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ORIGINAL ARTICLE

Development and evaluation of an indigenous *Salmonella gallinarum* ghost vaccine for the control of fowl typhoid in poultry

Asad Ullah Ghalib[1], Sajjad Hussain[2], Waseem Shahzad[2], Rashid Mir[3,4], Bushra Muneer[1*]

[1]*Prof. Dr. Ikram-ul-Haq Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan*
[2]*Veterinary Research Institute, Livestock and Dairy Development Department, Government of the Punjab, Lahore, Pakistan*
[3]*Prince Fahd Bin Sultan Research Chair for Biomedical Research, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Kingdom of Saudi Arabia*
[4]*Department of Medical Laboratory Technology, University of Tabuk, Tabuk, Kingdom of Saudi Arabia*

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ABSTRACT

Background and Objectives: *Salmonella gallinarum*, causes fowl typhoid, continues to be a significant limitation to poultry production in developing nations, with continuously rising antimicrobial resistance. The current research was design and test an indigenous *S. gallinarum* ghost vaccine and to measure its immunogenicity and protective effect on layer birds.
Materials and Methods: Bacteriological culture of suspected clinical cases of fowl typhoid was performed, and isolates were verified by PCR against the 16S rRNA gene, after which they were sequenced, and phylogenetic analysis was performed. To obtain bacterial ghost cells, a representative native isolate was subjected to a detergent-based chemical lysis procedure, followed up by scanning electron microscopy. 120-day-old layer chicks were divided into six groups randomly and immunized at the age of seven days using oral, intramuscular, air-spray, intramuscular with oil adjuvant or eye-drop routes. There was a 1st dose at 26 days of age in all vaccinated groups and the challenge of virulent *S. gallinarum* at 42 and 93 days of age. ELISA was used to test humoral immune response at different time points.
Results: The highest antibody titers were found in vaccinated groups compared to controls ($p < 0.05$), and the intramuscular route generated the best and most enduring response in systemic immunity. Mucosal pathways elicited not high but rather significant antibody responses and provided protection against clinical disease.
Conclusion: The research contributes to the feasibility of ghost-based vaccination in the role of a sustainable alternative to the disease control measures that are highly reliant on antibiotics in endemic poultry production systems.

Keywords: *Salmonella gallinarum* ghost; Fowl typhoid; Vaccine; Chicken; Efficacy

INTRODUCTION

Salmonellosis is a significant health and poultry production issue at a global scale, and both developed and developing nations continue to be impacted. *Salmonella* genus contains over 2,600 serovars, some of which can cause diseases to both livestock and humans (1). Poultry has been identified as one of

*Corresponding author: Bushra Muneer, Ph.D, Prof. Dr. Ikram-ul-Haq Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan. Tel: +92-321-4641552 Email: dr.bushramuneer@gcu.edu.pk; bushramuneer11@yahoo.co.uk

the most significant reservoirs of various serotypes of pathogenic organisms, which are transmitted via the food system. Salmonellosis has remained among the most common causes of food-borne disease in the world, even though hygiene, biosecurity and processing standards have been improved (2). Host-adapted serovars of *Salmonella*, including *Salmonella gallinarum*, continue to cause endemic disease in most areas, particularly those with high density production with less biosecurity pressure on the perpetuation of the pathogen (3).

Salmonellosis is an under-reported and major issue in commercial and home scale poultry systems in Pakistan, where climatic factors, large flock density and inconsistent farm management practices all play a role in perpetuating the disease. Multiple investigations have reported the presence of *S. gallinarum* and *S. pullorum* within breeder, layer and broiler flocks with both clinical and subclinical carrier conditions facilitating both vertical and horizontal infection (4). The lack of regular surveillance, irregular vaccination, and unrestricted mobility of birds contributes to even more complicated control. Extensive use of antimicrobials without proper sensitivity testing has facilitated the development of multi drug-resistant strains of *Salmonella* that pose risks to both the health of poultry and to the human health via possible food-borne channels (5).

