Journal of Animal and Veterinary Advances 4 (3): 332-334, 2005

Evaluation of the Efficacy of a Bacterin Against *Salmonella gallinarum* Infection

[1]M. M. Rahman, [2]Zahed U. M. Khan and [3]S.M. H. Rashid
[1]Department of Microbiology, Dinajpur Government Veterinary College, Dinajpur, Bangladesh
[2]Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh
[3]Department of Pathology and Parasitology, Dinajpur Government Veterinary College, Dinajpur, Bangladesh

Abstract: An inactivated *Salmonella* vaccine was prepared from 5 x 10^7 cells of *Salmonella gallinarum* to assess the potential protective efficacy of *Salmonella gallinarum* vaccine. Specific pathogen free(SPF) hens were vaccinated at 6 weeks and 10 weeks of age and challenged orally at 14 weeks of age with 10^7 cells of homologous *Salmonella gallinarum* strain. The nonvaccinated chickens were also challenged with same number of *Salmonella gallinarum* cells. The antibody titers of the vaccinated hens were detected by rapid serum plate agglutination test(RSPAT) and tube agglutination test. Non-vaccinated hens were observed for 28 days for their changes. Rapid serum plate agglutination test and tube agglutination test revealed that the antibody titers of vaccinated hens rose quickly reaching peak at 8th week post vaccination and were still high at 12th week post vaccination. After challenge, antibody titers were also high and were gradually decreasing upto 20 weeks post vaccination. No antibody titer was detected in non-vaccinated control group.

Key words: Inactivated, *Salmonella gallinarum*, Vaccine, Specific Pathogen free (SPF), Rapid serum plate agglutination Test(RSPAT), Antibody, Titer.

Introduction

Salmonella is a septicemia disease that affects primarily chickens and turkeys, although natural infections in ducks, pheasants, guinea fowls, peafowl, grouse, ostriches, wood pigeons, swans, sparrows, peacocks and quail have been reported (Shivaprasad, 1997). It can spread in several ways. Oral route of infection represents the normal route of inflection. It can pass through the faces of the affected birds and contaminate the poultry house with litter, feed, water and other environments. The egg transmission is the most frequent way in modern poultry industry to spread the *Salmonella* infection among birds, farms or countries. Vaccination was proposed as a new tool in *Salmonella* control in poultry on the basis of numerous experiments regarding the efficacy of live and inactivated salmonella vaccines(Barrow *et. al.* 1990 and Tan *et. al.* 1997). Information on the efficacy of vaccines from field trials is still very limited. Field trials give additional information with respect to efficacy of vaccines under field conditions. Various types of salmonella vaccines have been used to immunize chickens and protect against shedding of salmonella organism. Barbour *et. al.* (1993) indicated that 100% protection against shedding was not btained immediately after challenge. Gast *et. al.* (1992) reported that vaccination with oil emulsion bacterial vaccine did not affect percentage of hens that shed *Salmonella enteritidis* in feces after oral challenge with approxmately 10^9 cells of *Salmonella enteritidis*. Before introduction of the vaccination program for *Salmonella* control in commercial layer flocks based on a *Salmonella gallinarum* strain, an evaluation was done based on the efficacy and the safety of the vaccination program and the effect on the performance of *Salmonella* serologic tests (an indirect ELISA, two blocking
ELISAs and a rapid plate agglutination test [RPA test]) under field conditions (Feberwee *et. al.* 2001). Protective efficacy of the vaccine using pathogenic challenge with a infective dose of same bacterium is needed to declare a vaccine effective.
The objectives of the present study were to test the efficacy of formalin-inactivated oil emulsion vaccine with an antigen of *Salmonella gallinarum* using rapid serum plate agglutination (RSPAT) and tube agglutination test in vaccinated birds after challenge.

Materials and Methods

Chickens: At the age of 6 weeks birds of Fayoumi breed were taken from CPF, Mirpur, Dhaka were tested with specific antigen of *Salmonella* using rapid serum plate agglutination test. The birds which gave negative reaction to *Salmonella* antigen were selected for the experiment. 80 SPF chickens were housed in individual cage. The birds were divided into two experimental groups for vaccination study. Each group contained 40 birds. One group was used for vaccination and another group was kept as nonvaccinated control. They were provided separately with water and antibiotic free feed *ad libitum* throughout the experiments.

