Quantitative Assessment of Fimbriae-Specific Serum and Egg Yolk Antibodies Induced in Chicken Layers by a Newly Developed Live *Salmonella* Enteritidis Vaccine and Relationship to Infection

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ABSTRACT

The increase in *Salmonella* Enteritidis (SE) human outbreaks, mostly incriminating the poultry as the source of the infection, has increased the search to control this pathogen in chickens. The purpose of this study was to quantify the SE fimbriae (SEF)-specific serum and egg yolk antibodies following a controlled SE-challenge of laying hens previously immunized with a newly developed live SE vaccine versus a classical killed commercial vaccine. This work also aimed to determine the relationship between this quantitative assessment and the protection against SE invasiveness in the livers and spleens of the experimental hens. In the procedure, laying hens were divided into 4 different groups. Birds in group A were given orally a newly developed live SE vaccine at 222 days of age, while birds in group D were given subcutaneously in the neck a commercial killed SE vaccine at 40 and 130 days of age; birds of groups B and C were deprived of vaccination (control groups). Hens in groups A, B, and D were challenged orally at 237 days of age with a highly invasive SE strain, acquiring 14.1 and ~ 50 kb plasmids, at 1.5×10^{11} cfu/hen, while birds in group C were left without challenge. On one hand, the vaccinated-challenged groups A and D and unvaccinated-challenged group B showed quantitative serum antibodies spe-

cific to SE fimbriae SEF 14 and SEF 21, and egg yolk antibodies specific to SEF 21 at 7 days post challenge. On the other hand, only the live SE vaccinated-challenged group A showed quantitative egg yolk antibodies-specific to SEF 14 at 7 days post challenge. The SEF 14- and SEF 21-specific serum antibodies decayed consistently at 14 days post challenge in groups A, B, and D. On the contrary, there was an increase in egg yolk antibodies in groups A, B, and D specific to SEF 14 and a decrease to SEF 21 at 14 days post challenge. The highest level of SEF 14- and SEF 21-specific egg yolk antibodies at 7 and 14 days post challenge were recognized in group A administered the newly developed live SE vaccine; while hens of group D administered the killed SE vaccine had the highest level of SEF 14- and SEF 21-specific serum antibodies at 7 and 14 days post challenge. Hens in groups A and D did result in 100% protection against invasiveness of SE to livers and spleens. However, SE-challenged hens of group B, deprived of live or killed vaccine, showed a 40% and 20% invasiveness in the livers and spleens, respectively. The controlled unvaccinated and unchallenged group C didn't show any infection in livers or spleens by SE.

INTRODUCTION

The incidence of *Salmonella enterica* serovar Enteritidis (SE) isolation associated with food illness has dramatically increased in many countries since the mid to late 1980s.[1,2] The US Department of Agriculture Food Safety and Inspection Service (FSIS) data on SE in broiler chicken carcass rinses collected from 2000 through 2005 showed an annual number of isolates increased more than 4 fold and the proportion of establishments with SE-positive rinses increased nearly 3 fold.[3]

It is essential to reduce the public health risk associated with consumption of infected eggs by controlling SE infections in laying hens. Vaccination of laying hens against SE might be the most effective way to reduce egg contamination. Some live and inactivated vaccines have been proven to protect against SE infection in laying hens and to reduce egg contamination.[4]

The fimbriae structures, located external to the cell wall of bacteria, are considered important as they frequently have been shown to mediate adhesion of SE bacterium to the host tissues.[5-8] The correlation between the presence of fimbriae and bacterial virulence is established in many research works.[9-11] Type 1 fimbriae have been shown to contribute to the intestinal stage of SE infection of chickens and to play a role in the interaction of SE with oviduct and colonization of reproductive organs.[4] De Buck et al[12] suggested that SE adhesion to the isthmal secretions was mediated by type 1 fimbriae and that this binding could play a role in the contamination of eggs through incorporation of the bacteria in the shell membranes.