The clinical and subclinical diseases caused by *Salmonella* infections in poultry severely affect the productivity and welfare of the flocks. *S. gallinarum* causes fowl typhoid that is marked by septicemia, depression, anorexia, greenish diarrhea and high mortality especially in adult layers and breeder birds (6). There will also be decreased production and poor quality of eggs, and vulnerability to secondary infections in the infected flocks. Recovered birds are usually carriers and shed bacteria every now and then and keep on transmitting the bacteria at the farm level. Carrier birds are a significant challenge to eradication, and traditional hygienic and culling approaches are not able to address the challenges of the high-density production setting (7).

The economic effects of poultry salmonellosis are enormous and are not only related to death of the animals. The losses are due to reduced productivity, low feed ratio, higher veterinary expenses, extended production time and trade embargo on infected flocks (8). Chronic infections in layer operations lead to long term reductions in egg production and shell quality whereas breeder infections affect hatchability and quality of chicks. On a national scale, frequent outbreaks lead to decreased profitability of the poultry industry, higher production expenses and supply chain instability. Moreover, the presence of *Salmonella* in poultry products will lead to the rejection of export consignments and, thus, the international trade and the development of the national economy (9).

Vaccination has become an important alternative method to control *Salmonella* especially considering increased antimicrobial resistance. The conventional use of antibiotics has not only been ineffective in removing carrier states but has been an added factor in the selection of resistant strains and reduced treatment options and also created more zoonotic risk (10). Vaccines also provide a long-lasting solution to the problem by minimizing the colonization and shedding of the bacteria and the severity of the disease, which breaks the transmission cycles. Bacterial ghost vaccines have attracted interest as one of the new vaccine platforms because they maintain native antigenic structures whilst being entirely non-viable (11). These sterile bacterial envelopes can retain surface antigens that can stimulate humoral and cellular immune responses and can be used as strong candidates of safe and effective immunization strategies (12).

The current research seeks to construct and test an indigenous *S. gallinarum* ghost vaccine to control fowl typhoid in poultry using a layer bird as the experimental subject. The use of local strain of circulation increases the antigenic and possible field efficacy of vaccines. Controlled vaccination and challenge trials will determine the immunogenicity and protective efficacy of the ghost vaccine and humoral immune responses will be measured by enzyme-linked immunosorbent assay (ELISA). This study aims at developing evidence-based, scientifically proven, and locally flexible vaccination to decrease reliance on antimicrobials and form lasting mechanisms of controlling fowl typhoid in the Pakistani poultry sector.

MATERIALS AND METHODS

Sample collection. Birds with clinical evidence of fowl typhoid were chosen, and necropsy of clinically ill or recently dead birds was performed under asep-

tic conditions. Tissue samples (liver, spleen, lungs, intestine, and heart), which are primary target organs in systemic salmonellosis, were collected, placed in sterile bags, and transported to the laboratory under chilled conditions to preserve bacterial viability. Every sample was labeled appropriately with farm ID, type of tissue, and date and time of collection to allow traceability and data integrity (13).

Isolation of *Salmonella gallinarum*. *S. gallinarum* was isolated through standard bacteriological techniques. Each tissue sample was homogenized (25 g) in Buffered Peptone Water (BPW) and incubated at 37°C for 18-24 hours in primary enrichment. A subculture was then placed in Rappaport-Vassiliadis (RVS) broth and incubated at 42°C for 24 hours to selectively enrich. Bacteria were streaked on MacConkey agar, and non-lactose-fermenting isolates were subcultured and molecularly identified (14).

Molecular confirmation of *Salmonella gallinarum*. The confirmed isolates were used to extract genomic DNA using Qiagen DNeasy Mini Kit as instructed by the manufacturer. Conventional polymerase chain reaction (PCR) with the cyclic initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1.5 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min. The 50 µl of reaction mixture consisted of 50 ng of genomic DNA, 2.5 units of *Taq* polymerase, 5 µl of 10 X buffer (100 mM Tris-HCl, 500 mM KCl pH-8.3), 200 µM dNTPs, 1.5 mM $MgCl_2$ and 10 pmoles of each primer. The Forward 27F 5' (AGAGTTTGATCMTGGCTCAG) 3' and Reverse Primer 1492R 5' (TACGGYTACCTTGTTACGACT T) 3' were used. The amplified PCR products were analyzed by gel electrophoresis through a 1% agarose gel in TAE buffer. Bands were detected by ethidium bromide staining and UV transillumination. Amplified PCR products were then purified with QIAquick PCR purification kit (Cat no./ID. 28104) as per the manufacturer's instructions. Samples were sent for sequencing. Sequences were analyzed using the blast tool against the NCBI core nucleotide database with a similarity threshold of ≥90% for species-level identification. The molecular identity of the isolates was positive and confirmed *S. gallinarum* (15).

