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Preparation of Fowl Typhoid Vaccine from Field Isolates and Determination of Efficacy

Jannatun Nime[1,2], Mehedi Hasan[3*], Md. Abdus Sattar[2], Mst. Dilara Nuri[2], Md. Bahanur Rahman[2] and Afia Khatun[4]

[1]Department of Microbiology, Jhenaidah Government Veterinary College, Jhenaidah-7300, Bangladesh.
[2]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[3]Department of Animal Science, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh.
[4]Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi-6202, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JN and MBR designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors MH, MAS, MDN and AK managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was conducted to isolate and identify *Salmonella gallinarum* from field cases to prepare formalin killed vaccine and to determine the efficacy of experimentally prepared fowl typhoid vaccine. A total of 48 chickens were divided into six groups (group A, B, C, D, E and group of unvaccinated control chickens F) including 8 layer chickens of Sonali breed in each group. Chickens in group A, B, C, D and E were vaccinated primarily with experimentally prepared fowl typhoid vaccine with a dose 0.5 ml (4.7×10^7 CFU/ml) through subcutaneous route at the age of 9 weeks and booster dose at 14, 21, 28, 35, 42 days after primary vaccination with the same dose

**Corresponding author: E-mail: mehedivet@gmail.com;*

and route respectively. Blood samples were collected to obtain sera from each chicken after 15 days boostering for determination of antibody titre following using passive haemagglutination test. Highest mean antibody titres obtained from Group A, B, C, D and E was 96±12.04. Among the five groups the highest mean antibody titre of 96±12.04 was obtained when vaccine was given at 14, 21, 28 days after primary vaccination. The result of Challenge infection revealed that among the 8 birds of A, B, C, D and E all were protected from virulent challenge and all chickens were died from the group F. These results revealed that experimentally prepared Fowl typhoid vaccine provided 100% protection.

Keywords: Salmonella gallinarum; fowl typhoid; vaccine; infection; chicken.

1. INTRODUCTION

Fowl typhoid (FT) is an important systemic disease of poultry [1]. It is an acute or chronic septicemic disease that caused by *Salmonella (S.) gallinarum biovar Gallinarum* under the family Enterobacteriaceae [2]. It is an economically significant disease with mortality rates reaching 100 percent. The disease occurs sporadically or enzootically in most countries in the world including Bangladesh. FT losses often begin at hatching time and losses continue to laying age [3]. *S. gallinarum* are very important in poultry health because they are responsible for massive destruction of poultry [4]. The disease FT is of particular economic importance in those countries which are beginning to intensify their industry, e.g. countries in Latin America, South America, the Middle East, the Indian subcontinent and parts of Africa. FT seriously threatened the poultry industry in the early 1900s due to widespread outbreaks accompanied by high mortality [5].

Fowl typhoid is one of the major constraints of poultry industry in Bangladesh [6]. The disease is considered as OIE, list B disease [7]. Among the family, the genus Salmonella named for the eminent United States Department of Agriculture (USDA) Veterinarian and Bacteriologist Daniel E. Salmon, consists of more than 2300 serologically distinguishable variants [4].

Fowl typhoid is under control in many countries in Europe and North America however remains a major problem in countries where poultry husbandry was recently intensified or where the high ambient temperature causes difficulties to environmental hygiene. With great expansion of the poultry rearing and farming, FT has become wide and backyard poultry industry reveals that FT infection causes high morbidity and mortality in developing and growing poultry industry in Bangladesh, resulting alarming situation in chicken population and thus create a panic to the poultry raisers [8].

The major emphasis for preventing infections is to avoid introduction of pathogens into the farms by increased biosecurity [9] along with vaccination [10]. The vaccines available are both live (usually based on the Houghton 9R strain) and bacterins (killed/inactivated vaccine). The offspring of vaccinated birds are protected by maternal antibodies. If the parent birds are vaccinated against *S. gallinarum*, the chicks are protected by maternal antibodies in the hatchery.

Fowl typhoid vaccines of both live and killed are imported and marketed in Bangladesh by different commercial companies. It is necessary to monitor purity, safety and protective efficacy of any biologics or vaccines by respective controlling agency or an alternative agency prior to introduce it within the country for an extensive field use. As a preliminary study of *S. gallinarum* vaccine or FT vaccine manufactured by Department of Livestock Services (DLS) was studied by [11] covering the immunogenicity study without the study of purity, safety and protective efficacy against virulent FT organisms. FT in vaccinated birds have been reported from the fields that indicate insufficient protection conferred by the available imported FT vaccine (Personnel communication). Hence, a through investigation on protective efficacy of experimentally prepared FT vaccine was done in Sonali chicken.

2. MATERIALS AND METHODS

2.1 Sample Collection

The current study was conducted in Phenix hatchery Ltd. of Gazipur (24°00'00"N and 90°25'05"E) district and the Bacteriological laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural Univeristy,

Mymensingh, Bangladesh. The samples (heart, liver and spleen) were collected from dead birds of hatchery and transported through ice flask to the Bacteriological laboratory of the Department of Microbiology and Hygiene for isolation, identification, biochemical characterization and vaccine production.

