Use of live and inactivated *Salmonella enteritidis* phage type 4 vaccines to immunise laying hens against experimental infection

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**Summary:** Four groups of Dekalb Delta commercial layer hens (8 hens per group) were reared individually in cages in an isolation unit. At the age of 8 weeks, groups 1 and 2 were vaccinated with 9R *Salmonella gallinarum* live (9R live) vaccine. At the age of 18 weeks, group 1 was re-vaccinated with 9R live *S. enteritidis* vaccine, while groups 2 and 3 were vaccinated with *S. enteritidis* bacterin. At the age of 22 weeks, groups 2 and 3 were re-vaccinated with *S. enteritidis* bacterin. Group 4 was not vaccinated and was kept as a control group.

At 24, 27 and 30 weeks of age, the four groups were challenged using various concentrations of *S. enteritidis* phage type 4 and various routes of inoculation. Eggs, cloacal swabs and blood were collected weekly for bacteriological and serological examination.

Isolation of the challenge organism from the eggshells and egg contents of the vaccinated groups was significantly lower than with the control group. There was no correlation between humoral antibodies and the shedding of the challenge organism in eggs or cloacal swabs.

A combined vaccination programme of 9R live *S. enteritidis* vaccine and *S. enteritidis* bacterin provided better protection to laying hens than either vaccine administered alone. Vaccination should never be performed alone but in conjunction with other measures related to veterinary hygiene and good management.

**KEYWORDS:** Eggs – Hens – Inactivated vaccines – Live vaccines – Salmonella – *Salmonella enteritidis* – Vaccines.

**INTRODUCTION**

*Salmonella enteritidis* infection in laying hens has increased dramatically in recent years in Saudi Arabia and many other countries. A large proportion of human *S. enteritidis* outbreaks have been attributed to the consumption of contaminated eggs or food containing eggs (2, 6, 12, 13). In Saudi Arabia, *S. enteritidis* phage type 4 (PT4) was rarely diagnosed in laying flocks before 1988 (11, 12). *S. enteritidis* PT4 is highly invasive in laying hens, and a range of tissues can be infected (blood, liver, spleen, kidney,
ovary/ovules, oviduct and lung) often in the absence of faecal carriage (10). The bacterium would also appear to have a predilection for reproductive tissue; for example, *S. enteritidis* PT4 can be isolated from the oviduct within one hour of crop inoculation (10).

Egg-borne transmission of *S. enteritidis* to humans has become a major public health hazard, emphasising the need for programmes to reduce the incidence of *S. enteritidis* infections in commercial layer hens (7). The control of this serotype – by preventing both transmission from one generation of chickens to the next and horizontal spread within flocks – must be a vital step in the protection of public health (6). Improved hygienic conditions in poultry management, maximum biosecurity and feed decontamination have reduced the incidence of *Salmonella* infections, but re-infection of poultry farms from natural reservoirs (e.g. rodents) still occurs (17). Therefore, vaccination of hens to reduce susceptibility to *S. enteritidis* infection may provide a useful adjunct to the current control programmes.

Conflicting reports have been published on the efficacy of live recombinant *Salmonella* vaccines and killed *S. enteritidis* bacterins in protecting laying hens against *S. enteritidis* infection, and the interpretation of the published work was complicated. It was unclear whether recombinant *Salmonella* live vaccines produce better protection against *S. enteritidis* infection in laying hens than currently-available inactivated vaccines.

The objectives of this study were to assess the potential protective efficacy of the 9R live vaccine (vaccine A) and *S. enteritidis* bacterin (vaccine B) in a combined vaccination programme, and to compare this programme with separate administration of vaccine A or B. Efficacy was measured using the following parameters: intestinal colonisation, dissemination to internal organs, and production of contaminated eggs after challenge with *S. enteritidis* PT4 (known to be invasive). The study was also extended to examine the effect of the various vaccination programmes on humoral response and total egg production after challenge.