Bioinformatic analysis of *S. gallinarum*. Trimming and alignment of sequencing data on confirmed isolates were done with BioEdit software. The MEGA X software was used to conduct phylogenetic analysis to evaluate the genetic relatability of native isolates and standard strains of *Salmonella gallinarum*. The identity and diversity of strains were confirmed with phylogenetic trees developing with the help of the proper evolutionary models (16).

Indigenous *S. gallinarum* ghost cells preparation. The preparation of indigenous *S. gallinarum* ghost cells was done as a detergent based chemical lysis technique. Pure cultures were cultured in Luria-Bertani broth with 7% Tween-80 and left to incubate overnight at 37°C. The pH of the culture was then adjusted to 3.6 to cause gradual lysis of the membranes and maintenance of the outer membrane structures, after which the culture was standing at room temperature, one hour. Cells were centrifuged, and washed three times with half strength normal saline to remove cytoplasmic content and traces of detergent to ensure total inactivation and standardize the concentration of ghost cells (17).

Ghost formation confirmation using scanning electron microscopy. The scanning electron microscopy was used to verify the morphology of ghost cells. The samples were acetone dehydrated using graded dehydration in 2.5% glutaraldehyde followed by gold sputter coating. SEM imaging was also carried out to determine membrane integrity and the presence of transmembrane lysis pores which showed successful ghost formation (18).

Vaccine trials on layer chicks. To evaluate various routes and formulations of an indigenous *S. gallinarum* ghost vaccine, day-old layer chicks (n = 120) were randomly segregated into six groups (20 birds each). Group A was used as non-immunized control and Groups B to F were administered the vaccine through oral, intramuscular, air-spray, intramuscular with Montanide™ ISA 70 VG (SEPPIC) adjuvant and eye-drop routes respectively using 1×10^{10} ghost cells respectively (19). At 26 days following the primary vaccination, all groups receiving vaccination were boosted. Virulent *S. gallinarum* (1×10^{10} ghost cells) challenge on Day 42 and Day 93 was used to test early and long-term protection and monitored after the challenge to identify clinical manifestations, mortality and immune system as mentioned in Table 1.

Table 1. Experimental design of the chick vaccination trial

Group	Number of Chicks	Vaccination Route	Vaccine Dose	Adjuvant	Booster (Day 26)	Challenge Dose by live *oklu't-mn43*	Challenge Days
A – Control	20	None	None	None	No	1×10^{8} cells	Day 42 & Day 93
B – Oral	20	Oral administration	1×10^{10} cells	None	Yes	1×10^{8} cells	Day 42 & Day 93
C – Intra-muscular	20	IM injection	1×10^{10} cells	None	Yes	1×10^{8} cells	Day 42 & Day 93
D – Air Spray	20	Spray application	1×10^{10} cells	None	Yes	1×10^{8} cells	Day 42 & Day 93
E – IM + Oil Adjuvant	20	IM injection	1×10^{10} cells	Montanide™ ISA 70 VG	Yes	1×10^{8} cells	Day 42 & Day 93
F – Eye Drop	20	Eye-drop instillation	1×10^{10} cells	None	Yes	1×10^{8} cells	Day 42 & Day 93

*ELISA blood sampling was strategically included at:
Day 28 → One day after booster (Day 26)
Day 49 → One week post first challenge (Day 42 + 7 days)
Day 87 → One week before second challenge

Serological immune evaluation. A commercial ELISA kit was employed to determine humoral immunity on *Salmonella* spp. On Day 1 (post-vaccination), a week after 1st challenge and one week after 2nd challenge, blood samples were taken from the wing vein. The serum was centrifuged and kept at -20°C after which antibody titers were studied (20).

