Immunogenicity and protective efficacy of irradiated *Salmonella* Gallinarum against homologous challenge infection in Bovans brown chickens

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Abstract

Fowl typhoid is a systemic poultry disease caused by *Salmonella* Gallinarum (SG). It is responsible for significant economic loss, due to its severe morbidity and mortality. An irradiated vaccine is one of the possible alternatives to prevent and control fowl typhoid. This study aimed to evaluate the safety, immunogenicity, and protective efficacy of irradiated SG using a randomized control trial in chicken. A field strain of SG was exposed to different doses of gamma irradiation to determine its effect on the viability of SG. Safety and immunogenicity were assessed by administering irradiated SG orally to 3 groups (5 each) of 5 weeks old Bovans brown chickens at 2400, 2500, and 2600 gray (Gy). The protective efficacy of 10^8 colony forming units (CFU) of SG irradiated at 2400 Gy administered orally and subcutaneously was then evaluated using homologous challenge infection and compared with SG 9R commercial vaccine using 40, 5-week old Bovans brown chickens where the chickens were randomly assigned to 4 groups. Chickens in Group 1 were exposed to 10^8 CFU of irradiated SG orally; Group 2 to the same dose subcutaneously; Group 3 to SG 9R strain commercial vaccine subcutaneously, Group 4 to phosphate-buffered saline (PBS) orally. Data related to survival, antibody response, and pathological lesions were recorded. Mann-Whitney U-test, Kruskal-Wallis test, and Fisher's exact tests were used to examine for statistical significance. Irradiation at 2600 Gy caused complete inactivation of SG whereas SG exposed to 2400 Gy showed better immunogenicity and was safe for chickens. Antibody response in a group of chickens vaccinated with irradiated SG administered

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subcutaneously (SC) was significantly higher than those vaccinated with the SG 9R vaccine on day 7 (p=0.003) and day 14 (p=0.002) post-immunization. Comparative evaluation of the protective efficacy based on the mortality rate of chickens after challenge showed that 2400 Gy irradiated SG vaccine administered SC and SG 9R vaccine-induced equal protection of 50% while the irradiated vaccine administered orally protected only 10% of chickens against homologous challenge infection. SG was not isolated from the liver, spleen, and feces of chickens that survived challenge infection until the end of the experiment. Irradiated SG administered SC is shown to be a promising vaccine against fowl typhoid. Further studies using a large sample size involving tuning of irradiation dose to improve immunogenicity and use of booster vaccination are recommended.

Keywords: Chicken; Fowl typhoid; Gamma irradiation; *Salmonella* Gallinarum; Vaccine

Introduction

Fowl typhoid (FT) is a systemic disease of poultry caused by *Salmonella* Gallinarum (SG), which results in septicemia, high morbidity, and mortality as well as severe inflammation of internal organs such as liver and spleen. Chickens are the natural hosts of SG which is responsible for significant economic losses to the poultry industry worldwide (Barrow and Freitas, 2011). Although FT has been eradicated from commercial poultry farms in many developed countries through the isolation, removal of contaminated flocks, implementation of biosecurity, and hygienic practices; it is still a major problem of poultry production in many developing countries (Desin *et al.*, 2013; Revolledo, 2018). Previous studies conducted in different areas of Ethiopia showed that the overall prevalence of SG among chicken flocks ranges from 0.8% to 44.8% (Aragaw *et al.*, 2010; Alebachew and Mekonnen, 2013; Kumar *et al.*, 2014). Vaccination has been the most practical and effective strategy for the control of fowl typhoid in developing countries (Laniewski *et al.*, 2014).The most commonly used vaccine against FT is a commercial live vaccine derived from the stable rough strain of *S.* Gallinarum (SG 9R)(Revolledo, 2018). However, this vaccine has several drawbacks such as the presence of residual virulence, which is particularly serious in some strains of chickens, prolonged survival in feces, litter or dust, and its persistence in immunized chickens leading to transmission through eggs (Kwon and Cho, 2011). Therefore, there is a need for novel, safe and effective vaccines for prevention and control of FT.