**MATERIALS AND METHODS**

**Chickens**

Thirty-two day-old *Salmonella*-free Dekalb Delta commercial layer chicks were purchased from a local hatchery. The chicks were individually wing banded and randomly distributed into four groups of eight chicks each. Each group of chicks was reared in an isolation unit, in the lower tier of wire-bottom batteries (two chicks per cage), in pens which were separate but essentially identical. The isolation unit was supplied with filtered air, and the temperature and lighting were controlled. The chickens were periodically monitored, using cloacal swabs and serological tests, to ensure freedom from *Salmonella*.

At the age of 18 weeks, the pullets were transferred to the higher tier of the battery cages and reared individually on a sloped cage floor (allowing eggs to roll away, thus reducing faecal contamination).

**Feed**

The chickens were fed using a commercial feed for laying chickens which contained no antibiotics and was free from *Salmonella* (samples of feed were cultured bacteriologically to confirm freedom from *Salmonella*).
Salmonella vaccines

Two types of Salmonella vaccines available in the local market were used, as follows:

- Vaccine A consisted of 9R live vaccine (in freeze-dried form) against S. gallinarum (fowl typhoid) infections of chickens. Each dose of reconstituted vaccine contained not less than 20 million viable organisms. The vaccine was administered by subcutaneous injection into the back of the neck. The dosage volume was 0.5 ml and the age for priming vaccination was 8 weeks, with re-vaccination at 18 weeks of age.

- Vaccine B (S. enteritidis bacterin) was an inactivated vaccine emulsified in an oil adjuvant. The bacterin contained selected antigenic isolates of S. enteritidis. The dose was 0.5 ml injected subcutaneously into the back of the neck. The vaccination regime involved two injections administered four weeks apart: the first at 18 weeks and the second at 22 weeks.

Salmonella enteritidis PT4

S. enteritidis PT4 isolate was used as the challenge organism. This invasive strain of S. enteritidis was isolated from the yolk sac of a newly-hatched chick at the poultry disease laboratory in Riyadh, Saudi Arabia. The challenge inoculum consisted of a live overnight brain/heart infusion broth culture of S. enteritidis PT4.

Experimental design

The laying hens were divided into four groups (8 hens per group) and each hen was reared individually in a separate cage.

Hens in group 1 were 8 weeks old when first vaccinated subcutaneously with 0.5 ml of vaccine A, and were then re-vaccinated subcutaneously with 0.5 ml of the same vaccine at 18 weeks of age.

Hens in group 2 were 8 weeks old when first vaccinated subcutaneously with 0.5 ml of vaccine A. At 18 weeks of age the hens were vaccinated subcutaneously with 0.5 ml of vaccine B and then re-vaccinated with the same vaccine at the age of 22 weeks. This group of hens therefore received two different vaccines in a combined programme of live and killed vaccines.

Hens in group 3 were 18 weeks old when first vaccinated subcutaneously with 0.5 ml of vaccine B and were then re-vaccinated with the same vaccine at 22 weeks.

Hens in group 4 were not vaccinated and were kept as a control.

All hens in the four groups were challenged at 24, 27 and 30 weeks of age with an overnight brain/heart infusion broth culture of S. enteritidis PT4, using various concentrations and routes of administration.

At 24 weeks of age, all hens in the four groups were challenged orally with 1 ml of $10^5$ colony-forming units (CFU)/ml of S. enteritidis PT4. At 27 weeks of age, all hens were challenged orally with 1 ml of $10^5$ CFU/ml of the same challenge organism. At 30 weeks of age, all hens were challenged by intramuscular injection with 0.5 ml of $10^6$ CFU/ml of S. enteritidis PT4.

The number of eggs laid per day by each hen in each group was recorded, and the mean daily egg production was calculated over weekly intervals until the end of the experiment. Cloacal swabs and blood samples were collected weekly from each hen from 24 weeks of age until the end of the experiment. At the end of the experiment
(i.e. at 33 weeks of age), all hens were killed by neck dislocation, and the internal organs – liver, spleen, ovary, oviduct and caecal tonsils – were collected separately and aseptically in sterile polythene bags. The organs were then stored at -20°C until examined bacteriologically for the isolation of *Salmonella* organisms.

