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Immunogenicity of *Salmonella pullorum* killed vaccine in selected breeder flock

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ABSTRACT

Present study was carried out to study the immunogenicity of a commercial available formalin-killed alum-precipitated *Salmonella pullorum* vaccine manufactured by the Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) at the Department of Microbiology and Hygiene, BAU, Mymensingh. accination was carried out on Shaver brown chicken (parent stock) of the Phenix Poultry Ltd. Located at Bhaluka, Mymensingh. Birds belonging to the group A (n=11,000) were used for vaccination, while birds of group B (n=10) was used as unvaccinated control group. Each bird was vaccinated intramuscularly at 65 days of age with a subsequent booster dose after 35 days of primary vaccination. Following primary vaccination the mean value of Passive Hemagglutinating (PHA) antibody titers of vaccinated birds, at 15, 30 and 35 days were 116.00±12.00, 96.00±8.26 and 88.00±11.71 respectively. While, the subsequences mean value of PHA antibody titers of vaccinated birds at 15 and 30 days following the booster vaccination were 128.00±10.12 and 108.00±7.66, respectively. The mean PHA antibody titer in chickens of unvaccinated control birds were ≤ 4.25±0.51. The findings demonstrated that the level of PHA titer after primary vaccination decreased immediately; however, after booster vaccination the level of titer of PHA antibody increased and sustained. Present study indicated that the *S. pullorum* killed vaccine prepared at by LPVRPC; significantly ($p<0.01$)) increased the level of PHA antibody in vaccinated chickens compared to unvaccinated control as determine by the PHA test.

Keywords: *Salmonella pullorum*, killed vaccine, Immunogenicity, PHA antibody titers, On-farm study, Shaver Brown Chicken.

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INTRODUCTION

Salmonellosis is one of the most important bacterial diseases in poultry industry causing heavy economic loss through mortality and reduced productivity (Begum, 1992, Haque et al., 1997, Khan et al., 1998). Salmonellosis includes pullorum disease, fowl typhoid and other infections cause's varieties of clinical signs from acute systemic disease and gastrointestinal problems in poultry flocks to embryonic problem in hatchery (Gast, 1997). Pullorum disease also known as Bacillary White Diarrhoea caused by *Salmonella pullorum* usually remains confined to the first 2-3 weeks of age and occasionally occurs in adults (Shivaprashad, 1997). With great expansion of the poultry rearing and farming, pullorum disease and fowl typhoid have become widespread problem in Bangladesh like other areas of the world (Sarker, 1976 and Rahman et al., 1979) which causes heavy economic losses in broiler, layer and breeding flocks. Recently it has been reported that in Bangladesh the prevalence of *Salmonella* was 71.11% in broiler, 38.89% in layer and 25% in indigenous chicken (Naurin et al., 2012). Heavy economic loss occurs due to

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morbidity, mortality, reduced production and chick quality. Mortality may vary from negligible to 10% and upto 80% or higher in severe outbreaks (Kaura et al., 1990, Kleven and Yoder, 1998).

Poultry Biologics unit (PBU) recently renamed as "Livestock and poultry Vaccine Research and Production Centre" (LPVRPC) incorporated with its parent organization the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU) Mymensingh. LPVRPC produces vaccine against *S. pullorum* and *S. gallinarum* for distribution in the field. In the present study the immunogenicity of formalin-killed alum-precipitated *S. pullorum* vaccine produced by LPVRPC at BAU was studied in Shaver Brown chicken.

MATERIALS AND METHODS

Vaccination

The vaccine used in the study was a formalin-killed alum-precipitated *S. pullorum* killed vaccine.

Vaccination was carried out (on a farm study) intramuscularly in Shaver Brown chicken (Parent stock) (n=11,000) in the Phenix Poultry Ltd. Located at Bhaluka, Mymensingh; following manufacturer's instructions, 0.5 ml (0.625×10^{10} CFU) each and these birds were designated as Group A. Unvaccinated birds (n=10) were considered as control and designated as Group B (n=10). Primary vaccination was done at the age of 65 days to all groups of birds except control group. The booster vaccine was administered to the birds at the age of 100 days. Collection of sera were scheduled as prior to primary vaccination, 15 days after primary vaccination, 30 days after primary vaccination, prior to booster vaccination, 15 days after booster vaccination and 30 days after booster vaccination.