Salmonella Enteritidis fimbriae 14 (SEF 14) are defined as thin fimbriae produced by the *SefA* gene in SE and a few other related group D serovars.[13] The type 1 fimbriae or SEF 21 enables the bacterium to adhere to a wide variety of eukaryotic cells in vitro.[14] In studies on adherence and pathogenesis of SE in mice, it was reported that SE adherence to the mouse intestinal cells involves 2 types of fimbriae, thus emphasizing their role in pathogenesis of infection.[15]

It has been reported that immunization of hens with the 14 kDa fimbrial protein results in egg yolk antibodies specific to this protein. These antibodies were effective in inhibiting the attachment of SE to mouse intestinal epithelial cells.[16] The protective role of fimbriae proteins against SE infection needs to be examined in chickens.

Recently, it was shown that the early immune response in chicken to SE is not specific to any of the reported fimbriae proteins, which could explain the low immunogenicity of the fimbriae proteins leading to failure of protection in chicken against SE infection.[17] The greatest amount of interest in options for intervention to reduce the incidence of SE infection in chickens has been directed toward vaccination.[18] The costs or impracticality of improvements in hygiene

and management together with the increasing problems of antibiotic resistance suggest that vaccination in poultry against SE will become more attractive as an adjunct to existing control measures.[19,20]

Different preparations of classical killed vaccines for SE[14,21,22] and the recent immunopotentiation of killed SE vaccine by thymulin and Zn still showed incomplete protection against infection in chicken by SE organisms.[23] Live attenuated vaccines against salmonellosis have been more effective in reducing mortality and shedding in challenged birds than killed vaccines.[20] A primary aim of developing attenuated live SE vaccines is to protect the reproductive tract and prevent the vertical transmission of this serotype. Live vaccines may invade host cells, and their efficacy may be due to their particular distribution within the body, as well as to their capability of stimulation of the cell-mediated immunity.[20,24]

This is the first study that quantifies serum and egg yolk antibodies, specific to SEF 14 and SEF 21 in chicken layers in response to a live SE vaccine. It also examines the relationship of the quantified antibodies specific to the SEF to protection against a controlled challenge by a highly invasive SE possessing the 14.1 and ~ 50 kb plasmids.[25,26]

MATERIALS AND METHODS

Chicken Layers

A total of 20 brown feathered Babcock layers of 222 days of age were provided by the Agricultural Research and Education Center (AREC) in Bekaa, Lebanon. The hens were divided into 4 groups (5 hens/group); each group was put in a separate isolated room. All groups of hens were fed similarly according to the National Research Council 1994 standard requirement.[27] Cloacal swabs collected from all the hens and the laboratory culture results confirmed the hens as free from *Salmonella* infections.

Newly Developed SE Vaccine

The SE isolate used was chosen according to its prevalence; it had the most common plasmid profile among SE isolates recovered from 11 poultry broiler flock outbreaks.[25] The isolate had 2 plasmids located at different base pair positions, namely, 14.1 and ~50 kb. The attenuation of the field highly invasive SE strain acquiring 14.1 and ~50 kb plasmids was performed in a low concentration of formalin equivalent to 0.38×10^{-3}%. The contact time between the SE cells and highly diluted formalin, at room temperature, was 15 hours resulting in a viable count of the attenuated SE cells equivalent to 2.4×10^8 cfu/mL.

Vaccination

The 4 groups of hens were labeled as A, B, C, and D, and each group received a different treatment (Table 1). The hens in group A received orally 1 mL of the live SE vaccine (2.4×10^8 cfu/mL) followed by 3 mL of saline to wash in the vaccine. The hens in groups B and C were left without vaccination; however, hens of group D received a classical killed commercial SE vaccine (Hipra, Spain). The killed SE vaccine was administered to the hens subcutaneously through the neck at a level of 0.5 mL/hen and delivered twice, at 40 and 130 days of age, according to the manufacturer instructions.

