

Original Article

Determination of lowest immune protective titer against *Salmonella gallinarum* and *Salmonella pullorum* in chicken vaccinated with BAU-Salmonella bivalent vaccine

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ABSTRACT

Objective: The research work was conducted to determine the duration of protective efficacy and lowest immune protective titer of Salmonella bivalent vaccine containing *Salmonella gallinarum* and *Salmonella pullorum* prepared at the Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) of Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh.

Materials and methods: The experimental chickens were subdivided into four main groups (A, B, C and D). Group A and B were vaccinated with BAU-Salmonella bivalent vaccine with dosed 0.5 mL intramuscularly at the age of seven weeks followed by a booster vaccination at 12 weeks of age while group C and D served as unvaccinated control. The sera samples were obtained at 7, 12, 15, 18, 23, 27, 30, 32, 34, 36 and 41 weeks of age of birds.

Results: Significantly elevated level of immune response in terms of antibody production resulted from booster vaccination. Vaccinated chicken showed protective resistance following virulent challenge with isolates of *S. gallinarum* and *S. pullorum* (** $P < 0.01$) till 41 weeks, whereas unvaccinated control birds failed to resist the virulent challenge infection.

Conclusion: BAU-Salmonella bivalent vaccine showed lowest immune protective titer up to seven months following booster vaccination.

KEYWORDS

Antibody; PHA test; *Salmonella* vaccine; Titer; Vaccination

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INTRODUCTION

Despite tremendous progress of poultry industry in Bangladesh, it has been suffering from a number of infectious diseases such as avian influenza, Newcastle disease, fowl cholera, salmonellosis etc. The major constraints which lead to serious economic loss as well as discouraging poultry rearing in Bangladesh are the outbreaks of several devastating diseases ([Das et al., 2005](#); [Hamid et al., 2017](#); [Rahman et al., 2017](#); [Najmin et al., 2018](#)). Among the infectious diseases, salmonellosis is one of the top most important diseases in poultry that cause serious economic loss because of mortality and reduce egg production ([Khan et al., 1998](#); [Sun et al., 2016](#)). In Bangladesh, 16.9% prevalence of salmonellosis was reported in breeding flock ([Saleque et al., 2003](#)).

Salmonellae are Gram-negative bacteria classified under the family of Enterobacteriaceae which are short rods, non-spore forming, non-capsulated, aerobic and anaerobic organisms ([Parvej et al., 2016](#); [OIE, 2008](#)). Based on 67 somatic antigens more than 2500 serovars of salmonella exist (for nonmotile species) and a lot of "H" antigens (for motile species) identified so far ([Echeita et al., 2002](#)). The infection in human and animal caused by salmonella belong to subspecies enteric, serovar pullorum (pullorum disease), gallinarum (fowl typhoid) and paratyphoid have a very significant economic importance in poultry sector ([OIE, 2008](#); [Guo et al., 2016](#)).

Good farming, hygienic practices and test and slaughters of positive flocks from production farms are the main basis for the successful control of *Salmonella pullorum* and *S. gallinarum* infections ([Calnek et al., 1997](#)). Biosecurity and introducing clean chicks are important for prevention of those diseases ([Gifford et al., 1987](#)). As with other *Salmonellae*, recovered birds are resistant to the effects of infection but may remain carriers. As a preventive measure, vaccination is being practiced in commercial farms against *S. pullorum* and *S. gallinarum*. In Bangladesh, both live (Houghton 9R strain) and bacterins (Killed/inactivated vaccine) are available and is being used by the poultry farmers ([Choudhury et al., 1987](#); [Kamble et al., 2016](#)). Besides local manufacturers, Salmonella vaccines of both live and killed type are imported from foreign countries and are being marketed in Bangladesh by different commercial companies.