Inactivated vaccines produced by killing the pathogens with chemicals, heat, or radiation are believed to be more stable and safe compared to live vaccines (Rosenthal and Zimmerman, 2006). Vaccines developed by irradiating pathogens have been reported as strong inducers of cellular and humoral immune responses (Eberl *et al.*, 2001; Zorgi *et al.*, 2011). Gamma irradiation is capable of destroying nucleic acids without damaging the pathogen surface antigens and can also eradicate chemical contaminants (Seo, 2015). A previous study on the possible use of gamma-irradiated rotavirus showed a lack of infectivity of virus inactivated at 50 Kilo Gray(KGy) and induction of strong neutralizing antibody responses in mice (Shahrudin *et al.*, 2018). This study aimed to evaluate the safety, immunogenicity, and protective efficacy of irradiated SG and compare it with the existing commercial SG 9R vaccine.

Materials and methods

Experimental animals and experimental design

A total of 60 female Bovans brown breed of chickens at the age of 5 weeks were purchased from a small-scale poultry farm in Bishoftu town, located about 40 Km South of Addis Ababa. The farm receives and grows day-old chickens from a branch of EthioChicken Private Limited Company, one of the large chicken multiplication farms in Ethiopia located in Adama Town. The chickens were purchased twice, first 20 of them for investigation of safety and immunogenicity of irradiated SG, and on the second occasion, the other 40 chickens for assessment of protective efficacy of irradiated bacteria. The chickens were kept in cages with area of 1 meter by 1 meter and height of 90 cm. Five chickens were kept per cage in the animal house facility of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. They were provided with water and feed free of antibiotics. All chickens used in the current experiment were vaccinated against Marek's, Gumboro, and Newcastle disease while they were on multiplication farm. The complete randomized experimental design was used to group experimental animals into different treatment groups.

Bacterial strain and vaccine

A field strain of SG originally isolated from a poultry farm in Ethiopia during the previous epidemiological study of *Salmonella* in a poultry farm was used to assess the effect of different irradiation doses and to evaluate the protective efficacy of the irradiated vaccine. The isolate was confirmed with biochemical tests and serotype-specific PCR as described previously (Yang, *et al.,* 2014).

Live attenuated rough strain of SG (SG 9R) strain vaccine, produced at National Veterinary Institute, Bishoftu, Ethiopia (Batch no. Ft 02-18) containing $5x10^7$CFU per dose was employed as a control vaccine.

Determination of optimum bacterial titer and irradiation process

Three uniform isolated colonies of SG grown for 18 hrs. on Tryptone Soya Agar (Oxoid, UK) were inoculated into 5ml nutrient broth (Oxoid, UK) and cultured at 37°C for 6 hrs. One ml of the suspension was withdrawn every hour for five hrs. and serial dilution ranging from 10^{-1} to 10^{-6} was performed in 9 ml phosphate-buffered saline (PBS). Hundred µl of each dilution was inoculated to plate count agar (Oxoid, UK) using the spread plate technique. The number of CFU was counted after 24 hours of incubation at 37°C to determine the time required to reach 10^8 CFU/ml.

After determination of the time required to reach 10^8 CFU/ml, which was 5 hrs., 3 uniform colonies were inoculated into the nutrient broth and grown for 5 hrs. at 37°C. Then culture suspension was centrifuged at 6000 revolutions per minute at 4°C for 15 min. The supernatant was discarded and the pellet of cells was washed twice with PBS and re-suspended in 5ml PBS in 15 ml falcon tubes. It was then transported to the National Institute for Control and Eradication of Tsetse and Trypanosome (NICETT) at Kaliti, Addis Ababa, in an icebox where irradiation was conducted. Suspended bacterial cells were exposed to gamma irradiation with doses ranging from 500-3000 Gy using a cobalt 60 irradiation machine (MDS NORDION, Canada).

Viability, safety, and immunogenicity of irradiated SG

Viability of SG exposed to different irradiation doses was assessed by inoculating serial dilution of irradiated SG in PBS on plate count agar and incubating for 24-48 hrs. at 37°C. Then the number of CFU/ml of suspension and lethal irradiation dose of the bacteria were determined through counting visible colonies. Various irradiation doses were examined to find the lowest optimum irradiation at the margin of the lethal dose. Twenty, 5 weeks old Bovans brown chickens were acclimatized to the laboratory condition for 1 week before initiation of the experiment. During this time, the chickens were screened for the antibody of SG using slide agglutination test (SAT) according to Quinn *et al* (1999) and none of the experimental chickens were seropositive.