Collection and preservation of sera

About 1.5- 2 ml of blood samples were collected aseptically from the right jugular vein of each bird using 5 ml disposable plastic syringe. Collection of sera was accomplished according to Heddleston and Reisinger (1960). Finally the sera were collected into sterilized glass vials with rubber cap and stored at -20° C until use.

Passive haemagglutination (PHA) test

Immunogenicity of the vaccine was investigated by determining the production of antibody in the vaccinated chicken by PHA test as method described by Akter et al., (2012.).

Statistical analysis

A repeated measure ANOVA was performed for significant differences in PHA titers of different ages. P value at ≤ 0.05 was considered statistically significant. Paired t-test was initiated to locate significant differences between mean PHA titers. Package software SPSS 12.00 version was used to analyze all the data.

RESULTS AND DISCUSSION

The mean ± SE PHA titer of viccnated birds are presented in Table 1**.** The mean prevaccination PHA antibody titer was $\leq 4.25\pm0.51$ in chickens of all groups. After 15 days of primary vaccination the mean PHA antibody titers was found 116.00±12.00, after 30 days of primary vaccination was the titer was 96.00±8.26 and after 35 days of primary vaccination was 88.00±11.71 in vaccianated group. After 15 days of booster vaccination the mean PHA antibody titers was were 128.00±10.12 and after 30 days of booster vaccination the mean PHA antibody titers was 108.00±7. The mean PHA antibody titer in chickens of unvaccinated bird were $\leq 4.25\pm0.51$. After primary vaccination, the mean PHA titer of viccnated birds shows titer rised followed by descend quickly whereas following booster vaccination the PHA titer rised immediately and decrease slowly.

Table 1
PHA (Mean ± SE) titers of sera of Shaver Brown chickens vaccinated with *Salmonella pullorum* vaccine.

Prevaccinated PHA titer of all vaccinated and control birds (Mean ± SE)	PHA titer of all vaccinated birds after primary vaccination (Mean ± SE)	PHA titer of all vaccinated birds after 15 and 30days booster vaccination (Mean ± SE)	P value	Level of significance
4.25±0.51	116.00±12.00 (after 15 days of primary vaccination)		0.0001	**
4.25±0.51	96.00±8.26 (after 30 days of primary vaccination)		0.0001	**
4.25±0.51	88.00±11.71 (after 35 days of primary vaccination)		0.0001	**
4.25±0.51		128.00±10.12(15 days after booster vaccination)	0.0001	**
4.25±0.51		108.00±7.66(30 days after booster vaccination)	0.0001	**

** means significant at 1% level ($p<0.01$)

The study was aimed at on-farm investigation on the immunogenicity of a *S. pullorum* vaccine produced by LPVRPC at BAU, Mymensingh. The pre-vaccination PHA titer of sera samples of all vaccinated chickens were recorded as ≤ 4.25±0.51 which was closely related to the findings of Ferdous (2008) and Yeasmin (2010). Following primary vaccination the mean value of PHA antibody titers of vaccinated birds, at 15, 30 and 35 days were 116.00±12.00, 96.00±8.26 and 88.00±11.71 respectively. On the other hand, the subsequent mean value of PHA antibody titers of vaccinated birds at 15 and 30 days of booster vaccination were 128.00±10.12 and 108.00±7.66, respectively. So the level of titer after primary vaccination was decrease immediately; but after booster vaccination level of titer is rised within short period and sustained. The antibody titers in this study ranged from 64 to 128 after 30 days of booster vaccination.

Rahman et al. (2005) also used rapid serum plate agglutination test and tube agglutination test to determine the antibody titer of *S. gallinarum*. The authors stated that antibody titers of the vaccinated birds increased quickly and reached peak 4 weeks after vaccination. This ranges also similar to the finding of present study.

Recently the efficacy of the same vaccine was studied in detailed by Akter et al., (2012) who reported that the vaccine induced serum antibody titers that peaked in 2-week following both primary and booster vaccination ($P<0.05$), and started to decline following 4-week of both vaccinations. Both primary and booster vaccination induced detectable antibody responses that were able to react with whole cells *S. pullorum* as determined by passive haemagglutination test (PHA). However, present study differs from that of Akter et al., (2012) since it was carried out at on-farm level.

Based on the results of the present work, it could be concluded that the *S. pullorum* killed vaccine prepared at LPVRPC, Department of Microbiology and Hygiene, BAU, Mymensingh induced significantly high level of antibody in chickens as determine by PHA test conducted in an on-farm study.

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