Statistical analysis. All quantitative data, including ELISA titers, weight changes, and post-challenge bacterial loads, were analyzed using One-way ANOVA to compare differences among the six vaccinal groups at each sampling point (Table 1). When ANOVA indicated significant variation, Tukey's post hoc test was applied for pairwise comparison of group means. Results were expressed as mean ± SEM, and significance was considered at $p < 0.05$ (17).

RESULTS

Isolation and cultural identification of *Salmonella gallinarum*. Bacteriological culture yielded colonies consistent with *Salmonella* spp. On MacConkey agar. The isolates formed pale, non-lactose-fermenting colonies, remaining colorless to light yellow after incubation at 37°C, with smooth, round morphology and an approximate diameter of 1-3 mm (Fig. 1). These cultural features supported presumptive identification of *S. gallinarum* and enabled differentiation from lactose-fermenting Enterobacteriaceae commonly encountered in poultry samples.

Fig. 1. *Salmonella gallinarum* colonies on MacConkey Agar

Molecular confirmation and phylogenetic characterization. All of the purified isolates were identified as *S. gallinarum* by PCR using Forward and Reverse primer set, where all tested isolates amplified as expected, whereas controls performed. The amplified fragment was sequenced and found to have a 1415-bp consensus sequence that was deposited in NCBI and was assigned accession number CP116616 (described as *Salmonella enterica* subsp. *enterica* serovar Gallinarum). The local isolate was assigned to the *Salmonella enterica* subsp. *enterica* clade by phylogenetic analysis and clustered with reference Gallinarum strains containing more than 99% of the evolutionary similarity (Fig. 2). The phylogeny revealed that short branch lengths and low nucleotide divergence (scale bar 0.0002 substitutions per site) indicated that *S. gallinarum* is clustered very closely in genetics to the

reference *S. gallinarum* sequences and that there is no confusion with other *S. enterica* serovars that were incorporated in the study to guarantee comparison.

Ultrastructural verification of ghost cell development. The morphology of the chemically treated cells under the scanning electron microscopy showed morphological features related to ghost cell formation. Several discrete transmembrane pore holes were found on the bacterial surface (Fig. 3) and had a diameter of the pore below the micron range (say 500-1400 nm). The total cell envelope and outer architecture were also intact without any indication of the total breakdown or fragmentation, therefore, validating the formation of bacterial ghosts with retained surface structure.

Humoral immune response post-vaccination (pre-challenge). The humoral response of serum antibody titers at a specific age at 28 days of age varied significantly across groups, and it was apparent that humoral responses followed a route-dependent pattern after the *S. gallinarum* ghost vaccine was introduced (Fig. 4). The non-immunized control group had lower levels of antibodies than the vaccinated groups, and there was a significant difference between the different groups ($p<0.05$). Post-hoc multiple comparison testing separated the treatments into homogeneous subsets of tests by mean titers. Intramuscular route (Group C) had the highest mean antibody titer and had a distinct subset meaning that the responses were much stronger compared to other routes. The same responses were observed in the intramuscular formula-