Chickens were randomly assigned to four groups each containing 5 chickens. The first 3 groups G1, G2, and G3 were administered subcutaneously (SC) with 10^8 CFU of SG irradiated with 2400, 2500, 2600 Gy in 0.5 ml of PBS, respectively. The fourth group served as a negative control and administered with 0.5ml of PBS SC. Following vaccination, chickens were monitored daily for any unwanted reactions such as anorexia, depression, diarrhea, and death if any for 21 days. The liver and spleen of dead chickens were examined for the presence of SG by plating on general and selective media. For the detection of SG antibody using the SAT test, blood samples were collected from wing vein at day 7 and day 14 post vaccination (Quinn *et al.*, 1999). Safety was measured by injection site reactions such as pain and swelling, systemic reactions like fever and anorexia, and lesion in the liver and spleen.

At the end of the experiment, all chickens were sacrificed and a postmortem examination was conducted to assess gross pathology in each group. Chickens were euthanized by cervical dislocation. It was carried out by grasping the leg of the chicken and stretching the neck and pulling on the head through applying a ventrodorsal rotational force to the skull (AVMA, 2013). Gross lesions on the liver and spleen were given scores of "0" when there is no lesion, "1" for mild few necrotic foci in the liver "2" for enlarged liver, but gizzard not covered, or "3" when there is a severe lesion that covered the gizzard as described previously (Matsuda *et al.*, 2011; Adamu *et al.*, 2017). Samples of liver and spleen were ground and cultured for isolation of *Salmonella* strains from chickens sacrificed for postmortem examination at the end of the experiment.

Vaccination and challenge infection

Once the best immunization irradiation dose was established, i.e. 2400 Gy, forty, 5 weeks old chickens confirmed negative for the antibody of SG using SAT were randomly assigned into 4 groups, containing 10 chickens per group. Group 1 and Group 2 received 10^8 CFU of SG irradiated at 2400 Gy in 0.5 ml of PBS orally and subcutaneously, respectively. Group 3 received 0.2 ml of commercial SG 9R strain vaccine containing 10^7 CFU and Group 4 received 0.5 ml of PBS orally. Twenty-one days post-immunization; all chickens were challenged with 10^8 CFU of field strain SG suspended in 1 ml of PBS orally.

Chickens were observed daily for any clinical signs such as depression, loss of appetite, and diarrhea, and the number of sick and healthy chickens per each

group was recorded daily. Blood samples were collected from each chicken after immunization, at day 7, and 14 post-vaccination to determine the presence of specific antibody using SAT (OIE, 2018). Agglutination reaction was given scores of "0" for no agglutination, "1" for weak agglutination, "2" for moderate agglutination; and "3" for strong agglutination. Inventory of healthy, sick and dead chickens was recorded every day. Postmortem examination was conducted in all dead chickens during the challenge period and for all survivors, at the end of the study on day 21 post-challenge. The scoring of gross lesions observed was conducted as shown above. Liver and spleen samples were collected aseptically into a sterile plastic container for isolation of *Salmonella* from chickens that were sacrificed for postmortem examination at the end of the experiment (day 21). Vaccine protective efficacy was calculated using the formula developed previously (Orenstein *et al.*, 1985). Briefly, EV = [(ARU-ARV)/ARU]*100; where EV= Efficacy of vaccine; ARU=Attack rate (morbidity and/or mortality) of the unvaccinated group; and ARV= Attack rate (morbidity and/or mortality) of the vaccinated group.

Ethics consideration

This study was reviewed and approved by the Institutional Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Ref. No.: ALIPB/IRB/008/2015/16) prior to initiation of the experiment. Chickens were handled following the guiding principles for biomedical research involving animals and complies with the ARRIVE guideline of National Centre for Replacement, Refinement, and Reduction of Animals in Research (Kilkenny *et al.*, 2010). A trained veterinarian performed chicken handling and sample collection humanely.

Statistical analysis

Analysis of data was performed using Stata software version 14. Descriptive statistics were used to summarize clinical data (lesion score and mortality). Results were expressed as mean ± standard deviation and Mann-Whitney U-test and Kruskal-Wallis test were employed to examine for the significant difference in lesion scores and the immune responses among groups. The difference in mortality of chickens between the immunized and control groups was analyzed using the Fisher exact test. At all levels, *p*-value of less than 0.05 was considered as statistically significant.