A total of 20 samples (heart, liver and spleen) were collected from dead birds. The surface of the samples was seared with a hot spatula and was incised with sterile scalpel. An inoculating loop was inserted through the cut surface then it was smeared in Salmonella-Shigella (SS) agar. These were incubated at 37℃ for 24 hours for bacterial growth. All the samples were initially grown in these two media and then on different media.

2.2 Isolation and Identification

From the Salmonella-Shigella agar, subcultures were made on Brilliant green agar (BGA), Nutrient agar (NA), MacConkey agar, Triple sugar iron (TSI) agar and Nutrient broth (NB). Standard techniques were used for identification of the organisms as described by Merchant and Peaker [12] and Cheesbrough [13].

2.3 Morphology Study

Morphological characteristic of Salmonella colonies were studied by using Gram's stain according to the method described by Merchant and Peaker [12].

2.4 Biochemical Study

The isolated bacteria were subjected to different biochemical test. Five basic sugars (dextrose, sucrose, lactose, maltose, and mannitol) were used for fermentation test. Methyel Red test, Voges-Proskauer test, Indole test were performed for identification of the organisms following the procedure described by Merchant and Peaker [12] and Cheesbrough [13].

2.5 Vaccine Production

Isolates of *Salmonella gallinarum* was selected for the production of Fowl typhoid vaccine. Isolates of *S. gallinarum* were cultured in SS agar and kept in incubator at 37° for 24 hours. Isolated colonies were inoculated in nutrient broth added with yeast extract (2 gm/L) and beef extract (1 gm/L) and no growth was found. Later on, formalin was added in broth culture and after 24 hours allum was also added, dispensed in vials and stored at room temperature for future use.

2.6 Purity Test of Experimentally Prepared FT Vaccine

Five blood agar plates were inoculated with FT vaccine and incubated at 37℃ for 24 to 48 hours in the incubator for the growth of aerobic and anaerobic organism. Thus the collected FT vaccine which does not exhibit the growth of aerobic and anaerobic organism was used in the experiment [14].

2.7 Safety Test of Experimentally Prepared FT Vaccine

The safety test was carried out following the method of Matsumoto and Heifer [15]. Five mice were inoculated subcutaneously with 0.2 ml of each vaccine and the vaccine considered safe because of the inoculated mice remained alive and healthy during the observation period of 5 days.

2.8 Experimental Immunization

The experimental immunization of chickens was done with experimentally prepared inactivated "Fowl Typhoid Vaccine". The vaccine was administered through subcutaneous (SC) route and at the dose rate of 0.5 ml (4.7×10^7 CFU/ml) for each bird. Experimental chickens were divided into six groups namely A, B, C, D, E and F. The chickens of group A, B, C, D and E were vaccinated with experimentally prepared FT vaccine. The initial dose (0.5ml) of vaccine was administered to the chickens of group A, B, C, D and E at the age of 63 days (9 weeks) through the SC route. These birds were revaccinated with same dose of vaccine through same route respectively after 14, 21, 28, 35, 42 days of primary vaccination as booster. Chickens of group F were considered as control.

2.9 Collection and Preservation of Sera from the Vaccinated Birds

About 1.5-2 ml of blood samples were collected aseptically without anticoagulant from the wing vein of the vaccinated birds of each group using 5 ml disposable plastic syringe. The blood samples were allowed to clot in the syringe and the collection and preservation of serum were accomplished according to Heddleston and Reisinger [14].

2.10 Inactivation of Collected Chicken Sera

The stored serum samples were kept in water bath at 56℃ for half an hour in order to inactivate complements. This procedure was carried out according to Choudhury et al. [16]. After inactivation, sera were stored at -20℃ until use.

2.11 Challenge Exposure to Experimental Chicken

Both the vaccinated and unvaccinated groups of birds were subjected to challenge with virulent *Salmonella gallinarum* containing a dose 4.7×10^7 CFU/ml, through intramuscular after 15 days of boostering following the procedure described by Choudhury et al. [16].

2.12 Passive Haemagglutination Test

The test was used to determine the antibody titres in birds against *Salmonella gallinarum* after vaccination and followed the method described by Tripathy et al. [17] with slight modification. The modification of the tests was as follows:

Reagents/ Parameters	Tripathy et al. (1970) [17]	Present
PBS	P^H 6.4	P^H7.2
Tannic acid solution	1:25000	1:20000
Strength of Na_2HPO_4. 12 H_2O	0.15 M	0.2 M
Strength of KH_2PO_4. 2 H_2O	0.15 M	0.2 M

2.13 Statistical Analysis

A repeated measure ANOVA was performed for significant differences in PHA titres of different groups following vaccination and challenge infection at different ages. Least significant difference test was initiated to locate significant differences between mean PHA titres. Package software SPSS 10.0 version was used to analyze all the data.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella gallinarum* (SG)