Challenge Inoculum

A highly virulent and invasive SE isolate was used for the preparation of the challenge inoculum. The involvement of this SE isolate, acquiring plasmids 14.1 and ~ 50 Kb, in severe outbreaks of poultry has been report-

Table 1. Nature of treatments in the 4 groups of hens.

Hen Group	Nature of Vaccine	Challenged[a]
A	Live[b]	Yes
B	NA	Yes
C	NA	No
D	Killed[c]	Yes

NA = not applicable.

[a]Each challenged layer received orally 1.5×10^{11} cfu/mL/hen of an invasive SE strain acquiring 14.1 and ~50 Kb plasmids, at 237 days of age (14 days post live SE vaccination in hens of group A).

[b]A viable count of 2.4×10^8 cfu/mL of live attenuated SE vaccine-cells per hen was administered orally at 222 days of age.

[c]Group D hens were each given a killed commercial SE vaccine (Hipra, Spain). The vaccine was delivered in 0.5 mL/hen, subcutaneously in the neck, at 40 and 130 days of age.

ed previously to the World Animal Health Organization by the authors of this work.[25] Two passages in vivo were performed to the isolate in order to raise its virulence. In brief, 24-hour culture of SE colonies that were heavily grown on a 15-cm diameter Brilliant Green Agar (BGA) (HIMedia Laboratories, Mumbai, India) plate were scraped from one third the plate area. The cell mass was then reconstituted in 1 mL of sterile saline; 0.5 mL was delivered intravenously in the brachial vein of a 2-months-old white Pop Quail. The invasive SE was recovered from the spleen by culturing on BGA. The recovered colony was subcultured by heavy seeding on another BGA plate that was incubated for 24 hours at 37°C. One third of the seeded colonies were scraped from the BGA plate and reconstituted in 0.5 mL sterile saline. Another 2-months-old white Pop Quail was intravenously inoculated with 0.5 mL of the SE suspension in its brachial vein. The SE was recovered from the spleen of the infected white Pop Quail on a BGA plate that was incubated for 24 hours at 37°C. All the SE recovered colonies were scraped from the BGA plate and reconstituted in 100 mL of sterile Tryptose Phosphate Broth (HIMedia Laboratories, Mumbai, India). The SE count in the prepared challenge was 1.5×10^{11} cfu/mL.

Each hen in groups A, B, and D received orally 1 mL of the SE challenge (1.5×10^{11} cfu/mL/hen) at 237 days of age, followed by 3 mL of sterile saline to wash in the challenge, while group C remained unvaccinated and unchallenged as a negative control group.

Immunoblott Sera and Egg Yolk

Blood samples were collected from the brachial vein of all the hens in the 4 different groups at 7 and 14 days post challenge (244 and 251 days of age). The 5 serum samples collected from the 5 hens of the same age belonging to each group were pooled in equal portions. Each pooled sera was stored at –20°C for use in Western Immunoblotting.

The eggs were collected at 7 and 14 days post SE challenge, transported in refrigerated containers, cleaned with potable water using a detergent and a disinfectant, and then refrigerated in cleaned marked bags until the experimental testing was performed.

Before analysis, each collected egg was disinfected with 70% alcohol and a sterile cotton swab. A hole was then made in the eggshell at the aircell side using a sterile forceps and scissors. After removing a piece of the vitelline membrane, 1 mL of the yolk was obtained using a clean disposable pipette and mixed with 1 mL of phosphate buffered saline (PBS) and vortexed for 1 minute. The above mixture was centrifuged at 2,500 rpm for 30 minutes using an IEC centrifuge (HN-SII, International Equipment Company, Needham, Massachusetts, USA). The supernatant was collected. The supernatants of the egg yolk samples that were collected from hens of same age in each group were pooled in equal portions. Each pooled egg yolk sample was stored at –20°C to be used in Western Immunoblotting.