Bacterin Preparation: Oil adjuvant inactivated *Salmonella gallinarum* (Fowl typhoid) experimental vaccine in 100 doses vial prepared by Livestock Research Institute(LRI), Department of Livestock Services(DLS), Dhaka was used in chickens for the detection of antibody level of *Salmonella gallinarum* vaccine. For the preparation of inactivated *Salmonella* vaccine, the organism was cultured in nutrient broth and incubated for 24 hours at 37.5°C. Purity of culture was studied and inoculated in agar media in 10 Roux flasks at the rate of 3 ml broth per flask. The broth was spread on the surface of agar by gentle smearing of the flask. The flasks were incubated at 37°C for 24 hours. Then the growth was washed with saline water at the ratio 1:100. So ultimately 1000 ml washing was made. The harvested fluid that is, the washing was treated with following materials:

Washing fluid	70%	1000 ml
Almunium hydroxide gel	28%	400 ml
Glycol buffer	1%	14.3 ml
Formalin	1%	14.3 ml
Phenol red	0.5%	1 ml

The PH of the fluid was 7.4. The treated fluid appeared light pink colour and put to magnetic stirrer for 24 hours to make even suspension. The number of organisms per ml of final product was 40,00,000 *Salmonella gallinarum* bacteria.

Vaccination: 80 SPF chickens of aged 6 weeks were transferred to individual cage in the experimental house and divided into two equal groups. One group contained 40 chickens were vaccinated at 6 weeks of age with *Salmonella gallinarum* inactivated vaccine

manufactured at Livestock Research Institute, Mohakhali, Dhaka with a 1 ml dose intramuscularly into the thigh region and boosted with the same dose at 10 weeks of age. Another group was retained as non-vaccinated control. At the age of 14 weeks, the vaccinated and the non-vaccinated control groups were challenged with 10^7 CFU (infective dose) of *Salmonella gallinarum* field isolates. Each group was kept without physical contact with the others in the same room.

Monitoring Frequency and Examination Methods: The vaccinated flock was monitored at the time of vaccination and at 4,8 ,12,16,20 and 24 week post vaccination by routine serology. For serology, the blood samples were collected and tested by RSPAT and tube agglutination test. The non-vaccinated flocks were also monitored.

Blood Sample Collection and Processing: 2 ml of blood from each bird of vaccinated group was collected aseptically with sterile disposable syringe and needle by puncturing the wing vein. One drop of blood from each bird was used for RSPAT at the time of collection of blood and rest volume of blood was kept for 2 hour for separation of serum from the cellular part of blood. The collected serum was then exposed for heat treatment at 56°C for 30 minutes to destroy the complement and other nonspecific reaction producing components present in the sera. The sterile separated sera kept in individual vial and were then preserved at - 20°C for use. Blood samples were collected from each hen at the time of inoculation of vaccine and at 4 week intervals for 24 week period after 1st vaccination. Samples were assayed for the presence of specific antibodies by using RSPAT and tube agglutination tests.

Challenge : The challenge was conducted 8 weeks after the first vaccination. The challenge bacterium was pathogenic field isolate. The birds were challenged orally with 1 ml of a broth suspension of 10^7 cfu of *Salmonella gallinarum* per bird. The non-vaccinated control group of birds were also challenged orally with same dose of same isolate. Challenged birds were observed for 4 weeks after challenge. Postmortem examinations were conducted of all birds that died during the challenge period.
The vaccinated and non-vaccinated birds were observed throughout the experiment and recorded.

Observation of Non-vaccinated Birds: The affected and dead birds of non-vaccinated group were observed. The affected birds showed the typical signs of *Salmonella* infection. The lesions of the dead bird were examined on post mortem and was found typical lesions to salmonellosis in liver and spleen.
Treatment was not performed for the affected birds. On 28 day post challenge, only 7 birds remained healthy but rest of the birds died during the observation periods.

Results and Discussion

Immune response of the *Salmonella gallinarum* vaccine: No hen was detected as seropositive just before inoculation of *Salmonella gallinarum* inactivated vaccine. The results of the sera of the vaccinated birds has been presented in Table 1. The percentages of hens detected as seropositive after vaccination rising from 80% at 4th week post vaccination to peak values of 95% by 8 weeks post vaccination. The antibody response of hens was found to be decreased at 12th week post vaccination (60%) followed by 27.5% at 16 week post vaccination and 7.5% at 20 weeks post vaccination. No antibody was detectable at 24th week post vaccination. Sera of the group of birds vaccinated with *Salmonella gallinarum* were monitored with RSPAT. Antibody response showed peak (95%) at 8-week post vaccination and a gradual reduction curve reaching 7.5% positive within 20-week post vaccination. Results of the present study supports the findings of Yamane *et al.* (2000), Barrow *et al.* (1990). In RSPAT only some very weak reactions were seen which might also be non-specific. These results in the RSPAT confirm previous observations of no detectable amount of agglutinin present after use of vaccine based on a rough mutant *Salmonella gallinarum* and these result of this study closely agree with the findings of Madhuri *et al.* (1999), Silva *et al.* (1980). None of the vaccinated bird were showing any symptoms of salmonellosis.