Livestock and Poultry Vaccine Research and Production Centre (LPVRPC), a vaccine research and production center of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh produces a bivalent vaccine named "BAU-Salmonella"

consisting of *S. gallinarum* and *S. pullorum* which are distributed for field use. The "BAU-Salmonella" is recommended to be vaccinated at the 5-6 weeks age of chicken and then booster at 11 weeks of age of birds. BAU-Salmonella bivalent vaccine induce protective antibody titer and protect the immunized chicken against virulent challenge infection following primary and booster vaccination at 4 and 8 weeks, respectively ([Basak and Amin, 2013](#); [Modak et al., 2012](#); [Akter et al., 2013](#)). But the duration of protective immune responses following vaccination with BAU-Salmonella has not been investigated so far. The present work was undertaken with the objectives to determine the duration of protective efficacy along with determination of Passive Hemagglutination Assay (PHA) antibody titer of sera obtained from the vaccinated birds. Hence, a thorough investigation on duration of protective efficacy and lowest immune protective titer of experimentally prepared salmonella bivalent vaccine (*S. gallinarum* and *S. pullorum*) was performed in BLRI developed shorna strain of layer birds.

MATERIALS AND METHODS

Ethical approval: This study was approved by the Animal Welfare and Ethics Committee, Bangladesh Agricultural University (No. 2018/03/AWEC/BAU).

Vaccine used: Salmonella bivalent vaccine prepared by LPVRPC, BAU, Mymensingh was used in this study.

Experimental chicken: A total of 304 apparently healthy birds (specific pathogen free) of BLRI developed strain "Shorna" of either sex of 6 (six) weeks of ages were used for this research. The chickens were subdivided into four main groups namely A, B, C and D, each consisting of 76 birds.

Experimental immunization of chicken: Birds of group-A and B were vaccinated with BAU-Salmonella bivalent vaccine prepared by LPVRPC at 7 weeks of age and booster dose of vaccine was administered at 12 weeks of age. The birds of groups C and D kept as unvaccinated control. The sera samples were collected at 7 (pre-vaccinated), 12 (after primary vaccination) and 15, 18, 23, 27, 30, 32, 34, 36 and 41 weeks of age (after booster vaccination) from both vaccinated and unvaccinated birds. All the sera samples were preserved at -20°C until used.

Virulent challenge exposure to vaccinated chicken: Birds of each group either vaccinated or unvaccinated were subdivided into nine different subgroups containing 8 birds (**Figure 2-3**). Chickens of different subgroup

were challenged either with *S. gallinarum* (4.68×10^{12} CFU/mL) or *S. pullorum* (5.26×10^{12} CFU/mL) at 15, 18, 23, 27, 30, 32, 34, 36 and 41 weeks of age after final immunization. Re-isolation of *S. gallinarum* and *S. pullorum* was done from dead control chicken following challenge infection.

Passive hemagglutination (PHA) test: The immune response in terms of antibody titers of pre-vaccination and post vaccination sera was evaluated by passive haemagglutination (PHA) test (Choudhury et al., 1987; Mondal et al., 1988; Sarker et al., 1992; Siddique et al., 1997).

Statistical analysis: Statistical analysis was performed using SPSS software. The PHA titers were analyzed by using *t*-test to determine the differences between vaccinated and unvaccinated control birds. Survival rate following challenge exposure was evaluated by Mantel Cox log rank test. *P* value of ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In Bangladesh poultry industry is growing very rapidly and salmonellosis is a great burden for the commercial poultry raisers. There is huge shortage of Salmonella vaccine production in this country so a good number of commercial companies importing *Salmonella* killed vaccine for marketing. Such imported vaccines are being used in the field without any field trial in Bangladesh which should have been mandatory in terms of testing of efficacy. The present study was performed to determine the protective efficacy and lowest immune protective titer against *S. gallinarum* and *S. pullorum* in chickens vaccinated with BAU-Salmonella bivalent vaccine. To determine immunogenicity against BAU-Salmonella bivalent vaccine in chicken, humoral immune response was measured by PHA test.