Results

Viability of *S. Gallinarum* exposed to different irradiation doses

An exponential decrease in viability of SG was observed while increasing the dose of gamma irradiation (Figure 1). *Salmonella* Gallinarum exposed to doses greater than or equal to 2600 Gy of irradiation was completely inactivated and no growth was observed after culturing for 48 hrs. Therefore, the lethal dose (2600 Gy) and two doses close to lethal dose 2400, 2500 Gy were selected to assess the safety and immunogenicity of irradiated SG in chicken.
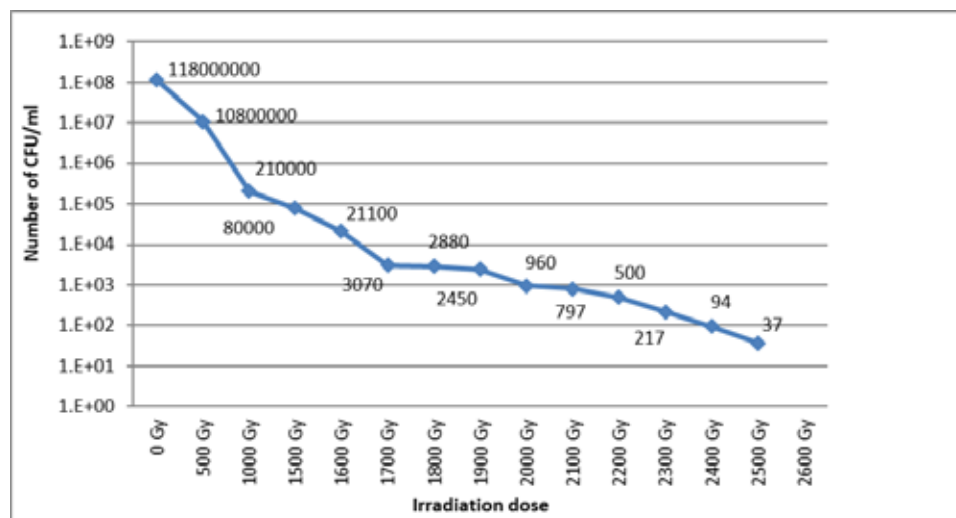

Figure 1: Effect of different irradiation doses on the viability of S. Gallinarum

Immunogenicity and safety of irradiated bacteria

The proportion of SG seropositive chickens 7-14 days after immunization with irradiated SG at 2400 Gy and 2500 Gy was 100%. In chickens immunized with irradiated SG at 2600 Gy, the percentage of seropositive chickens was 80% on day 7 and 100% on day 14, while all chickens in G 4 (negative control) were seronegative both on 7th and 14th days. According to the SAT score, chickens immunized with SG irradiated with 2400 Gy showed a better antibody response compared to the other two higher doses. Overall, it appears that increasing irradiation dose from 2400 Gy to higher doses is associated with a decrease in the level of mean antibody production (Table 1). All vaccinated chickens did

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not show any clinical signs of disease, injection site reactions, and pain. No lesion was observed in the liver and spleen of chickens in all groups. Based on the immunogenicity and safety data, irradiation at 2400 Gy was selected for further investigation.

Table 1: **Antibody response of chickens after immunization with *Salmonella* Gallinarum irradiated at various doses**

Group (n=5)	Irradiation dose (Gy)	Immunization route	Weeks after immunization	Positive after vaccination (%)	Mean agglut ination score ± SD
Group1	2400	SC	1	5(100)	2.8±0.2
			2	5(100)	3.0±0
Group2	2500	SC	1	5(100)	2.4±0.4
			2	5(100)	2.8±0.2
Group3	2600	SC	1	4(80)	1.8±0.6
			2	5(100)	2.4±0.4
Group4	PBS	SC	1	0(0)	0
			2	0(0)	0

SC=Subcutaneous; PBS=Phosphate buffered saline

Immunogenicity of 2400 Gy irradiated SG and SG 9R commercial vaccine

The proportion of seropositive chickens after vaccination was 100% in the irradiated SG immunized orally and subcutaneously on day 7 and day 14 post-vaccination. On the other hand, the rate of seropositivity in those chickens vaccinated with SG 9R was 80% on day 7 and 90% on day 14 post-vaccination. The highest mean SAT score was recorded in chickens immunized subcutaneously at day 14 post-vaccination and the second mean score was also recorded in this group at day 7 of vaccination. The mean SAT score for chickens immunized with irradiated SG subcutaneously and orally was higher than those immunized with commercial SG 9R vaccine. None of the chickens in the unimmunized group were positive for the SG antibody on day 7 and day 14 post-immunization (Table 2).