**Bacteriological examination**

**Eggs**

**Egg contents**

The eggs laid by each hen (3-7 eggs each week) were collected at weekly intervals in a sterile plastic bag. Each egg was broken to release the contents into a sterile beaker, and the eggshells were placed in a sterile mortar. The contents of all eggs laid by each hen during the week were pooled together in a sterile beaker. The egg contents were thoroughly mixed by manual swirling with a sterile glass rod. After mixing, 10 ml portions of each pool were added to 90 ml of lactose broth and incubated at 37°C for 18-24 h. A 1 ml portion of each lactose broth culture was then transferred to 9 ml of selenite broth and incubated at 37°C for 18-24 h. A small amount of selenite broth culture was then extracted using a loop, streaked onto brilliant green agar and incubated at 37°C for 18-24 h. The identity of prospective *Salmonella* colonies was confirmed biochemically and serologically.

**Eggshells**

After the egg contents were released, the eggshells were pooled in a sterile mortar and ground together. Then 90 ml of lactose broth was thoroughly mixed with the ground eggshells in the mortar. The entire mixture was incubated at 37°C for 18-24 h. A 1 ml portion of each lactose broth culture was then transferred to 9 ml of selenite broth and incubated at 37°C for 18-24 h. A small amount of selenite broth culture was extracted using a loop, streaked onto brilliant green agar and incubated at 37°C for 18-24 h. Biochemical and serological tests were performed to confirm the identity of the suspected *Salmonella* isolates.

**Cloacal swabs**

Cloacal swabs were obtained from each hen in the four groups immediately prior to challenge and at weekly intervals for 9 weeks post-challenge, to monitor the establishment and persistence of *S. enteritidis* in the intestinal tract. A sterile cotton swab was inserted into the cloaca of each bird and rotated gently to collect a sample. The swab was transferred to a 9 ml universal bottle of selenite broth and this was incubated overnight at 37°C. A small amount of broth was then extracted using a loop, and streaked onto brilliant green agar for *Salmonella* isolation. The identity of suspected *S. enteritidis* isolates was confirmed biochemically and serologically.

**Internal organs**

At the end of the experiment, all hens were killed by neck dislocation. After post-mortem examination, the internal organs were collected aseptically from each hen in the following order: liver, spleen, ovary, oviduct and caecal tonsils (with the caeci). Each organ was kept in a sterile plastic bag and stored at -20°C until examination. Liver, ovary and oviduct were separately homogenised in a sterile mortar and mixed with 90 ml of lactose broth, while the homogenised spleen and caecal tonsils were mixed separately with 9 ml of lactose broth and kept overnight at 37°C. A 1 ml portion of the lactose broth culture from each organ was then transferred to 9 ml of selenite broth and
incubated at 37°C for 18-24 h. A small amount of selenite broth culture was then 
extracted using a loop, streaked onto brilliant green agar and incubated at 37°C for 
18-24 h. The identity of suspected *Salmonella* isolates was confirmed biochemically and 
serologically.

**Serological tests of blood serum**

Blood was collected from the brachial vein of each bird at weekly intervals following 
the first challenge at 24 weeks of age until the end of the experiment at 33 weeks of age. 
Blood samples were stored in a refrigerator (at 4-8°C) for 24 h. Serum was harvested 
individually from each blood sample and kept in a small glass tube in the refrigerator 
until examined for the presence of specific antibodies against *Salmonella* by slide 
agglutination and enzyme-linked immunosorbent assay (ELISA).

*Slide agglutination*

This test was performed on serum samples using stained *S. pullorum* antigen and 
stained *S. enteritidis* antigen as described by Cooper *et al.* (5).

*ELISA*

For this experiment, an *S. enteritidis* antibody test kit (an ELISA for the detection of 
antibodies to *S. enteritidis* in avian serum) was used. For the detection of antibody 
subsequent to vaccination, the serum sample was diluted 1/500.

