian Journal Comparative Microbiology, Immunology and Infectious Diseases*. 22(2): 131-133.

Siddique, A.B., Rahman, M.B., Amin, M.M. and Rahman, M.M. 1997. Antibody titres in chicks following pigeon poxvirus inoculation. *Bangladesh Veterinarian*. 14 (2): 12-14.

Vodas, K. 1978. Correlation of agglutinin production with age in fowls experimentally infected with *Salmonella gallinarum. Veterinary Bulletin,* 48 (2): 758.

Wambura, P.N., Makuri, H.G., Hoza, A.S., Tuntufye, H. and Mellau, L.S.B. 2006. Application of microagglutination test for detection of antibodies to *Salmonella gallinarum* in commercial layer chickens. *Bulletin of Animal Health and Production in Africa.* 54 (2): 124-130.