Table 2: Antibody response of chickens immunized with *S.*Gallinarum irradiated at 2400 Gy

Group	Type of vaccine	Route of administration	Week Post-vaccination	No. positive (%)	Mean agglutination ± SD
G1	Irradiated SG	Oral	1	10(100)	2.0±0.26
			2	10(100)	2.3±0.21
G2	Irradiated SG	SC	1	10(100)	2.7±0.15
			2	10(100)	3.0±0.0
G3	9R strain SG	SC	1	8(80)	1.4±0.31
			2	9(90)	1.8±0.33
G4	PBS	Oral	1	0	

SC= subcutaneous, PBS= Phosphate-buffered saline

Analysis of the level of antibody production using the Kruskal-Wallis test between the three vaccine groups revealed that the mean agglutination value of the three groups of vaccinated chickens was significantly different at day 7 (p=0.007) and day 14 (p= 0.005) post-vaccination. Mann-Whitney U-test analysis showed that there was no significant difference between the mean agglutination value of groups of chickens vaccinated orally with the irradiated vaccine (G1) and those vaccinated with commercial SG 9R (G3) vaccine at day 7 and 14 post-vaccination. On the other hand, there was a significant difference between those immunized with irradiated vaccine orally (G1) and subcutaneously (G2) on day 7 (p=0.042) and day 14 (p=0.005). Similarly, there was a significantly higher mean serum agglutination value of chickens in G2 compared to those chickens in G3 at both day 7 (p=0.003) and day 14 (p=0.002) (Table 3).

Table 3: Comparative serum antibody response among chickens immunized with irradiated *S.* Gallinarum and SG9R vaccine using Kruskal-Wallis and Mann-Whitney U-tests

Among group	Type of test	p-value	
		First week	Second week
G1 Vs G2 Vs G3	Kruskal-Wallis test	0.007	0.005
G1 Vs G2	Mann-Whitney U-test	0.042	0.005
G1Vs G3	Mann-Whitney U-test	0.176	0.261
G2 Vs G3	Mann-Whitney U-test	0.003	0.002

G1= Immunized with irradiated SG orally, G2= Immunized with irradiated SG subcutaneously, G3= Immunized with SG9R vaccine.

Morbidity and mortality of chickenspost-challenge infection

The number of apparently healthy, sick, and dead chickens during 21 days following challenge infection for vaccinated and unvaccinated control groups is presented in Figure 2. Typical signs of morbidity like depression, loss of appetite, and yellowish diarrhea were seen as of day 3 post-challenge in the unvaccinated group (Group 4), and on day 5 in chickens vaccinated with irradiated SG orally (Group 1). The course of disease establishment in the unvaccinated group was acute and all the chickens died within 7 days of the challenge with gross lesions on liver and spleen at postmortem examination. In chickens vaccinated with irradiated SG orally (Group 1), 5 (50%) of them got sick and died in the first 6 days of infection and on day 10, 9 (90%) died from the group with gross pathology in internal organs. Morbidity and mortality rates in groups of chickens vaccinated with irradiated SG subcutaneously (Group 2) and SG 9R commercial vaccine (Group 3) were similar. In both groups, 10 to 20% of chickens were found sick on days 5, 6, and 7 post-challenge, and a total of 5 (50%) of chickens died on day 9 and day 10 from Group 2 and Group 3, respectively. All the remaining 50% of chickens from both groups were apparently healthy until the end of the experiment. There was no statistically significant difference in the mortality rate of chickens vaccinated with irradiated vaccine orally and unvaccinated group ($p > 0.05$). However, more chickens survived in those vaccinated with the irradiated vaccine administered SC and SG 9R vaccine compared to the unvaccinated group ($p < 0.05$). Both of them induced 50% protection compared to mortality recorded in the unvaccinated control group (Table 4).
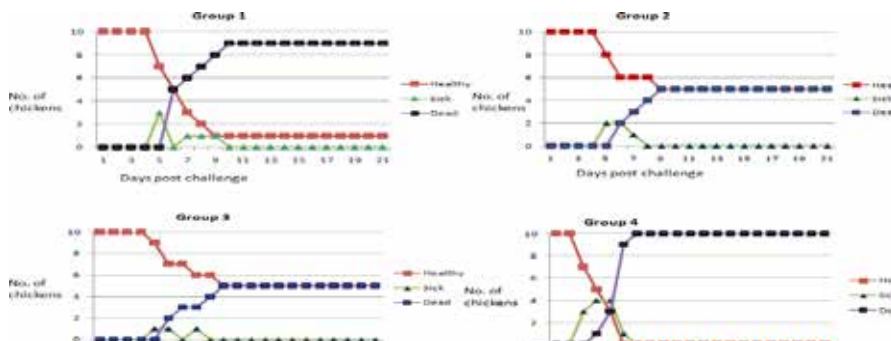

