

## Immunogenicity of BAU-Bivalent Salmonella and Fowl Cholera Vaccines in Shuvra Chickens

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### ABSTRACT

The study was performed to investigate the immunogenicity of Salmonella and Fowl cholera vaccine prepared at BAU using Shuvra strain of chickens administered with BAU Salmonella bivalent and BAU Fowl cholera vaccines prepared by "Livestock and Poultry Vaccine Research and Production Centre", (LPVRPC). A total of 60 apparently healthy BLRI strains Shuvra were used in this study. The birds were divided into three groups, consisting of 20 birds in each group. Shuvra- A group birds were vaccinated with BAU Salmonella bivalent vaccine, Shuvra-B group birds with BAU Fowl cholera vaccines and Shuvra-C group birds were used as unvaccinated control. The birds, except the ones in group C, were vaccinated intramuscularly (0.5ml) at 8 weeks of age followed by a booster dose at 12 weeks of age. Sera samples were collected on day 56, 85, 105 days and after challenge at 140 days age of birds. Secondary (booster) vaccination ( $P < 0.05$ ) exhibited a significant level of antibody response. The antibody titre of these birds slowly descended till four weeks of challenge test. It was observed that a dependable level of immunogenicity of BAU Salmonella Bivalent and BAU Fowl cholera vaccine was observed when administered under this schedule followed in Shuvra strain of chickens.

**Keywords:** Shuvra, BAU Salmonella bivalent vaccine, BAU Fowl cholera vaccine, efficacy.

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### Introduction

Life and land are closely entwined in Bangladesh of which the total agriculture including livestock is a glaring example. In case of livestock, poultry has by now turned out to be close to an industry (Aklilu *et al.*, 2008). However, there was a growing concern for a type of chicken that would be well-suited to rural Bangladesh along with increased production quality with a comparable feed conversion rate (CFR). In view of this, Bangladesh Livestock Research Institute (BLRI) after a long and strenuous research achieved a proud product with the support from Japan International Cooperation Agency (JICA) and very recently released a strain of poultry named 'Shuvra' indicating whiteness of the color of body (UNB, 2011). The new strain of chicken has, by now, been handed over to commercial poultry farm to rear and study of production factors as well as to distribute to rural areas. At this junction, the question of disease resistance and study of immunogenic capacity of birds came for discussion and scientific investigation.

Infectious diseases are a major cause of poultry death worldwide. Of the bacterial diseases causing tremendous loss of poultry, Salmonellosis and Fowl cholera are noteworthy. The former ailment is due to the pathogen *Salmonella* in poultry and is termed as Pullorum disease (PD) and Fowl typhoid (FT) (Shivaprasad and Barrow, 2008). Salmonellosis in poultry causes heavy economic loss of poultry rearing and the mortality rate is

as high as 100% (Khan *et al.*, 1998), and reduced production (Klein, 1889; Retteger, 1900) and the morbidity rate is often significantly higher than the mortality rate (Kottwitz *et al.*, 2010). On the other hand, Pasteurellosis (Fowl cholera) is also a bacterial disease of economic importance that account for 25 % to 30 % chicken mortality all over Bangladesh (Choudhury *et al.*, 1985).

A number of research programme has been carried out in the Department of Microbiology and Hygiene, BAU, Mymensingh, on the immunogenic performance of Salmonella bivalent (Ferdous, 2008; Khatun, 2011). But reports on immune response of newly developed Shuvra were very limited. This investigation is a primary one on the immune response of this type of birds following vaccination with Salmonella bivalent and Fowl cholera vaccines prepared by "Livestock and Poultry Vaccine Research and Production Centre", (LPVRPC) of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh.

### Materials and Methods

#### Study location and duration

The present research was carried out at the Department of Microbiology and Hygiene, BAU, Mymensingh during the period of May, 2011 to April, 2012.

#### Vaccines used

Salmonella bivalent and Fowl cholera vaccines produced by LPVRPC, BAU, Mymensingh were used in this study.