**Statistical analysis**

The ‘Statistix’ computer programme was used for statistical analysis. Significant 
differences (*P* < 0.05) in the frequency of isolation of *S. enteritidis* PT4 from eggshells, 
egg contents and cloacal swabs of the four groups of hens were determined by the 
application of the ‘least significant difference’ (*t*) test. This test was also used to 
determine the mean egg production of the four groups of hens after challenge.

**RESULTS**

Both vaccines were safe and caused no mortality or clinical symptoms in the hens, 
either when administered in a combined vaccination programme or when each vaccine 
was administered alone.

**Isolation of the challenge organism from eggshells, egg contents and cloacal swabs**

When the laying hens were aged 24 weeks, no *S. enteritidis* PT4 was isolated from the 
eggshells, egg contents and cloacal swabs of the four groups. This confirms that the four 
groups of hens were free from *S. enteritidis* PT4 before challenge (Table I).

During the first three weeks following oral challenge with a dose of $10^5$ CFU/bird of 
*S. enteritidis* PT4, the frequency of isolation of the challenge organism from eggshells, 
egg contents and cloacal swabs in the vaccinated groups was considerably less than in 
the non-vaccinated group (group 4). The highest frequency of isolation of the challenge 
organism from group 4 was from eggshells (6 of 8 hens: 75%), egg contents (3 of 8 hens: 
37.5%) and cloacal swabs (3 of 8 hens: 37.5%) (Table I). The highest frequency of 
isolation from the vaccinated groups (1, 2 and 3) was from the eggshells of group 3 at 
2 weeks after challenge (3 of 8 hens: 37.5%), but the challenge organism was not 
isolated from the eggshells, egg contents and cloacal swabs of group 2 during this period 
(Table I). Vaccine A strain was isolated from eggshells (one of 8 hens: 12.5%) of group 2
### Table I

*Isolation of Salmonella enteritidis phage type 4 from eggshells, egg contents and cloacal swabs of laying hens after challenge at various ages*

<table>
<thead>
<tr>
<th>No. of weeks post-challenge</th>
<th>Age (weeks)</th>
<th>Group *</th>
<th>S. enteritidis-positive samples/total (%)</th>
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<td></td>
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<td></td>
<td>Eggshells</td>
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<td>3</td>
<td>0/8 (0)</td>
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<td>4</td>
<td>0/8 (0)</td>
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<td>25</td>
<td>1</td>
<td>0/8 (0)</td>
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<td>3</td>
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<td>4</td>
<td>6/8 (75)</td>
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<tr>
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<td>5/8 (62.5)</td>
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<td>29</td>
<td>1</td>
<td>2/8 (25)</td>
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<td>6 **</td>
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<td>1</td>
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<td>0/8 (0)</td>
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<td>3</td>
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<td>1/8 (12.5)</td>
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<td>9</td>
<td>33</td>
<td>1</td>
<td>1/8 (12.5)</td>
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<td>2</td>
<td>0/8 (0)</td>
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<td>3</td>
<td>0/8 (0)</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>1/8 (12.5)</td>
</tr>
</tbody>
</table>

* groups 1, 2 and 3 vaccinated; group 4 non-vaccinated control

** challenge with *S. enteritidis* phage type 4

*** 9R strain of *S. gallinarum* live vaccine was isolated
at 26 weeks of age, 2 weeks after the first challenge. This confirms the potential vertical transmission of vaccine A strain in laying hens.

After increasing the oral challenge dose to $10^9$ CFU/bird at 27 weeks of age, frequency of isolation of the challenge organism from the eggshells increased in groups 1 and 2 (3 of 8 hens: 37.5%) and group 4 (5 of 8 hens: 62.5%), while frequency of isolation from egg contents of the vaccinated and non-vaccinated groups was zero (Table I). Faecal excretion increased considerably in the four groups by 28 weeks of age, one week after the second challenge. Shedding of the challenge organism continued in the four groups until the end of the experiment (Table I).