Isolation of SE Fimbriae 14 and 21 KDa

Salmonella Enteritidis was grown statically in 2 L of Colonization Factor Antigen medium (Difco, Becton Dickinson, Sparks, Maryland, USA) at 37°C for 60 hours. The SE culture was harvested by centrifugation at 3000 rpm (180×g) for 10 minutes using a GLC-2B centrifuge (Du Pont Instruments, Sorvall®, Newtown, Connecticut, USA) then suspended in 120 mL of 0.15 M ethanolamine buffer, pH 10.5.[13] Fimbriae were separated from the SE cells at room temperature (25°C) by shearing them in a blender (mixer Blender 3, Mammonlex, Model 242, Taiwan) for 3 cycles (1 minute/cycle). The cells and cellular debris were removed by centrifugation at 12,000×g for 15 minutes at 4°C using RC2-B Automatic Refrigerated Super Speed Centrifuge by Sorvall®. The supernatant (fraction 1) was centrifuged at 100,000×g for 1 hour at 4°C using OTD 65B ultracentrifuge by Sorvall® in order to remove the membrane vesicles. The result is a clarified supernatant (fraction 2) that was dialyzed overnight in the fridge against 10 mM Tris HCl (pH 7.5) containing 0.2% SDS

in a dialysis bag (Spectrum Lab., Rancho Dominguez, California, USA) of molecular weight pores cutoff between 6-8 KDa, in order to precipitate the SEF 14. The SEF 14 fimbriae were pelleted by centrifugation at 15,000×g for 15 minutes at 4°C using RC-5B centrifuge by Sorvall®, thus separating the precipitated SEF 14 from the SEF 21, which remained in the supernatant as fraction 3. The SEF 14 pellets were then reconstituted in 5 mL of sterile saline. Fraction 3 containing the SEF 21 fimbriae (75 mL) was concentrated to approximately 25 mL by dialysis against 30% polyethylene glycol 20,000 (Sigma, St. Louis, Missouri, USA) for around 2 hours followed by precipitation of SEF 21 with 37.5 mL of ice-cold acetone. The precipitated SEF 21 was recovered by centrifugation at 15,000×g for 20 minutes at 4°C. The pellet (fraction 4), containing the SEF 21, was suspended in 4 mL of Laemmli buffer and boiled for 5 minutes to solubilize the constituents.[13]

SDS-PAGE of Isolated Fimbriae

The banding of the SEF 14 and 21 KDa fimbriae was done by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), using the discontinuous buffer system.[28] The weight of protein of SEF 14 applied in 20 µL volume per lane was 1.75 µg, while that of SEF 21 was 3.5 µg. The molecular weight marker was diluted 1:20 with SDS reducing buffer and applied on gel in 10 µl volume. A 12% separating gel was allowed to polymerize for 45 minutes in a mini-protean II electrophoresis cell (Bio-Rad Lab., Richmond, California, USA). The electric current in the gel was run at 60 mA for 45 minutes.

Serum and Egg Yolk Antibodies to Fimbriae

The detection of chicken serum and egg yolk antibodies to SEF 14 and SEF 21 was performed by Western immunoblotting.[29] Briefly, the fimbriae of SE resolved on SDS-PAGE gels were electrophoretically transferred onto a nitrocellulose membrane (NCM) (Bio-Rad Lab., Richmond, California, USA). The electrophoretic transfer was performed in a transblot cell (Bio-Rad Lab., Richmond, California, USA) for 1 hour at 0.25 A and 100 V. Blocking of the active sites was performed by immersion of the NCM in 5% gelatin-Tris-Buffer Saline for 2 hours at 37°C. The pooled sera and egg yolk samples collected from each group of hens at a specific time (7 and 14 days post SE challenge) were diluted to 1:250 and 1:50, respectively, using 1% gelatin-Tris-Tween-Buffer Saline (TTBS). The lanes for SEF 14 and SEF 21 were individually reacted to each pooled sera and egg yolk samples for 10 hours at 37°C. A goat anti-chicken IgG (H+L) peroxidase conjugate (Sigma, St. Louis, Missouri, USA) was diluted to 1:1000 in 1% gelatin-TTBS and then added to NCM and incubated at 37°C for 1 hour. The substrate used was 3,3'-DAB peroxidase (Sigma, St. Louis, Missouri, USA), added to NCM for 30 minutes at 37°C. The NCM containing the formed brown colored bands was rinsed with distilled water and dried over a filter paper. The dried NCM bands were scanned using Scanjet 6300C (Hewlett-Packard Development Company, L.P., Palo Alto, California, USA) with setting at high Sharpen level, and output resolution of 300.