The sterility and safety test of the concerned vaccine was performed as per methods described in OIE (OIE, 2008). The evaluation of the sterility of the vaccine was

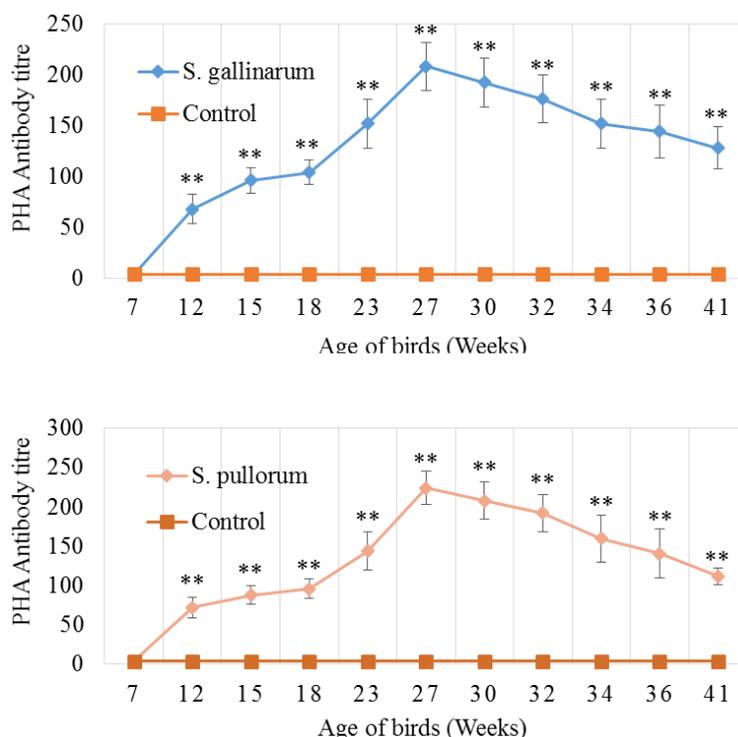


Figure 1. PHA titers with standard error of sera of chickens against *S. gallinarum* (upper panel) and against *S. pullorum* (lower panel) vaccinated with BAU-Salmonella bivalent vaccine. Chickens were immunized with BAU-Salmonella bivalent vaccine at 7 weeks of age as primary vaccination and 12 weeks of age as secondary vaccination via IM route dosed 0.5 mL/bird. Sera were collected after 35 (12 weeks) days of primary vaccination and after 21 (15 weeks), 42 (18 weeks), 77 (23 weeks), 105 (27 weeks), 126 (30 weeks), 143 (32 weeks), 154 (34 weeks), 168 (36 weeks) and 203 (41 weeks) days of secondary vaccination. Sera antibody titers against each collection were evaluated by PHA test. The results shows the mean \pm SE values ($n=8$). Level of significance was determined by comparing the titer of antibody of vaccinated birds and control birds in the mentioned day of collection. SE=standard error of mean; **, $P < 0.01$.

