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# CONTROL OF *SALMONELLA* ENTERICA SEROVAR ENTERITIDIS IN LAYING HENS BY INACTIVATED *SALMONELLA* ENTERITIDIS VACCINES

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## ABSTRACT

*Salmonella* Enteritidis is one of the agents that is responsible for outbreaks of human foodborne salmonellosis caused by *Salmonella* Enteritidis and is generally associated with the consumption of poultry products. Inactivated *Salmonella* Enteritidis cell vaccine is one of the available methods to control *Salmonella* Enteritidis in breeders and laying hens, however results in terms of efficacy vary. This vaccine has never been tested in Brazil, therefore, the present work was carried out to assess three commercial inactivated *Salmonella* Enteritidis vaccines allowed in Brazil. Four hundred white light variety commercial laying hens were obtained at one-day-of age. At eight weeks old, the birds were divided into four groups with one hundred animals each. Birds from three groups ($V_1$, $V_2$ and $V_3$) received different intramuscular vaccines, followed by a booster dose at 16 weeks of age. Birds from another group (CG) were not vaccinated. When the laying hens were 20, 25 and 31 weeks old, 13 from each group were transferred to another room and were challenged by inoculating 2 mL neat culture of *Salmonella* Enteritidis. On the second day after each challenge, the caecal contents, spleen, liver and ovary of three birds from each group were analyzed for the presence of *Salmonella* Enteritidis. Twice a week a cloacal swab of each bird was taken and all eggs laid were examined for the presence of *Salmonella* Enteritidis. After four consecutive negative cloacal swabs in all the groups, the birds were sacrificed so as to examine the liver, caecal contents and ovaries. Overall, the inactivated vaccine used in group $V_3$ reduced *Salmonella* Enteritidis in the feces and eggs. A very small amount of *Salmonella* was found in the spleen, liver, ovary and caeca of the birds in the four groups during the whole experiment. In general, inactivated *Salmonella* Enteritidis vaccines was able to decrease the presence of *Salmonella* Enteritidis in the birds and in the eggs as well. Nevertheless, they must be associated with general hygiene and disinfection practices in poultry husbandry.

**Key-words:** *Salmonella* Enteritidis, oil-emulsion inactivated vaccines, control, laying hen

## INTRODUCTION

In many countries, outbreaks of human salmonellosis have been occurring since the 1980s. These events are mostly related to the consumption of poultry products, specially eggs and food containing raw eggs contaminated by *Salmonella enterica* serovar Enteritidis (3,26,28,35).

*Salmonella* Enteritidis was introduced in poultry flocks mainly by vertical transmission. Once *Salmonella* Enteritidis reaches a flock of birds it is easily disseminated through the feces (11,34) and remains in the environment (16,40). Therefore, commercial birds may be contaminated throughout their lives. The *Salmonella* control program should pay attention to the vertical via in addition to other measures taken during the birds'

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life. Inactivated *Salmonella* Enteritidis vaccines have been used in several countries (9,21,22,37,39) and in some of them they have been used in breeder flocks (13,30,39).

The inconvenience of using inactivated *Salmonella* Enteritidis vaccines is the need for individual application and the reaction caused by the lipopolysaccharide bacterium plus the adjuvant vaccine. Nevertheless, there is a chance of including these vaccine antigens in other polyvalent inactivated preparations that have already been adopted. In addition, since they do not have live *Salmonella* Enteritidis cells there is no harm to public health.

The efficacy of vaccine preparation is judged by the level of intestinal and systemic colonization, morbidity and mortality rates after vaccination and experimental infection using the oral or parenteral routes of administration. However, the level of protection depends on the challenge strain, the route of administration, infection dose, bird age and species/line/breed. Consequently, it is difficult to compare the efficacy of the currently available vaccine preparations precisely (37).