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### Experimental chickens and laboratory animals

A total number of 60 aged eight (8) weeks of newly developed strain of chicken named Shuvra were used for this experiment. These birds were obtained as day-old chicks with the courtesy of Bangladesh Livestock Research and Institute (BLRI). A total of 74 healthy suckling day-old mice were collected for the determination of LD<sub>50</sub> and safety test of the vaccines. A total of 4 apparently healthy adult rabbits were used for the collection of sera for PHA test.

### LD<sub>50</sub> determination in mice

*S. gallinarum*, *S. pullorum* and *P. multocida* were cultured separately in nutrient broth overnight at 37°C in a shaker incubator and after 12 hours growth, they were centrifuged at 1500 rpm for 5 minutes. The supernatant was decanted off, and the sediment was washed thrice with PBS. The pellet was dissolved with sufficient amount of PBS and measured the OD value at 550 nm and adjust the OD value at 2.83. 250 µl and 100 µl, 50 µl and 25 µl of bacterial suspension was injected intraperitoneally respectively in 4 groups of mice, each group containing 8 and same amount of PBS was injected in each corresponding control group. Death pattern was observed until 10 days. A 10 fold dilution was made and spread on NA media for CFU count, *P. multocida* 250µl of 2.43×10<sup>8</sup> CFU/ml, *Salmonella gallinarum* 8.6×10<sup>13</sup> CFU/ml and *Salmonella pullorum* 8.9×10<sup>13</sup> CFU/ml contain LD<sub>50</sub> dose in mice.

### Experimental immunization of chicken

Experimental chickens were divided equally into three groups; Shuvra-A, Shuvra-B and Shuvra-C. The chickens in group Shuvra-A (n=20) were vaccinated with 0.5 ml (4.7 ×10<sup>7</sup>CFU/ml) of experimentally prepared BAU Salmonella bivalent vaccine. Similarly, group Shuvra-B (n=20) were vaccinated with 0.5 ml (2.43×10<sup>8</sup> CFU/ml) of BAU Fowl cholera vaccine while the remaining non-immunized was kept as control group Shuvra-C (n=20). The initial dose (primary vaccination) of Salmonella bivalent and Fowl cholera vaccine were given to the birds of Shuvra-A and Shuvra-B respectively through IM route at the age of 8 weeks. These birds were revaccinated with same dose of vaccines through IM after four weeks of primary vaccination as booster (12 wks of age).

### Collection and preservation of sera

The collection and preservation of sera were accomplished according to the method described by Heddleston and Reisinger (1960). The sera samples were collected on day 56 (8 weeks), 85 (12 weeks), 105 days (15 weeks) and after challenge at 140 days (20 weeks) age of the experimental birds. Sera was also collected from the control birds at 8, 12 and 15 weeks of age and all the

sera were kept in sterilized glass vials with rubber cap, rapped with para film and preserved at - 20°C until used.

### Challenge exposure to the vaccinated chicken

Test group of Shuvra-A, Shuvra-B and control group Shuvra-C were used for the protection test. After 7 days of final immunization the chickens of Shuvra-A were challenged with LD<sub>50</sub> of *S. gallinarum* and *S. pullorum* (8.9 × 10<sup>13</sup> CFU/ml of *S. gallinarum* and 8. 6x 10<sup>13</sup> CFU/ml of *S. pullorum*) and chickens of Shuvra-B were challenged with LD<sub>50</sub> of *P. multocida* (250µl containing 2.43×10<sup>8</sup> CFU/ml). Death pattern was observed upto 10 days.

### Passive haemagglutination (PHA) test

The serological analysis was conducted by passive haemagglutination (PHA) test to determine antibody titres of the sera collected from both vaccinated and non vaccinated chickens following the method described by Tripathy *et al.* (1970).

### Statistical analysis

The antibody titers of vaccinated and control birds were analyzed by students t - test. Significance of survival rates of chicken were analyzed by Mantel Cox log rank test.