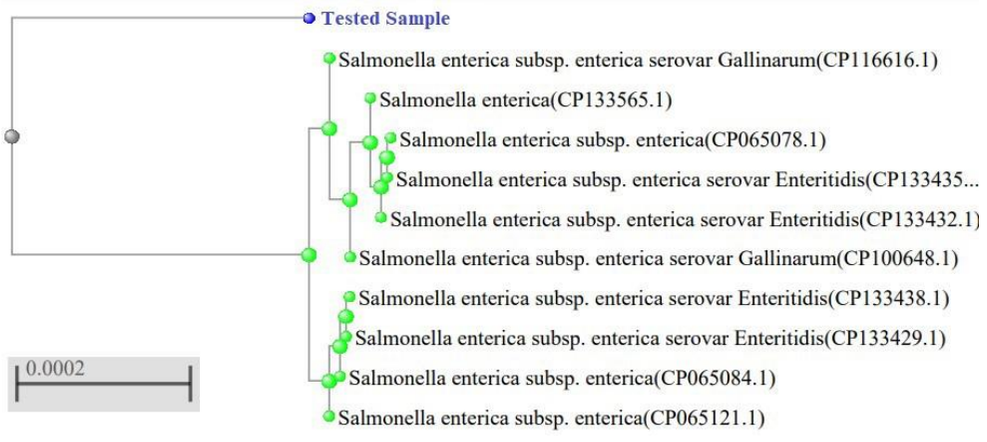

Fig. 2. Phylogenetic analysis of the tested isolate showing its close genetic relationship with *Salmonella enterica* subsp. *enterica* serovar Gallinarum.

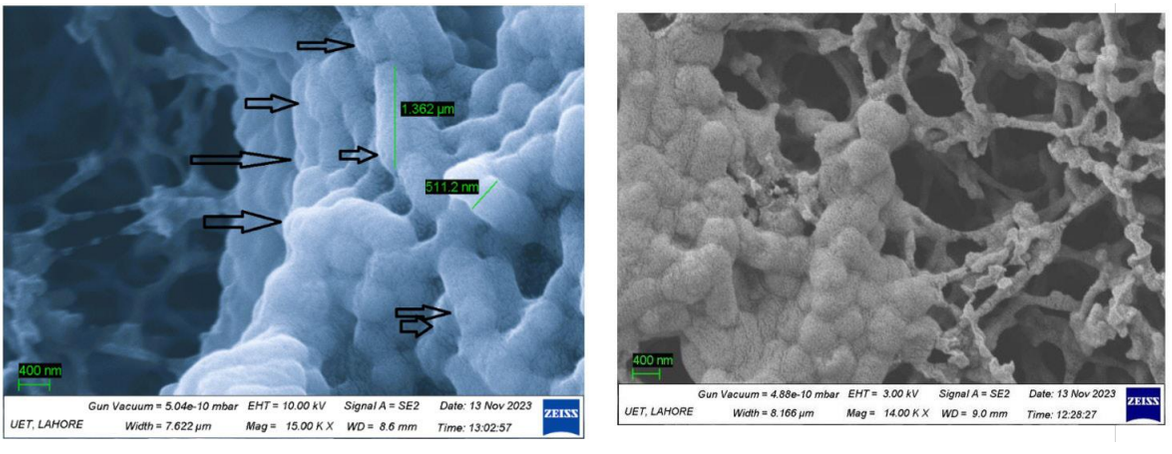

Fig. 3. A. Scanning electron micrograph of *Salmonella gallinarum* ghost cells. Arrows indicate the presence of transmembrane micro-holes on the bacterial cell surface, representing controlled pore formation during ghost cell generation.
B. Scanning electron micrograph of normal non-treated *Salmonella gallinarum*

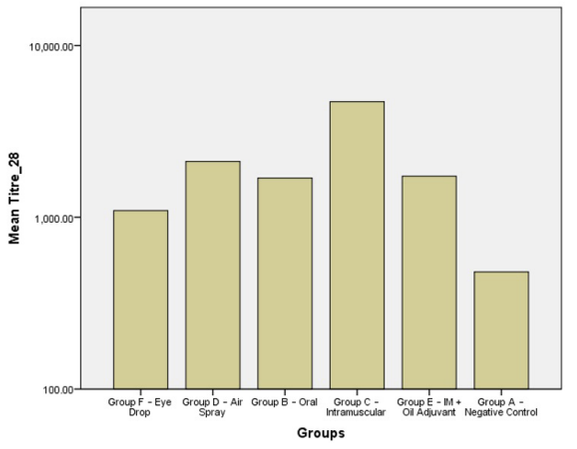

Fig. 4. Mean antibody titers measured at 28 days post-vaccination in birds immunized with the *Salmonella gallinarum* ghost vaccine at 7 days of age via different administration routes (eye drop, air spray, oral, intramuscular, and intramuscular with oil adjuvant), compared with a negative control group