Despite changing the route of inoculation at 30 weeks to begin intramuscular inoculation, the challenge organism was subsequently isolated only occasionally from eggshells of groups 1 and 4 but was not isolated from the egg contents of any of the four groups (Table I).

Throughout the experimental period, the frequency of isolation of the challenge organism from eggshells of the non-vaccinated control group (group 4) was higher than for the vaccinated groups, and there were significant differences ($P < 0.05$) between vaccinated and non-vaccinated groups (Table II). Frequency of isolation of the challenge organism from the egg contents of the non-vaccinated control group (group 4) was higher than in the vaccinated groups 1, 2 and 3 (Table II). Frequency of isolation of the challenge organism from cloacal swabs of group 1 was higher than the other groups and there were significant differences ($P < 0.05$) between group 1 and groups 2 and 3, but not between groups 1 and 4 (Table II).

**Isolation of the challenge organism from organ samples**

As shown in Table III, the challenge organism was isolated from the liver and caecal tonsils of group 1 and from the liver, spleen and caecal tonsils of group 4, but not from the ovary or oviduct of these groups. The challenge organism was not isolated from any organ of groups 2 and 3.

**Serum antibody titres**

At 24 weeks of age, before challenge with *S. enteritidis* PT4, groups 2 and 3 tested 100% positive for seroconversion by the slide agglutination test, whereas groups 1 and 4 tested negative (Table IV). This indicates that vaccine A does not produce antibodies in the

**Table II**

<table>
<thead>
<tr>
<th>Group *</th>
<th>Eggshells</th>
<th>Egg contents</th>
<th>Cloacal swabs</th>
<th>Mean egg production</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 a</td>
<td>1 ab</td>
<td>36 b</td>
<td>61 a</td>
</tr>
<tr>
<td>2</td>
<td>3 a</td>
<td>0 a</td>
<td>18 a</td>
<td>63 a</td>
</tr>
<tr>
<td>3</td>
<td>4 a</td>
<td>1 ab</td>
<td>18 a</td>
<td>63 a</td>
</tr>
<tr>
<td>4</td>
<td>16 b</td>
<td>4 b</td>
<td>21 ab</td>
<td>63 a</td>
</tr>
</tbody>
</table>

* groups 1, 2 and 3 vaccinated; group 4 non-vaccinated control

Note: within each column, results marked a are significantly different from those marked b ($P < 0.05$); those marked ab are not significantly different from those marked either a or b.
TABLE III

Isolation of the challenge organism (Salmonella enteritidis phage type 4) from internal organs of laying hens at the end of the experiment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group *</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Oviduct</td>
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<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Caecal tonsil</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>

* groups 1, 2 and 3 vaccinated; group 4 non-vaccinated control

blood, while vaccine B does produce such antibodies. These antibodies can interfere with serological tests for the detection of field infection with S. enteritidis in laying hens. The principal O antigen of S. pullorum is present in S. enteritidis, and therefore antibodies to these somatic antigens of S. enteritidis can be detected using S. pullorum antigen.

One week after challenge with S. enteritidis PT4, the antibody response was first detected in group 1 by S. pullorum stained antigen (50% seroconversion). After two weeks, the seroconversion rate had risen to 100%. However, with S. enteritidis stained antigen, the antibody response was first detected after two weeks (50% seroconversion), and 100% seroconversion occurred only after four weeks. The seroconversion rate of the control group increased rapidly after challenge and reached the level of the vaccinated

TABLE IV

Presence of antibodies in serum following vaccination and/or challenge, tested by slide agglutination using Salmonella pullorum and S. enteritidis stained antigen (SP and SE, respectively)