The colony characters of *Salmonella gallinarum* (SG) on SS agar was lentil, raised, round, smooth, glistening, opaque, black and transparent. On nutrient agar circular, smooth, opaque, translucent, on BGA pale, pink color, on MacConkey agar colorless, smooth, pale and on TSI agar black color colonies against a yellowish background. The colony characters of SG in SS agar, TSI agar and BGA were corresponded with [12,18]. In Gram's staining the bacteria appeared as short plump rod shaped pink color gram negative and arranged in single or paired that is supported the result of [19,20]. All isolates of SG fermented dextrose, maltose and mannitol and produced acid but no gas and did not ferment lactose and sucrose which satisfy the statement of [18,21]. All SG were MR positive but VP and indole were negative. Similar findings were also reported by [12]. However, local isolate of SG was used for the vaccine production against fowl typhoid.

3.2 Results of Purity Test

About 0.1 ml of FT vaccine was inoculated onto Blood agar (BA) medium. After incubation for 24 to 48 hours at 37℃ in the incubator growth of organisms were checked. No growth of organisms was detected, which indicated that the vaccine was inactivated and biologically pure [14].

3.3 Results of Safety Test

After inoculation of 0.2 ml of FT in to the mice subcutaneously, the mice were kept under observation for five days. No clinical sign or mortality was detected within the observation period. The results revealed that the vaccine was safe for vaccination [15].

Table 1. Mean PHA titers with standard error of sera of chickens vaccinated with experimentally prepared FT vaccine

Groups	Prevaccination titre	After booster vaccination
A	<4±0.00	96±12.04
B	<4±0.00	96±12.04
C	<4±0.00	96±12.04
D	<4±0.00	88±11.71
E	<4±0.00	88±11.71
P value		0.620*

** means P>0.5, Values are statistically non significant*

3.4 PHA Antibody Titre

All groups of chicken showed 4±0.00 prevaccination mean PHA titre with standard error (SE) (±). After 15 days of boostering the

Table 2. Antibody titres of group A, B, C, D, E and F by PHA after boostering

Prevaccination antibody titres							Antibody titres after 15 days of boostering					
Tag no.	Groups						Tag no.	Groups				
	A	B	C	D	E	F		A	B	C	D	E
1	≤4	≤4	≤4	≤4	≤4	≤4	1	128	64	64	64	128
2	≤4	≤4	≤4	≤4	≤4	≤4	2	64	128	64	128	64
3	≤4	≤4	≤4	≤4	≤4	≤4	3	128	128	128	64	64
4	≤4	≤4	≤4	≤4	≤4	≤4	4	64	64	64	64	64
5	≤4	≤4	≤4	≤4	≤4	≤4	5	64	128	128	128	128
6	≤4	≤4	≤4	≤4	≤4	≤4	6	128	64	64	64	128
7	≤4	≤4	≤4	≤4	≤4	≤4	7	128	64	128	128	64
8	≤4	≤4	≤4	≤4	≤4	≤4	8	64	128	128	64	64

Table 3. Rate of survivality at challenge infection performed after 15 days of booster infection

Group	Route of vaccination	Total birds	No. of birds survive	No. of birds died	Percentage of survivality	Percentage of died
A	SC	8	8	0	100%	0%
B	SC	8	8	0	100%	0%
C	SC	8	8	0	100%	0%
D	SC	8	8	0	100%	0%
E	SC	8	8	0	100%	0%
F	Unvaccinated	8	0	8	0%	100%

mean PHA antibody titres were 96±12.04, 96±12.04, 96±12.04, 88±11.71 and 88±11.71 in group A, B, C, D and E respectively. The highest Mean ± SE titre was 96±12.04, when booster is given at 14, 21 and 28 days after primary vaccination (Table 1). This finding is similar to [22,23]. The antibody titre ranges from 64 to 128 after 15 days of boostering. The lowest antibody titre was 64. The highest antibody titre was 128. The mean PHA titres in birds of unvaccinated control group F were always <4±0.00 (Table 2 above). The result also satisfies statement of [24].

3.5 Result of Challenge Infection

Challenge infection at the rate of 0.5 ml (4.7×10^7 CFU/ml) was given to the chickens of group A, B, C, D, E and F (Unvaccinated). Birds of the vaccinated groups were resisted to virulent challenge exposure. All birds of F (control group) were died within 7 days of post challenge. This indicated that experimentally prepared FT provided 100% protection. These results were in agreement with [25]. The rate of survivality at challenge infection performed after 15 days of booster infection are presented in Table 3 above.

4. CONCLUSION

The study had proved that experimentally prepared fowl typhoid vaccine produces satisfactory level of antibody in chickens and it is very effective for controlling *Salmonella gallinarum* infection. Since this is small scale study (8 birds in each group), the large scaled studies are required to evaluate efficacy of candidate vaccine.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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