tion with oil adjuvant (Group E) and oral route (Group B) and they clustered into the same subset. The air-spray route (Group D) had the highest titers than oral and eye-drop routes and the eye-drop route (Group F) had the lowest titers in both vaccinated groups though still higher as compared to control. The lowest titers were always obtained in the control group (Group A). Humoral immune response 1 week after first challenge. The antibody titers collected at 49 days of age (Day 49) rose in the vaccinated groups following challenge at 42 days of age and were significantly different among treatments (overall $p < 0.05$; Fig. 5). The intramuscularly vaccinated (Group C) birds demonstrated the highest mean antibody titer and represented a separate homogenous group in a post-hoc analysis, showing responses at a significantly higher level than that of all other groups. Intermediate antibody responses were detected in the air-spray group (Group D), intramuscular with oil adjuvant group (Group E) and oral group (Group B) as they formed similar homogeneous subsets, indicating similar post-challenge humoral responses between these routes. Group F (eye-drop) recorded lower titers compared to the intermediate groups, and the control group (Group A) was lowest. At the end of the first challenge, there were no deaths in any group observed.

Humoral immune response before second challenge and clinical observations. Before the second challenge (93 days of age) antibody titers measured at 87 days (Day 87) across groups were found to be significantly different (overall $p < 0.05$; Fig. 6). The intramuscular group (Group C) had the best mean titer and was found to be statistically superior to other routes in post-hoc comparisons. The intermediate titers were observed in the air-spray (Group D), intramuscular with oil adjuvant (Group E) and oral (Group B) groups with an overlapping homogeneous subset,

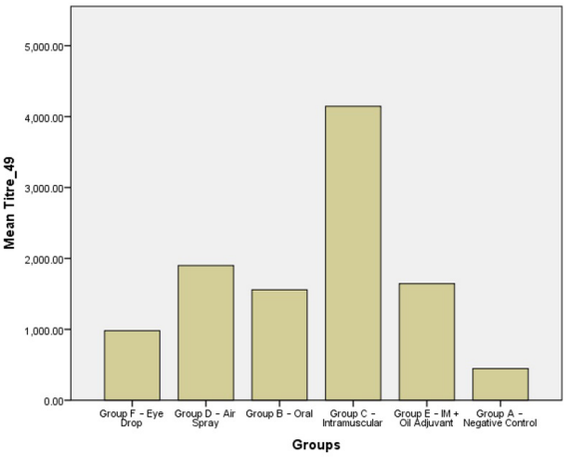

Fig. 5. Mean antibody titers measured at 49 days of age (one week post-challenge) in birds vaccinated with the *Salmonella gallinarum* ghost vaccine at 7 days of age via different administration routes (eye drop, air spray, oral, intramuscular, and intramuscular with oil adjuvant), compared with a negative control group.

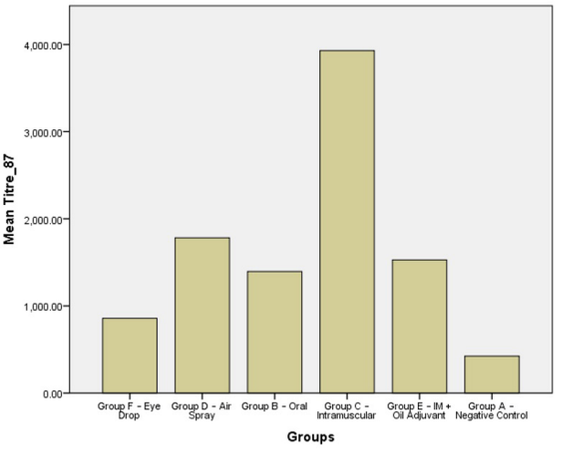

Fig. 6. Mean antibody titers measured at 87 days of age in birds vaccinated with the *Salmonella gallinarum* ghost vaccine at 7 days of age via different administration routes (eye drop, air spray, oral, intramuscular, and intramuscular with oil adjuvant), following the second challenge at 93 days of age.

but lower titers but persistent in the eye-drop group (Group F). The lowest titers were again registered in the control group (Group A). After the second challenge, vaccinated birds had mild loose droppings that spontaneously resolved in two days and none of them died.