Figure 2: Inventory of apparently healthy, sick and dead chickens immunized with irradiated SG at 2400 Gy and SG 9R followed by homologous challenge infection compared to the unvaccinated control group (G1=vaccinated with irradiated SG orally, G2 = vaccinated with irradiated SG subcutaneously, G3 vaccinated with SG 9R vaccine; G4 unvaccinated control group). All chickens were challenged with 108 CFU of SG 21 days post-vaccination orally and were followed for the next 21 days.

Salmonella Gallinarum was isolated from liver and spleen of 6 (66.7%) of 9 chickens that died from G1, 3(60%) of 5 chickens from G2, 4 (80%) of 5 chickens from G3 and 9 (90%) of 10 chickens from G4. On the other hand, SG was not isolated from the intestinal contents of dead chickens from all groups. *Salmonella* Gallinarum was not isolated from the liver, spleen, and intestinal contents of all vaccinated chickens that survived the challenge infection upon postmortem examination at the end of the experiment on day 21. The mean lesion score of all chickens in the immunized group was significantly lower compared to those in the unimmunized group (Table 4).

Table 4: Protection efficacy of *S*. Gallinarum irradiated at 2400 Gy, lesion score and recovery of bacteria after oral homologous challenge with 10^8CFU of *S*. Gallinarum

Group (n=10)	No. dead (%)	P-value[b]	Efficacy (%)	Bacteria recovery from liver and spleen (%) [a]	Mean lesion score ±SD	*p*-value[c]
G1	9(90)	1	10	6(66.7)	1.6±0.31	0.002
G2	5(50)	0.033	50	3(60)	1.1±0.38	0.001
G3	5(50)	0.033	50	4(80)	1±0.33	0.000
G4	10(100)		-	9(90)	2.9±0.1	

G1 = vaccinated with irradiated *S*. Gallinarum orally, G2 = vaccinated with irradiated *S*. Gallinarum subcutaneously, G3 = irradiated with SG9R vaccine; G4 = unvaccinated but challenged with 10^8 CFU of *S*. Gallinarum, [b]Fischer's exact test; [c]Mann-Whitney U-test, [a]only for those chickens that died during the experiment

Discussion

Commercial poultry farming is one of the fastest-growing sectors in most developing countries. However, it has several challenges including poultry diseases of which fowl typhoid is known to cause heavy economic loss through mortality and reduced productivity (Shivaprasad, 2000). Vaccination is the best way of protecting poultry from this disease. Adequate balance between safety and immunogenicity is crucial for designing effective vaccines (OIE, 2018). However, safety is particularly a major concern when using live attenuated vaccines due to their residual virulence and long persistence in the internal organs of immunized hosts, which may cause adverse effects in immunized hosts and contaminate the environment via shedding of live attenuated strains through feces (Silva *et al.*, 1981; Kwon and Cho, 2011).