Quantitative Assessment of Antibodies

The antibodies specific to SEF 14 and SEF 21 fimbriae as colorimetrically formed on NCM were quantitatively measured by reading the mean absorbance (intensity of color) of the scanned bands using a new computerized program developed by the National Institute of Health, USA, namely the NIH Image 1.62 program. This program is available on the Internet at http://rsb.info.nih.gov/nih-image powered by Executor for Windows, which is available also on the Internet at http://www.ardi.com. The mean intensity values obtained for antibodies specific to the banded fimbriae of SE in groups A, B, C, and D were subtracted from the background mean intensity of the control group "C" at each specific age.

Protection

To assess the invasiveness of the highly

virulent field SE isolate acquiring 14.1 and ~50 kb plasmids in the laying hens that were orally challenged, SE was attempted to be recovered from the livers and spleens of those hens. All the laying hens in the 4 different groups were sacrificed by cervical dislocation at 14 days post SE challenge (251 days of age). An area of 0.75 × 1.0 cm2 was cut aseptically from each organ (livers and spleens) and cultured in 5 mL of sterile enrichment Selenite Broth. The Selenite cultures were incubated at 41.5°C for 20-24 hours. Individual subcultures from selenite to BGA plates were performed. The BGA culture was incubated at 37°C for 24 hr. Suspected *Salmonella* colonies on BGA were tested biochemically for conformation of identity using H_2S gas production and glucose fermentation in Triple Sugar Iron (TSI) agar (HIMedia Laboratories, Mumbai, India) and the lack of urease production in Urea Agar Base medium.[30]

RESULTS

Table 2 shows the quantified chicken serum and egg yolk antibodies specific to SEF 14 fimbriae at different times post SE challenge. An apparent consistent decay in the SEF 14 serum antibodies and an increase in the SEF 14 egg yolk antibodies in groups A, B, and D was noticed at 14 days post challenge in comparison to that at 7 days post challenge. The hens in group D that were administered 2 doses of killed SE bacterin showed the highest level of serum antibodies to SEF 14 at 7 and 14 days post challenge in comparison to hens of group A (administered a live vaccine and a challenge) and hens of group B (unvaccinated and challenged). Conversely, the hens of group A showed the highest level of egg yolk antibodies to SEF 14 at 7 and 14 days post challenge in comparison to hens in groups B and D.

Serum antibodies to SEF 14 were detected earlier after challenge and in higher quantities in the hens of groups A, B, and D compared to that of their respective egg yolk antibodies. Moreover, only the hens of group A had SEF 14 egg yolk antibodies detected in small amounts at 7 days post challenge. In addition, 14 days post challenge, SEF 14 egg yolk antibodies in group A were in higher quantities than those detected in the sera.

Table 3 shows the quantified chicken serum and egg yolk antibodies specific to SEF 21 at different times following the SE challenge. The serum antibodies specific to SEF 21 decayed consistently in groups A, B, and D with time post challenge (Table 3), similar to the consistent decay with time shown for serum antibodies specific to SEF 14 (Table 2). However, the yolk antibodies to SEF 21 rose with time post challenge (Table 3), while the yolk antibodies to SEF 14 dropped with time (Table 2).

The hens in group D that were given 2 doses of a killed SE bacterin and then challenged showed the highest level of serum

Table 2. Quantitative assessment[a] of chicken serum and egg yolk antibodies specific to SEF 14 in response to challenge.