Repeated measures analysis. Repeated measures ANOVA showed that time has a significant main effect on antibody titers at various sampling points showing that antibody levels vary significantly throughout the study. The test of the sphericity was significant ($p = 0.009$), and the corrections by Greenhouse-Geisser and Huynh-Feldt were made; the effect of time was highly significant after correction ($p < 0.05$). Multivariate tests ('Pillai's Trace, Wilks' Lambda', Hotelling Trace and Roy Largest Root) were repeatedly significant to show that there was significant temporal variation ($p < 0.05$). There was also a significant time x group interaction ($p < 0.05$) which implies that there was a difference in the pattern of change in antibody with time in the vaccination strategies. The analysis of the results using the technique of the analysis of variance demonstrated that the linear trend was significant with a significant quadratic component, and it indicates a progressive change in the antibody titers per group over time with varying dynamics.

DISCUSSION

The current research has shown effective development and testing of a native *S. gallinarum* ghost vaccine and its potential to induce a robust and prolonged humoral response in layer birds upon administration via various routes. The isolation and the molecular confirmation of the *S. gallinarum* strains circulating locally and then the phylogenetic validation ensured that the antigen vaccine was representative of the strains in the fields making it more potentially applicable in practical disease control in the endemic poultry systems (21).

Cultural and molecular identification of the isolates revealed that it was *Salmonella enterica* subsp. *enterica* serovar Gallinarum with a high genetic similarity of more than 99% sequence similarity of the isolates to reference strains. The preserved character of the 16S rRNA gene among the *S. gallinarum* strains has also been largely documented and represents the host-adapted and clonal population structure of this serovar (22). This genetic stability benefits the vaccine design since the antigenic targets run less risk of showing a great range of variations in the geographical locations and, therefore, a vaccine developed locally is generally applicable across the broad regions of endemics (23). Patterns of phylogenetic clustering have been carried out before by researchers in Asia and Africa, showing that there is little divergence between Gallinarum isolates across different parts of the world (24).

The scanning electron microscopy confirmed successful ghost cell formation in the form of intact cell envelopes with individual transmembrane pores. This resistance is a hallmark of bacterial ghost technology and is essential in the preservation of indigenous surface antigens needed in successful immune detection (25). The controlled release of cytoplasmic contents through the presence of controlled pores maintains pathogen-associated molecular patterns including lipopolysaccharides and outer membrane proteins which are natural adjuvants (26). These results confirm the detergent-based lysis technique as a safe, efficient, and efficient method of producing ghost vaccines without genetic manipulation, indicating earlier studies that indentured ghosts can be produced by using chemicals to produce a vaccine with immunogenic and structurally intact bacterial envelopes (27).

Several pieces of humoral data immune response evidence showed that the route of vaccination had a significant impact on the production of antibodies. Birds inoculated through the intramuscular route had the highest antibody titers in all sampling locations, pre- and post-challenge, which showed a high level of systemic immune stimulation (28). This finding concurs with previous reports of higher IgY production of ghost vaccines administered through parenteral routes than through mucosal routes as a result of effective adsorption of antigens by professional antigen-presenting cells in muscle tissue. The intramuscular route enhances the deposition of the antigens and antigen exposure with resultant increased B-cell stimulation and memory development that could be the reason behind the sustained antibody titers observed in the present study as long as the age of the layers was up to 87 days (29).

Interestingly, intramuscular vaccination using oil adjuvant failed to result in significantly higher antibody titers than intramuscular vaccination in the absence of adjuvant and this indicates that the ghost

cells themselves are inherently adjuvant (30). Bacterial ghosts have not lost various pathogen-associated molecular patterns that are capable of triggering innate immune receptors, including Toll-like receptors, and thus increase antigen presentation and immune response. This inherent immunostimulatory property can decrease the use of exogenous adjuvants which is beneficial to vaccine safety and cost (31).