### Results

The prevaccination mean PHA titre was ≤ 4.0±0.00 in chickens of all groups (both vaccinated and unvaccinated). Each group contain 20 birds. Chickens of group Shuvra-A were vaccinated with BAU Salmonella bivalent (SP+SG) vaccine and Shuvra-B were vaccinated with BAU Fowl cholera vaccine at day 56 (primary vaccination) and then at 85 days of age (secondary vaccination). The sera samples were collected at day 56 (8 weeks), 85 (12 weeks), 105 (15 weeks) and after challenge at 140 (20 weeks) days age of birds for conducting PHA test.

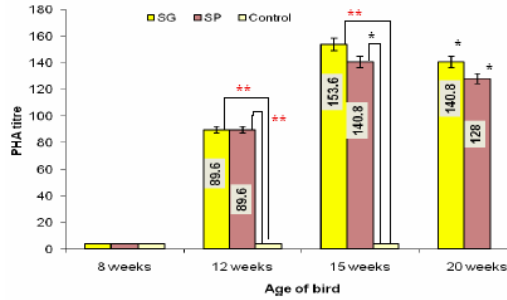
### Results of passive haemagglutination assay (PHA) in chicken

The prevaccination mean PHA titre was ≤ 4.0±0.00 in chickens of all groups (both vaccinated and unvaccinated). In Shuvra-A, The mean PHA test conducted with both *S. gallinarum* and *S. pullorum* antigen revealed titre of 89.60±15.677 and 89.60±15.677 on 29 days of post primary vaccination, 153.60±25.60 and 140.80±31.353 on 20 DSV, 140.80±31.353 and 128.00±35.054 on 55DSV (after challenge) (Table 1 and Fig. 1). In Shuvra-B, the mean PHA test performed with Fowl cholera vaccine expressed titre was 70.4±15.677 on 29 DPV, 153.60±25.60 on 20 DSV and 140.80±31.353 on 55 DSV (after challenge) (Table 1 and Fig. 2).

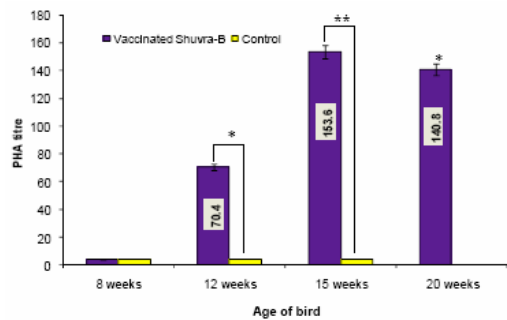
**Table 1. Results of mean PHA titre with standard error of sera of BLRI strain Shuvra-A and Shuvra-B chickens vaccinated with BAU Salmonella bivalent and BAU Fowl cholera vaccines**

Weeks of vaccination	Pre-vaccinated Antibody titre of all vaccinated and control (UVC) Birds, (Mean± SE)	Shuvra-A strains of chicken vaccinated with Salmonella bivalent (SP+SG) vaccine (Mean± SE)	Level of significance, when compared with control	Shuvra-B strains of chicken vaccinated with Fowl cholera Vaccine (Mean± SE)	Level of significance, when compared with control	Age of vaccination	
						1 <sup>st</sup> dose	2 <sup>nd</sup> dose
12 weeks (29 DPV)	≤4±0.00	<i>S. gallinarum</i> , 89.60±15.677	**	70.4±15.677	*	1 <sup>st</sup> dose	2 <sup>nd</sup> dose
		<i>S. pullorum</i> , 89.60±15.677	**				
15 weeks (20 DSV)	≤4±0.00	<i>S. gallinarum</i> , 153.60±25.60	**	153.60±25.60	*	8 weeks / 56 days	12 weeks / 85 days
		<i>S. pullorum</i> , 140.80±31.353	*				
20 weeks (55 DSV)	≤4±0.00	<i>S. gallinarum</i> , 140.80±31.353	*	140.80±31.353	*		
		<i>S. pullorum</i> , 128.00±35.054	*				