	Mean Intensity of Antibody Response[c] to Banded SEF 14 (days post SE challenge/age in days)			
	Serum		Egg Yolk	
Hen Group[b]	7/244	14/251	7/244	14/251
A	+12.2	-11.9	+0.3	+13.4
B	+58.2	+10.1	-9.3	-3.9
C	0.0	0.0	0.0	0.0
D	+126.5	+18.4	-5.3	+12.9

[a]Assessed by scanning of bands found on NCM followed by reading intensities of the bands using NIH Image 1.62 computer program.

[b]Hen groups or treatments are defined in Table 1.

[c]The means of intensity of SEF 14 bands reacted to serum of hens in groups A, B, C, and D is subtracted from the background mean obtained by hens of the control group "C" at each specific age.

Table 3. Quantitative assessment[a] of chicken serum and egg yolk antibodies specific to SEF 21 in response to challenge.

	Mean Intensity of Antibody Response[c] to Banded SEF 21 (days post SE challenge/age in days)			
	Serum		Egg Yolk	
Hen Group[b]	7/244	14/251	7/244	14/251
A	+23.2	+16.7	+18.6	+2.3
B	+15.5	+10.7	+6.4	-0.1
C	0.0	0.0	0.0	0.0
D	+66.2	+37.2	+3.49	+1.3

[a]Assessed by scanning of bands found on NCM followed by reading intensities of the bands using NIH Image 1.62 computer program.

[b]Hen groups or treatments are defined in Table 1.

[c]The means of intensity of SEF 21 bands reacted to serum of hens in groups A, B, C, and D is subtracted from the background mean obtained by hens of the control group "C" at each specific age.

antibodies to SEF 21 at 7 and 14 days post challenge in comparison to the hens in group A that were given the live vaccine and challenged and the hens in group B that were unvaccinated and challenged. Conversely, the hens of group A showed the highest level of egg yolk antibodies to SEF 21 at 7 and 14 days post challenge in comparison to the hens in groups B and D.

The SEF 21-specific antibodies were detected in both the serum and egg yolk of hens in groups A, B, and D as early as 7 days post challenge (Table 3); however, higher quantities of antibodies were found in the serum rather than in their respective egg yolks.

The protection effect against SE invasiveness into the visceral organs (livers and spleens) at 14 days post challenge is shown in Table 4. The live vaccine given to hens of group A, and the killed vaccine given to hens of group D did result in 100% protection against invasiveness of SE to livers and spleens. However, the hens in group B, deprived of live or killed vaccine, showed a 40% and 20% invasiveness in the livers and spleens, respectively. The hens of the control group C were free of *Salmonella* infections in their livers and spleens, a reflection of the animal room environment standard, indicating proper biosecurity and compliance to isolation requirements.

DISCUSSION

The quantitative assessment of the serum antibodies specific to SEF 14 (Table 2) in the various vaccinated hens reveals the ability of both the newly developed live SE vaccine and the commercial killed SE vaccine to induce enough protective SEF 14 antibodies at 7 days post challenge as evidenced in the hens of groups A and D, respectively. This effect was maintained in spite of the neutralization by the invading challenging organism. Such a finding is in agreement with prior research on the ability of vaccines to maintain immunity during neutralization by the invading organisms.[31,32]

Conversely, the early yolk antibody response at 7 days post challenge to SEF 14 was only evident in eggs of group A that were given the live SE vaccine. This finding disagrees with other previous observations,[33,34] in which early detection of egg yolk antibodies was absent. This early immune response to SEF 14 may be due to a difference in the nature of the newly developed vaccine and/or the genotype of the experimental hens.[35,36]

The consistent decay of the serum antibodies specific to SEF 14 by time (14 days versus 7 days post challenge) in groups A, B, and D may be contributed to the short half life of chicken antibodies and/or due to the neutralization effect by the invading SE organism.[37] In contrast, the increase in the