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>No. of weeks post-challenge</th>
<th>1 SP</th>
<th>2 SE</th>
<th>3 SP</th>
<th>4 SE</th>
<th>5 SP</th>
<th>6 SE</th>
<th>7 SP</th>
<th>8 SE</th>
<th>9 SP</th>
<th>10 SE</th>
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</thead>
<tbody>
<tr>
<td>24</td>
<td>0 **</td>
<td>0/8</td>
<td>0/8</td>
<td>8/8</td>
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<td>25</td>
<td>1</td>
<td>4/8</td>
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<td>27</td>
<td>3 **</td>
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<td>5/8</td>
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* groups 1, 2 and 3 vaccinated; group 4 non-vaccinated control

** challenge with S. enteritidis phage type 4
groups within two weeks. The 100% seroconversion of all four groups lasted for nine weeks after the first challenge, probably due to repeated challenge every three weeks. The results clearly show that *S. pullorum* stained antigen is more sensitive than *S. enteritidis* stained antigen for the detection of serum antibodies in laying hens. The serological picture of the ELISA test was similar for all four groups and correlated with the results of the slide agglutination test using *S. pullorum* stained antigen.

**Egg production**

The level of egg production in all four groups decreased after challenge at 24 weeks of age. At 26 weeks, egg production returned to normal. The mean total daily egg production per hen was calculated statistically at weekly intervals, and there were no significant differences between the four groups in the mean egg production of the laying hens at any point during the experiment (Table II).

**DISCUSSION**

The following observations indicated protection of the vaccinated groups over the first three weeks after oral challenge with $10^5$ CFU/bird of *S. enteritidis* PT4:

- **a)** complete elimination of the challenge organism from eggshells and egg contents of group 2
- **b)** considerable reduction in the recovery of the challenge organism from eggshells and egg contents of groups 1 and 3
- **c)** complete prevention of shedding of the challenge organism in faecal excretions of the three vaccinated groups.

Vaccine A gives good protection not only against the homologous fowl typhoid infection (3, 9, 14, 15, 18), but also against the heterologous *S. enteritidis* PT4 infection (3). The challenge organism isolated from eggshells during this period came from the oviduct and not from faecal contamination, as the challenge organism was not isolated from the cloacal swabs during this period. The oviduct can be infected with *S. enteritidis* PT4 in the absence of faecal carriage (10). Full protection of birds from shedding the challenge organism in eggs or faecal excretion, and the reduction of contaminated eggshells, requires cell-mediated, mucosal and humoral immunities (17).

Potential egg-borne transmission of vaccine A strain following vaccination was indicated by the isolation of this strain from the eggshells of one hen from group 2 at two weeks after the first challenge. This is in agreement with the findings of other research workers (8, 14) who demonstrated the occasional vertical transmission of vaccine A strain in chickens.

After increasing the oral challenge dose to $10^9$ CFU/bird, the mucosal immunity was overwhelmed by the large challenge dose and the challenge organism was isolated from the cloacal swabs. Parenteral vaccine administration may not evoke an extensive mucosal immune response (1). The recovery of the challenge organism from eggshells and cloacal swabs was probably a result of the large challenge dose used, rather than an indication of failure of the vaccine (16). This challenge dose was relatively high and much greater than any dose which might be expected under natural conditions; such a dose rarely occurs under field conditions (17).
At 30 weeks of age, a third challenge dose of $10^6$ CFU/bird of *S. enteritidis* PT4 was administered intramuscularly, to study the effect of administration route on isolation of the challenge bacterium. The challenge organism was occasionally isolated from eggshells of groups 1 and 4, but was not isolated from the egg contents of either the vaccinated groups or the non-vaccinated group. This was due to the repeated challenge of all groups with *S. enteritidis* PT4, which induced immunity and some protection in all groups. Thus, it is not possible to separate the effects of immunisation and challenge in either vaccinated or non-vaccinated groups. Vaccine B elicited a rapid and strong humoral antibody response in the immunised birds. There was no correlation between humoral antibodies and shedding of the challenge organism in the faeces or egg contents (7). Vaccine B (either alone or in combination with vaccine A) completely eliminated the recovery of the challenge organism from internal organs at the end of the experiment (at age 33 weeks). However, the recovery of the challenge organism from the internal organs of hens vaccinated with vaccine A alone indicated that the level of immunity produced by this vaccine was not sufficient to protect the liver and caecal tonsils from the challenge organism, while protection against various systemic consequences of *Salmonella* infection after immunisation with *S. enteritidis* has been reported on several occasions (4, 16).