Vaccinated chickens were immunized at 8 weeks of age and boosted at 12 weeks of age via I/M route at the dose rate of 0.5 ml of *Salmonella* bivalent: 4.7 ×10<sup>7</sup>CFU/ml and Fowl cholera: 2.43×10<sup>8</sup> CFU/ml in birds respectively. Blood was drawn at 8, 12, 15 and 20 weeks (after challenge) of age. Serum antibody titre was determined by PHA test. The result is expressed as mean ±SE values (n = 5). \*\* = indicates significant at 1% level of significance (P<0.01); \* = indicates significant at 5% level of significance (P<0.05)



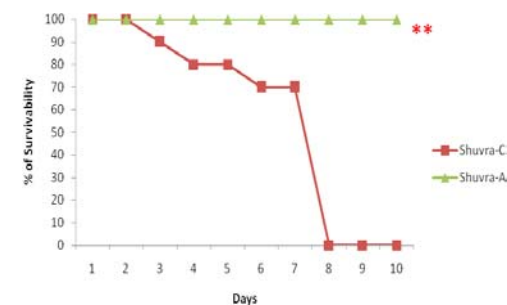
**Fig. 1. Histogram showing of PHA titre (mean±SE) of sera of Shuvra-A chicken vaccinated with BAU *Salmonella* bivalent vaccine.** Shuvra-A chicken was immunized at 8 weeks age and boosted at 12 weeks via I/M route at the dose rate of 0.5 ml/bird ( $4.7 \times 10^7$ CFU/ml). Blood was drawn at 8, 12, 15 and 20 weeks (after challenge) of age. Serum antibody titre was determined by PHA test. The result is expressed as mean ±SE (n = 10 chicken/group). P value is determined by Student's t-test. \*\* =  $P < 0.01$ , \* =  $P < 0.05$



**Fig. 2. Histogram showing PHA titre (mean±SE) of sera of Shuvra-B chicken vaccinated with BAU Fowl cholera vaccine.** Shuvra-B chicken was immunized at 8 weeks age and boosted at 12 weeks via I/M route at the dose rate of 0.5 ml/bird ( $2.43 \times 10^8$  CFU/ml). Blood was drawn at 8, 12, 15 and 20 weeks (after challenge) of age. Serum antibody titre was determined by PHA test. The result is expressed as mean ±SE (n = 10 chicken/group). P is determined by Student's t-test. \*\* =  $P < 0.01$ , \* =  $P < 0.05$ .

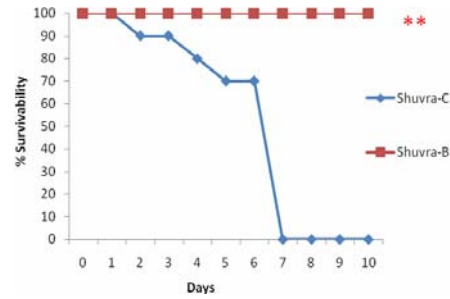
**Survivability at challenge experiment**

In post-challenge period, mortality rate was monitored for subsequent 10 days. Control group (Shuvra- C) died within 7 days where all the other vaccinated birds survived and conferred 100% protection against challenge infection (Fig. 3 and 4).



**Fig. 3. Survival rate of Shuvra-A chicken challenged intramuscularly with virulent isolate of *S. gallinarum* and *S. pullorum*.** Chickens were immunized twice with *Salmonella* bivalent vaccine at 8 week and boosted at 12 week of age of birds through i/m route at the dose rate of 0.5 ml/bird ( $4.7 \times 10^7$ CFU/ml). Control chickens were injected twice with 0.5 ml PBS/bird.

Survival rate of chicken was monitored following i/m challenge with LD<sub>50</sub> dose (250µl) of mice. The survival rate was monitored for subsequent 10 days. (n=10 chicken per group). \*\* =  $P < 0.01$  by Mantel-Cox Log rank test.