S. gallinarum

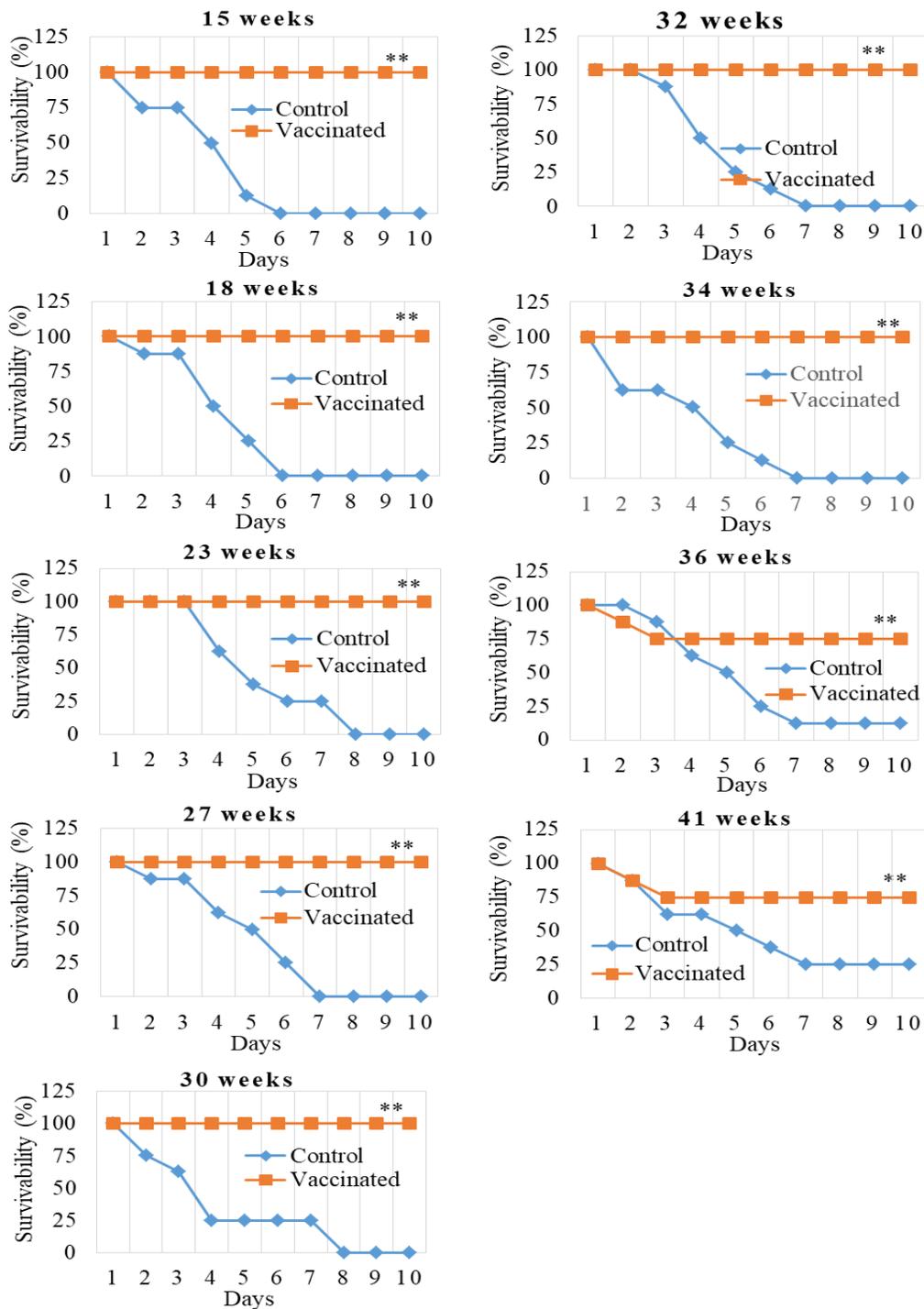


Figure 2. Survival rate of chicken challenged intramuscularly with virulent isolates of *S. gallinarum*. Chicken were immunized with BAU-Salmonella bivalent vaccine at 7 weeks of age as primary vaccination and 12 weeks of age as secondary vaccination via IM route dosed at 0.5 mL in each bird. Chickens were challenged through IM with 0.55 mL of virulent strain of *S. gallinarum* (4.68×10^{12} CFU/mL) at 15, 18, 23, 27, 30, 32, 34, 36 and 41 weeks of age, and the mortality was observed for the subsequent 10 days. ** $P < 0.01$, by Mantel-Cox logrank test.