Table 1: Detection of antibody response of birds with *Salmonella gallinarum* vaccine by Rapid Serum Plate Agglutination Test (RSPAT)

Time intervals	No. of tested sample	No. of positive sample	% positive
0	40	0	0
4 weeks	40	32	80
8 weeks	40	38	95
12 weeks	40	24	60
16 weeks	40	11	27.5
20 weeks	40	3	7.5
24 weeks	40	-	-

Table 2: Determination of antibody titer in chicken blood after vaccination with *Salmonella gallinarum* vaccine

Group of birds	No. of birds tested	Agglutination titer						
		Weeks post vaccination						
		0	4	8	12	16	20	24
Vaccinated group	40	0	8	32	28	4	2	0
Non vaccinated group		0	0	0	0	0	0	0

(* Mean titer of 40 birds)

Serum Antibody Titre by Tube Agglutination Test: The results of the antibody titer of the vaccinated birds has been presented in Table 2. It was evident that the antibody titers of the vaccinated hens rose quickly to reach peak at 8th week after vaccination and immediately before challenge. At 12th week after 1st vaccination, antibody titre of vaccinated birds were still high. The antibody titer was found 8 at 4th week post vaccination and at 16th week post vaccination the titer was found 4 and it was found 2 at 20th week post vaccination. Antibody titer was not found in non-vaccinated control birds. After challenge exposures antibody titers of vaccinated hens were gradually decreased. The results of the present study closely agreed with the observations of Babour *et al.* 1993, and Gast *et al.*1992. The findings of recent study much lower than that reported in a previous study (Nakamura *et al.*, 1994). They found the level of antibody titers were

Table 3: Post challenge study on nonvaccinated flock

Time interval of observation	Total No. of birds	Dead birds		Affected birds		Healthy birds	
		Number	%	Number	%	Number	%
1st week	40	2	10	25	28	70	
2nd week		11	15			12	
3rd week		13	7			7	
4th week		7	-			7	
At the end of observation		33	82.5			7	17.5

On 2nd week post challenged observation period, 11 birds died due to salmonellosis, 15 birds showed typical signs of salmonellosis and 12 birds remained healthy. On 3rd week post challenged period, 13 birds died due to salmonellosis, 7 birds were affected with *Salmonella* infection and 7 birds were healthy. On 4th week observation period, 7 birds were died and 7 birds remained healthy. Salmonellosis was confirmed by typical signs of affected birds, lesions after PM examination dead birds, cultural characteristic of bacteriological media and serological test. At the end of 4 weeks observation periods of non-vaccinated birds, only 7 birds remained healthy. This may be due to individual variation.

still high of vaccinated hens after challenge exposure. The results of the present study revealed that the efficacy of the vaccine which protect the birds within short period of time. Barbour *et al.* (1993) compared the efficacy of six *S. enteritidis* vaccine prepared with different diluents content and inactivation procedures. There have been many reports about killed *Salmonella* vaccine (Barbour *et al.*, 1993, Cooper *et al.*, 1993, Ghosh, 1989). Because these vaccines were prepared with different advents and different inactivation procedures, it is difficult to assess their potential protective efficacy. Rate of antibody response after challenge exposure of vaccinated and nonvaccinated hens indicated that hens were better protected when vaccine contained *Salmonella gallinarum* inactivated by formalin Those workers concluded that the nature of adjuvant and the method of inactivation of *Salmonella* play a role in protective efficacy of formulated vaccine against *Salmonella* infection in egg laying hens. The high level of antibody response in vaccinated hens was considered to be due to high agglutinability of the bacterial cells. Vaccination is not expected to confer complete protection against infection in the farm. A maximum effect of vaccination can be expected if infection pressure is kept low by good hygiene (Alderton *et al.*, 1991, Barow *et al.*, 1990, Gast and Stone, 1993, Gast *et al.*, 1992). Therefore, we initially intended to vaccinate only flocks that were placed on farms with an specific pathogen free (SPF). These results also support the argument that a positive effect of vaccination can be expected if good biosecurity standards are maintained.