Therefore, vaccinating with a combined vaccination programme of vaccine A and vaccine B in association with improved hygienic conditions, maximum biosecurity and rodent control, should provide an excellent comprehensive programme to poultry farmers for the control of *S. enteritidis* infection in laying hens.

ACKNOWLEDGEMENT

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* * * * *


Résumé : Quatre groupes de poules pondeuses de souches commerciales Dekalb Delta (huit poules par groupe) ont été élevés en cages individuelles dans une unité d’isolement. À l’âge de huit semaines, les groupes 1 et 2 ont reçu le vaccin contenant la souche vivante *Salmonella gallinarum* 9R (« 9R vivant »). À dix-huit semaines, le groupe 1 a été revacciné avec le « 9R vivant », tandis que les groupes 2 et 3 recevaient le vaccin à *S. enteritidis* tuées, au même âge, puis un rappel à 22 semaines. Aucun vaccin n’a été administré au groupe 4 servant de groupe témoin.

À 24, 27 et 30 semaines, les quatre groupes ont été éprouvés par différentes doses de *S. enteritidis* phage type 4 selon diverses voies d’administration. Des œufs, écouvillonnages cloacaux et des prélèvements sanguins ont été collectés chaque semaine pour analyse bactériologique et sérologique.
L'isolement de l'organisme responsable à partir des coquilles d'œufs et de leur contenu chez les groupes vaccinés était nettement plus rare qu'à partir de ceux du groupe témoin. Aucune corrélation n'a été observée entre le taux d'anticorps humoraux et l'excrétion d'organismes pathogènes dans les œufs ou écouvillonnages cloacaux.

Un programme de vaccination combinant le vaccin 9R à S. enteritidis vivantes et le vaccin à S. enteritidis tuées a conféré une immunité plus solide aux poules pondeuses que l'un ou l'autre de ces vaccins, administré seul. Une vaccination ne doit jamais être effectuée de manière isolée mais en association avec d'autres mesures d'hygiène vétérinaire et une conduite rationnelle de l'élevage.


Resumen: Cuatro grupos, formados cada uno por ocho gallinas ponedoras comerciales Dekalb Delta, fueron criados en jaulas individuales en unidades aisladas. A las ocho semanas de vida, se inoculó a los grupos N° 1 y N° 2 la vacuna con la cepa viva Salmonella gallinarum 9R («9R viva»). A las dieciocho semanas, el grupo N° 1 fue revacunado con S. enteritidis «9R viva», mientras que a los grupos N° 2 y N° 3 se les inoculaba la vacuna con S. enteritidis inactivada. A las veintidós semanas, los grupos N° 2 y N° 3 fueron revacunados con la misma vacuna. Para que pudiera servir como testigo, no se vacunó al grupo N° 4.

A las 24, 27 y 30 semanas, se probaron los cuatro grupos utilizando varias concentraciones de S. enteritidis faga tipo 4, inoculadas por vías diversas. Cada semana se colectaron huevos, muestras cloacales y muestras de sangre para realizar análisis bacteriológicos y serológicos.

El aislamiento del organismo inoculado a partir de las cáscaras de huevos y de su contenido en los grupos vacunados resultó notoriamente más escaso que en el grupo testigo. No se observó ninguna correlación entre el porcentaje de anticuerpos humorales y la excreción del organismo inoculado en los huevos o muestras cloacales.

El programa de vacunación que combinó la vacuna viva 9R y la vacuna inactivada inmunizó mejor a las gallinas que la inoculación de una sola de estas dos vacunas. Nunca debe utilizarse la vacuna sola, sino en combinación con medidas de higiene veterinaria y en el marco de un buen manejo.

REFERENCES