S. pullorum

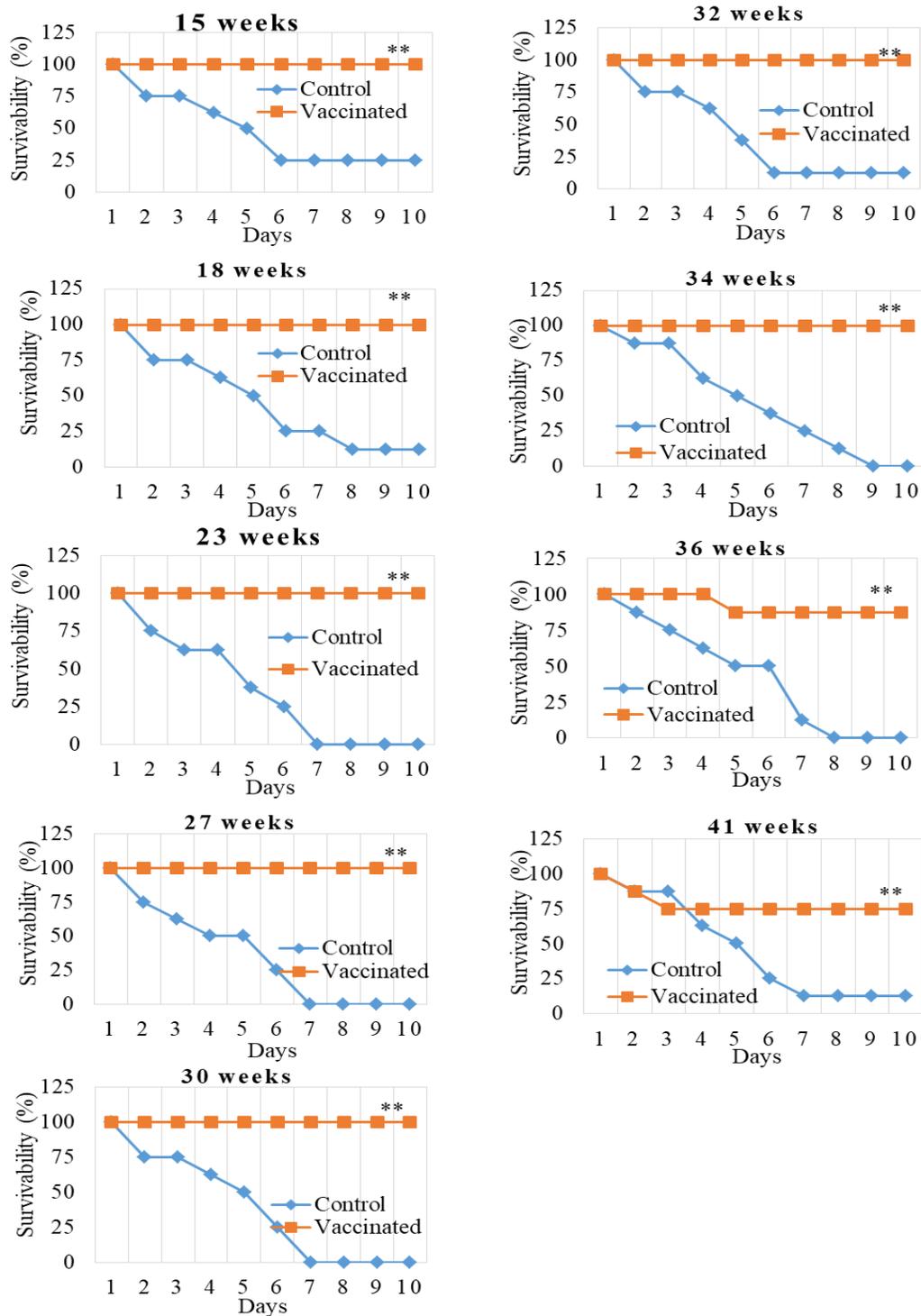


Figure 3. Survival rate of chicken challenged intramuscularly with virulent isolates of *S. pullorum*. Chicken were immunized with BAU-Salmonella bivalent vaccine at 7 weeks of age as primary vaccination and 12 weeks of age as secondary vaccination via IM route dosed at 0.5 mL in each bird. Chickens were challenged through IM with 0.55 mL of virulent strain of *S. pullorum* (5.26×10^{12} CFU/mL) at 15, 18, 23, 27, 30, 32, 34, 36 and 41 weeks of age and the mortality was observed for the subsequent 10 days. ** $P < 0.01$, by Mantel-Cox logrank test.

done by inoculating 0.1 mL of vaccine into blood agar media. As no growth of the organisms was observed after inoculation into the media after inoculation at 37°C for 24-48 h indicated the vaccine was biologically pure. For safety concern, 0.5 mL of vaccine was inoculated subcutaneously to each mouse to a group of 5 mice and the mice were monitored for subsequent 10 days. No clinical signs or mortality was observed within the monitoring and observation period which revealed that the vaccine was pure and safe for vaccination ([OIE, 2008](#)).