**Fig. 4. Survival rate of Shuvra-B chicken challenged intramuscularly with virulent isolate of *P. multocida*.** Chickens were immunized twice with BAU-FCV at 8 week and boosted at 12 week of age of birds through i/m route at the dose rate of 0.5 ml/bird ( $2.43 \times 10^8$  CFU/ml). Control chickens were injected twice with 0.5 ml PBS/bird. Survival rate of chicken was monitored following i/m challenge with LD<sub>50</sub> dose (250µl) of mice. The survival rate was monitored for subsequent 10 days. (n=10 chicken per group). \*\* =  $P < 0.01$  by Mantel-Cox Log rank test.

**Results of challenge exposure**

Challenge infection at the dose rate of 1 ml (*S. pullorum*:  $8.6 \times 10^{13}$  CFU/ml, *S. gallinarum*:  $8.9 \times 10^{13}$  CFU/ml and *P. multocida*:  $2.43 \times 10^8$  CFU/ml) was given to the vaccinated and unvaccinated chickens. All the vaccinated birds (Shuvra-A, Shuvra-B) were refractory to virulent challenge exposure showing alertness and were physically healthy. Birds of the control group Shuvra-C showed sign and symptoms of infection within 1-2 days of challenge infection.

**Discussion**

The present study was undertaken to determine the efficacy of BAU *Salmonella* bivalent and BAU Fowl cholera vaccines produced at LPVRPC, BAU, Mymensingh. As an essential part of efficacy test, the LD<sub>50</sub> of virulent *Salmonella gallinarum*, *Salmonella pullorum* and *Pasteurella multocida* was also determined individually in this study. During preparation of two types of vaccines, sterility and safety tests were individually carried out as per recommendation for OIE (2008). The pre-vaccinated PHA titre of sera *Salmonella* bivalent and BAU Fowl cholera vaccinated chickens were recorded as  $\leq 4 \pm 0$  which was closely related to the findings of Ferdous (2008), Khatun (2011) and Mondal *et al.* (1988).

In Shuvra-A, the mean PHA antibody titre on day 85 (29 DPV) was recorded as  $89.60 \pm 15.677$  with *S. gallinarum* antigen and  $89.60 \pm 15.677$  with *S. pullorum* antigen respectively. Following vaccination with BAU Fowl cholera the mean PHA titre at same days  $70.4 \pm 15.677$ , in case of Shuvra-B chicken. Subsequent secondary (booster dose) vaccination in Shuvra-A chicken, the mean PHA titre  $153.60 \pm 25.60$  with *S. gallinarum* antigen and  $140.80 \pm 31.353$  with *S. pullorum* respectively. On similar period, the mean PHA titre of birds having BAU-FCV vaccine ascended to  $153.60 \pm 25.60$  in sera samples obtained from Shuvra-B group of chickens. In case of challenge experiment conducted at 55 days of secondary vaccination, in Shuvra-A, the serum mean PHA titre of  $140.80 \pm 31.353$  with *S. gallinarum* and  $128.00 \pm 35.054$  with *S. pullorum* antigen. Similarly, sera samples from Shuvra-B having BAU Fowl cholera vaccine, the serum mean PHA titre was  $140.80 \pm 31.353$ . Thus, an overall review reflected a significant level of PHA titre following secondary (booster) vaccination at 12 weeks produced better immune response ( $P < .05$ ) and showed a highest Mean ± SE titre.

Chickens of both the groups (vaccinated and control) were challenged with virulent field isolate of *S. pullorum*: ( $8.6 \times 10^{13}$  CFU/ml) and *S. gallinarum*: ( $8.9 \times 10^{13}$  CFU/ml) and *P. multocida* ( $2.43 \times 10^9$  CFU/ml) with dose rate 250 $\mu$ l LD<sub>50</sub> of mice. It was observed that all the vaccinated birds were 100% resistant to virulent challenge exposure. Birds of the control group showed signs of disease within 1 days of challenge and all of them died within 7 days.

It may be noted that, newly developed Shuvra strain of chicken was for the first time exposed to field vaccine. However, the study would have been more appreciable if the study be performed with further details.

## Conclusion

It was demonstrated that immunogenicity of both the vaccines used in Shuvra strain of chicken was dependable and a protection of 100% was observed and the vaccines were safe.

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