Observation of experimental post challenge non-vaccinated birds: The results of the post challenge study on non-vaccinated flock has been presented in Table 3. On 1st week after inoculation, 2 birds died due to salmonellosis, 10 birds were affected and showed typical signs of *Salmonella* infection and 28 birds remained healthy.

References

Alderton, M. R., K. J. Fahey and P. J. Coloe, 1991. Humoral responses and *Salmonella* protection in chickens given a vitamin dependent *Salmonella typhimurium*. Avian Dis., 35: 435-442

Barbour, E. K., W. M. Frerichs, N. H. Nabbut, P. E. Poss and M. K. Brinton, 1993. Evaluation of bacterins containing three predominant phage types of *Salmonella enteriltidis* for prevention of infection in egg laying chickens. Am. J. Vet. Res., 54: 1306 – 1309

Barrow, P. A., J. O. Hassan, M. A. Lovell and A. Berchieri, 1990. Vaccination of chickens with aroA and other mutants of *Salmonella typhimurum* and enteritidis. Res. Microbiol., 14: 851-853

Barrow, P. A., M. A. Lovell and A. Berchieri, 1990. Immunization of laying hens against *Salmonella enteritidis* with live attenuated vaccines. Vet. Rec.,126: 241 –242

Cooper, G. I., I. M. Venables, R. A. J. Nicholas, G. A. Cullen and C. E. Hormaeche, 1993. Further studies of the application of live *Salmonella enteritidis* aroA vaccines in chickens. Vet. Rec., 133: 31-36

Feberwee, A. T. S. de Vries, E. G. Hartman, de Wit, J. J., A. R. W. Elbers and W. A. de Jong, 2001. Vaccination against *Salmonella enteritidis* in dutch commercial layer flocks with a vaccine based on a live *Salmonella gallinarum* 9R strain: evaluation of efficacy, safety, and performance of serologic *Salmonella* test. Avian Diseases, 45: 83-91

Gast, R. K., H. D. Stone, P. S. Holt, and C. W. Beard, 1992. Evaluation of the efficacy of an oil emulsion bacterin for protecting chickens against *Salmonella enteritidis*. Avian Dis., 36: 992 – 999

Gast, R. K. and H. D. Stone, 1993. Evaluation of the efficacy of oil-emulsion bacterins for reducing fecal shedding of *Salmonella enteritidis* by laying hens. Avain Dis., 37: 1085-1091

Ghosh, S. S., 1989. Comparative efficacy of four vaccines against *Salmonella virchow* in chicks in India. Res. Vet. Sci., 47: 280-282

Madhuri, D. and J. R. Puran Chandand Sadana, 1999. Comparison of a dot immunobinding assay and the serum agglutination test for detecting serological responses in vaccinated and unvaccinated chickens following challenge with *Salmonella gallinarum*. Avian Pathol., 28: 98-101

Nakamura, M., Nagamine, N., Takahashi, T., Suzuki, S., and Sato, S., 1994. Evaluation of the efficacy of a bacterin against *Salmonella enteritidis* infection and the effect of stress after vaccination. *Avian Diseases*.38: 717-724

Shivaprasad. H.L., 1997. Pullorum disease and fowl typhoid, In Calnek B.W.,.Barnes, H.J., Beard, C.W., McDougald, L.R., and Saif, Y. M. (Eds.).*Disease of Poultry 10th Edition*(pp.82-96). Ames, IA: Iowa State University Press

Silva, E.N.. Soneyenbos, G.H.,. Weinack O.M and Smyser, C.F., 1980. Studies on the use of 9R stain of *Salmonella gallinarum* as a vaccine in chickens. *Avian Dis*.25:38-52

Tan, S., Gyles, C. L., and Wilkie, B. N., 1997. Evaluation of aroA mutant *Salmonella typhimurium* vaccine in chickens using modified semisolid Rappaport Vassiliadis medium to monitor faecal shedding. *Vet Microbiol.* 54: 247-254

Yamane, Y., Leonard, J. D., Kobatake, R., Awamura, N., Toyota, Y., Ohta, H., Otsuki, K., and Inoue, T., 2000. A case study on *Salmonella enteritidis* (SE) origin at three egg laying farms and its control with an *S. enteritidis* bacterin. *Avian Diseases*. 44: 519 – 526