PHA test was used to evaluate the sera which were collected from the chickens of both vaccinated and unvaccinated group at different time points of vaccination. The PHA antibody titers of all vaccinated and unvaccinated birds at different age are presented in **Figure 1**. However, the prevaccination PHA titer of sera samples of all vaccinated and control birds were minimum level as tested by PHA which was closely related with many previous reports ([Ferdous, 2008](#); [Modak et al., 2012](#); [Basak and Amin, 2013](#); [Nime et al., 2016](#); [Guo et al., 2016](#)). The PHA antibody titers of vaccinated birds significantly increased after primary and booster vaccination with the advancement of age whereas titers of chickens of group B remained at $\leq 4.0 \pm 0.00$ (**Figure 1**). Most importantly, the PHA antibody titers of vaccinated chickens started to increase after primary and booster vaccination and gone to pick level at 27 weeks of age against both the organisms, after that the titers are started to decrease until 41 weeks of age ([Bhattacharya et al., 2004](#)) of the observation period.

Next we checked the protective potential of the vaccinated chickens at different ages after vaccination whether the vaccine induced immune response protect the chickens from challenge exposure with virulent *S. gallinarum* and *S. pullorum* bacteria. Therefore, challenge infection was given to the chickens of all groups separately containing 8 birds at 15, 18, 23, 27, 30, 32, 34, 36, and 41 weeks of ages after primary and secondary vaccination. Birds of all vaccinated groups challenged with the virulent bacteria resisted 100% against infection until 34 weeks of age (against both *S. pullorum* and *S. gallinarum*). Protection level of the birds against challenge infection reduced up to 75% at 36 and 41 weeks of age after vaccination though antibody titers reduced a little bit (**Figure 2-3**). Therefore, the minimum PHA antibody tires required for 100% protection against challenge infection were 152.00 ± 24.00 and 160.00 ± 29.63 for *S. gallinarum* and *S. pullorum* respectively. However, none of the birds of unvaccinated groups protected from

challenge infection. The controls birds showed specific signs and symptoms of Salmonella infection within 1-2 days of challenge and died within 7 days ([Andino and Hanning, 2015](#); [Langridge et al., 2015](#)).

To confirm whether, the control birds were died due to challenge infection, we further investigated the post mortem lesions of the dead birds. Enlarged and bronzed greenish tint of liver, enlarged spleen, hemorrhagic, misshapen and discolored ova were found in dead chickens that further confirmed the death due to *Salmonella* infections ([Wigley et al., 2001](#); [Hossain et al., 2006](#)). Therefore, the results revealed that the vaccine have the potentiality to protect the chicken ($P < 0.01$) up to 7 months following booster vaccination. In summary, a significant level of antibody response was induced in chicken vaccinated with BAU-Salmonella bivalent vaccine via intramuscular route. In addition, immune protective titer persisted in the serum up to 7 months after secondary or booster vaccination. So, chickens should be revaccinated after 6 months.

CONCLUSION

From the present study, it may be concluded that formalin killed BAU-Salmonella bivalent vaccine prepared in Livestock and Poultry Vaccine Research and Production Center (LPVRPC), BAU, Mymensingh worked satisfactory in terms of survivability pattern against *S. gallinarum* and *S. pullorum* infection up to 7 months following booster vaccination.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

EI, RS carried out the experiments, analyzed the data and wrote the initial draft of the manuscript. MGH modified the figures and revised the manuscript. SMAR helped to

design the research study. SS and MMA designed and supervised research work, rewrite and finalized the manuscript. All authors read and approved the manuscript before submission.

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