SEF 14-specific egg yolk antibodies by time, in groups A, B, and D, may perhaps reflect the time necessary for antibodies to transit from serum to the egg yolk during the egg formation.[38]

The 2 groups of hens, B and D, that showed the highest levels of SEF 14-specific serum antibodies at 7 days post challenge were able to keep these highest levels at 14 days post challenge. The killed vaccine administered to hens of group D was able to induce after SE challenge a higher antibody level specific to SEF 14 than the serum antibody levels obtained by group A hens that received the live attenuated SE vaccine (Table 2). It has been noted in previous studies the inability of attenuated live enteric vaccines to induce a significant humoral response compared with that caused by a killed vaccine.[20,39] However, the hens in group A that were given the live SE vaccine were able to keep a much higher level of egg yolk antibodies at 14 days post challenge in comparison to that of groups B and D. This finding is supported by some earlier studies, showing stronger and long-lasting local immunity induction against *Salmonella* infection in layers by live attenuated vaccines when compared with killed bacterins.[21,40-43]

The serum and egg yolk antibody response to SEF 21 was evident in groups A, B, and D at 7 days post challenge (Table 3). This result could indicate a higher immunogenicity of the 21-KDa fimbriae compared to the 14-KDa fimbriae. In a previous study, it was indicated that as the molecular weight of the polypeptide antigen increases, its immunogenicity increases accordingly.[17] The SEF 21-specific serum and egg yolk antibodies in the hens of group B that were not vaccinated but challenged were lower than those obtained in the vaccinated and challenged groups (A and D) at all times. This significantly indicates the importance of vaccination (sensitization) that induces a secondary immune response post challenge.[37,40,41,43]

Hens in group D, given the killed SE bacterin, had the highest level of serum antibodies to SEF 21 in comparison to the other groups of hens; this is in agreement with previous reports comparing the killed to live vaccines in induction of humoral immunities.[20] Hens in group A, given the newly developed live vaccine, had the highest level of egg yolk antibodies to SEF 21 when compared with the other groups; this observation is in agreement with previous findings in which the live vaccines induced better local immunities than the killed bacterins.[14,21,40-43]

The above results confirm the ability of both the newly developed live SE vaccine and the commercial killed SE vaccine to maintain adequate SEF 14 and SEF 21 serum antibodies at the critical period post challenge (Tables 2 and 3). This may have helped in the neutralization of the invasive SE organisms that were used in challenging the hens, thus leading to clearance of infection from the livers and spleens of hens in groups A and D (Table 4). It was previously noted that the presence of protective antibodies could intercept with infection and invasion of microorganisms in the host.[21,22] Hens in group B that were deprived of vaccination were able to induce antibod-

Table 4. Protection against SE invasiveness in visceral organs at 2 weeks (251 days of age) post challenge of the different groups.

	% (frequency[b]) of Layers With SE Infection in Different Organs	
Hen Group[a]	Liver	Spleen
A	0 (0/5)	0 (0/5)
B	40 (2/5)	20 (1/5)
C	0 (0/5)	0 (0/5)
D	0 (0/5)	0 (0/5)

[a]The nature of the treatment in each of the 4 groups of layers is defined in Table 1.

[b]Frequency is the number of layers with SE recovery from the specific organ divided by the number of layers in a treatment.

ies against SEF 14 and SEF 21 (Tables 2 and 3) as a result of the infection caused by the challenging organisms; however, this infection titer was not able to protect against invasiveness of SE organisms in this group of hens (Table 4). A reason for this could be the absence of memory cells to SE due to deprivation from vaccination[44,45]; more specifically, the absence of sensitized T-helper and T-delayed hypersensitivity cells that are helpful in cell-mediated immune responses against intracellular infections, such as that caused by SE organisms.[20]

In conclusion, both the newly developed live vaccine and the commercial classical killed SE vaccine were able to maintain SEF antibodies during the critical period of challenge. This contributed to the protectiveness against the invasion of visceral organs by the highly virulent SE used in the challenge.

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