The least lethal dose of 10^8 CFU/ml of SG (suspended in 5ml of PBS) recorded in the current study was 2600 Gy of gamma irradiation. In a previous study, it was shown that a dose of 3000 Gy was sufficient to inactivate *Salmonella* serotypes and *Vibrio parahaemolyticus* (Jakabi *et al.*, 2003). The minimum effective dose of irradiation required for SG inactivation varies depending on the volume of liquid in which bacteria are suspended and the concentration of bacteria. Therefore, optimization of irradiation dose is required under different conditions.

Although the SG 9R vaccine was also administered subcutaneously, the intensity and proportion of antibody production were less than the one produced by irradiated vaccine administered subcutaneously. The percentage of chickens detected seropositive after vaccination reached 100% in those vaccinated with irradiated SG while in chickens vaccinated with SG 9R, it reached only 90% at day 14 post-vaccination. This could be due to the high immunogenicity of irradiated vaccine than the live rough avirulent strain of SG. The avirulent strain may lack some of the antigens present in the virulent strain of SG and could still be retained in irradiated vaccines. Kwon and Cho (2011) previously demonstrated that SG 9R has rough lipopolysaccharide (LPS) with defective O-side chain repeats. Further investigation is required in this area. In the present study, antibody production by chickens inoculated with irradiated SG at 2400 Gy subcutaneously was higher compared to those inoculated with the same doses orally. This could be due to the fact that orally administered vaccines need to pass through different barriers of the gastrointestinal tract, which delay absorption as compared to subcutaneous administration and enzymatic degradation in the gastrointestinal tract (Davitt and Lavelle, 2015). Previous work also demonstrated less protection of the SG 9R strain vaccine through oral than subcutaneous vaccination (Silva *et al.*, 1981).

The observed seroconversion and protection against challenge infection in chickens vaccinated with irradiated SG is in agreement with the previous report where irradiated *Listeria monocytogenes* efficiently activated dendritic cells via Toll-like receptors and was capable of inducing protective T-cell responses in mice(Datta *et al.*, 2006). A previous study showed that gamma-irradiated toxid produced from *Salmonella* Typhimurium to be effective in protecting poultry against challenge with *S.* Typhimurium and *S.* Gallinarum (Begum *et al.,* 2011).Another study also showed that irradiated *Mannheimia hemolytica* protected rabbits from challenge infection (Ahmed *et al.*, 2016).

Similarly irradiated *Brucella abortus* was shown to induce better protective efficacy compared to strain 19 *B. abortus* vaccine in mice (Mahmoud *et al.*, 2016). Despite eliciting different immune responses compared to heat-killed or chemically killed vaccines, the efforts to gamma irradiation technology for production of vaccine is increasing because of its ability to penetrate different cells and can damage both double and single-stranded DNA without affecting surface antigens (Seo, 2015).

Irradiated SG vaccine administered subcutaneously showed comparable protection with the SG 9R vaccine against homologous challenge infection. Despite the high rate of antibody production demonstrated by strong slide agglutination reaction, only 50% protection from the mortality induced by homologous challenge infection was recorded in chickens vaccinated with irradiated SG subcutaneously. In the current study, the SG 9R vaccine also showed only 50% protection against challenge infection in Bovans brown breed of chickens. This is closely in line with the report of Silva *et al* (1981) where SG 9R vaccine gave 64.3% protection in the immunized group against SG wild type challenge infection at the dose of 10^8CFU/ml in meat-breed of chickens. However, the current finding is contrary to the report of Adamu *et al* (2017) where SG 9R vaccine showed 100% protection in the immunized group and 53.4% mortality rate was recorded in the unvaccinated control group in White leghorn breed of chickens unlike that of 100% mortality in the unvaccinated challenged group in the current study. This disparity may be due to differences in the level of susceptibility of breed of chickens used in the two experiments. It has been previously shown that level of protection of SG 9R varies with types of breed of chickens involved (Kwon and Cho, 2011). Besides, the difference in the dose of challenge bacteria used could have also contributed to the observed variation.

Conclusion

SG Irradiated at 2400 Gy administered subcutaneously induced good antibody response and provided comparable protection with SG 9R strain commercial vaccine against homologous challenge infection indicating its potential of being an effective vaccine against fowl typhoid. A further detailed study involving optimization of irradiation dose, quantification, and characterization of immunological responses, assessment of the effect of booster vaccination on the protective efficacy using a large sample size is recommended.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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