Orally administered, air-spray and eye-drop vaccination based on the mucosal route had generated moderate yet significant antibodies in comparison to controls. Despite lower levels of systemic immunoglobulins than those induced during intramuscular vaccination, mucosal immunization is preferentially known to stimulate local immune responses in respiratory and gastrointestinal mucosa which are prime entry points of *Salmonella* infections (32). Previous research has indicated that mucosal vaccines could offer viable protection with low serum antibody titers because of induction of secretory IgA and cell-mediated immune against mucosal surfaces. The current experiment had not determined the level of mucosal antibodies and cellular immune markers and this could have been the reason why protection was achieved even in the groups with comparatively low systemic antibody levels (33).

After exposure to challenge, no mortality was ever seen in all the vaccinated groups, and only transient, mild clinical manifestations were seen in the second challenge. This observation shows that the ghost vaccine was effective in offering protection against virulent *S. gallinarum* challenge in all routes of vaccination (34). Other ghost vaccine experiments have shown similar protective effects, with vaccinated birds having reduced levels of bacteria colonization and low clinical disease despite intermediate levels of the systemic antibody. These findings indicate that humoral and cellular immune responses might be important in protection against fowl typhoid as well as indicate that antibody titers, in and of themselves, might not be entirely predictive of protective efficacy (35).

The repeated measures test corroborated the presence of time effects as well as significant interaction of time and vaccination route, whereby the immune kinetics varied in response to the mode of antigen delivery. The increasing and sustaining of antibody titers following challenge indicates the formation of immunological memory, which is necessary in offering lasting protection in layer flocks that stay in production over a long period (36). The recorded quadratic curve of antibody responses is an initial priming period, with subsequent amplification upon returning to antigens, which is in line with classical adaptive immune responses that have been documented in the literature of vaccinating poultry (37).

In terms of disease control, the findings are especially applicable to the regions where fowl typhoid is still endemic, and the antimicrobial resistance becomes more and more restrictive to the control measures based on antibiotics. The *Salmonella* strains that are multidrug-resistant are common in the South Asian poultry systems such as Pakistan and they pose danger to the health of both animals and people (38). Ghost-based platforms have a potential to be used as an alternative to vaccination that would help to cut down the bacteria load, minimize environmental pollution, and decrease antibiotic usage. Additionally, indigenous strains could be better antigenic matches to circulating field isolates, and this could add to better real-world vaccine efficacy (39).

A significant benefit of ghost vaccines is that they have a safety profile as they are non-replicative and cannot revert to virulence, unlike other live attenuated vaccines. This renders them especially suitable in breeder and layer business, where vertical dissemination and carriage conditions are of key issues (40). Moreover, the capability of delivering ghost vaccines through a mass administration methodology like spray and oral routes has practical benefits in large-scale poultry production whereby a single individual injection is expensive and labour intensive. Even though highest antibody titers were obtained by intramuscular vaccination, air-spray and oral routes also provided protection demonstrating their possible value in field vaccination efforts (38).

The research anyway has some limitations, even though the findings are promising. There was no assessment of cellular immune responses, mucosal levels of antibodies and shedding of bacterial which would give a better picture of the mechanisms. Moreover, field tests would be required to test the performance of the vaccines under commercial conditions over a long period to ensure its reliability to natural exposure and fluctuating environmental pressure. Future research that includes cytokine profiling, intestinal colonization assays and transmission dynamics would enhance the evidence base to practice the use of this vaccine platform. A notable limitation of this study is the absence of fecal bacterial shed-

ding quantification post-challenge, which precluded assessment of whether vaccination reduced transmission risk and mucosal clearance of *Salmonella*.

CONCLUSION

The current study indicates that a native *S. gallinarum* ghost vaccine is scalable and elicits strong and prolonged immune reaction in layer birds, and it can protect against experimental fowl typhoid obstruction. The strongest humoral responses were obtained by intramuscular delivery and mucosal routes also offered protective immunity with logistical benefit to mass vaccination. These results justify the possibility of ghost-based vaccines to be safe, efficient, and sustainable to fowl typhoid control measures that rely on antimicrobials, especially in endemic areas of poultry production.

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