Inactivated vaccines have been used to control non-specific host *Salmonella* infections in poultry with varying success (9,17,21,22,30,33,36). Thus, several publications are favorable to their application due to the reduction of fecal shedding and decrease in organ colonization and contamination of eggs. Single oral or intramuscular immunization with formalin-inactivated *S*. Enteritidis at 2 weeks of age decreases fecal shedding and organ colonization of *Salmonella* Enteritidis after oral infection with $10^9$ colony forming units (CFU) at 6 weeks of age (29). The vaccination of hens with oil-emulsion inactivated *S*. Enteritidis vaccine reduced fecal shedding of *Salmonella* Enteritidis after the challenge. In vaccinated hens, 58% of fecal samples were positive, while in unvaccinated hens, 81% were positive (22). Laying hens vaccinated intramuscularly with a commercial inactivated *Salmonella* Enteritidis vaccine and challenged intravenously with *Salmonella* Enteritidis culture, produced less *Salmonella* Enteritidis positive eggs (54/439 batches of eggs) than the unvaccinated ones (99/252 batches) (39).

The immunization of 38 week old laying hens with an inactivated S. Enteritidis vaccine, followed by a booster four weeks later, reduced colonization of ovary, spleen and fecal shedding of *Salmonella* Enteritidis after intravaginal challenge. After the challenge, 19% of the eggs laid by vaccinated hens were positive, resulting in a significantly lower frequency than in unvaccinated hens (37%) (33). On the other hand, in a field trial conducted in 10 laying hen flocks, there was no difference in the recovering of *Salmonella* Enteritidis from bird organs and the environment, despite the administration of inactivated *Salmonella* Enteritidis vaccine (17). In another field study, in which inactivated *Salmonella* Enteritidis vaccine was administered to laying hens, *Salmonella* Enteritidis although not completely eliminated, was reduced in the flocks (15). According to Inoue (27) broiler chicks from vaccinated breeder flock shedded less *Salmonella* Enteritidis than those from unvaccinated breeder flock after experimental challenge on the first day of life.

Publications on *Salmonella* control by vaccines present approaches which are much more suitable to experimental conditions than to the real situation in the field. In this study, three commercial vaccines containing inactivated *Salmonella* Enteritidis cells in oil-emulsion were assessed, trying to simulate field conditions.

## MATERIAL AND METHODS

### Bacteria

The challenge was carried out with a mutant strain of *Salmonella* Enteritidis PT4 resistant to nalidixic acid and spectinomycin (SE $Nal^r/Spec^r$). Bacterial cultures were set in 10 mL LB broth (Difico-244620) incubated in a shaking incubator (100 rev/min) at 37ºC overnight. This culture contained approximately 2.13 x $10^9$ CFU/mL.

### Experimental animals

The experiment was carried out with a white light variety of commercial laying hens (Hyline W-36). Four-hundred birds were obtained at one-day-of age. They were reared and fed according to producer recommendations. At 8-weeks they were divided into four groups ($V_1$, $V_2$, $V_3$ and CG) with 100 birds each.

On arrival, the birds were inspected for *Salmonella* sp according to Zancan *et al.* (40).

### Vaccines

Three commercial vaccines ($V_1$, $V_2$ and $V_3$) were used which are produced by different companies allowed for use in breeder and commercial laying hen flocks. They contained inactivated *Salmonella* Enteritidis cells in oil-emulsion. At 8 and 16 weeks of age, the birds in each group were vaccinated intramuscularly as recommended by the manufacturer.

### Experimental design

Groups $V_1$, $V_2$ and $V_3$ were vaccinated with different vaccines and CG group received no vaccine.

When birds were 20, 25 and 31 weeks old, 13 from each group were transferred to another room and were challenged by being inoculating with 2 mL neat culture of *Salmonella* Enteritidis $Nal^r/Spec^r$.

On the second day after each challenge, the caecal contents, spleen, liver and ovary of three birds from each group were analyzed for *Salmonella* Enteritidis $Nal^r/Spec^r$. Twice a week a cloacal swab was taken from each bird and all eggs laid were examined for the presence of *Salmonella* Enteritidis $Nal^r/Spec^r$. Birds were sacrificed for the examination of liver, caecal contents and ovaries after four consecutive negative results of cloacal swab examination in all groups.

**Bacteriological analysis**

The bacteriological analysis was carried out as described by Barrow & Lovell (10) with some modification. The *Salmonella* Enteritidis $Nal^r/Spec^r$ fecal shedding was inspected by cloacal swabs, which were placed in selenite broth (CM 395, Oxoid) containing novobiocin (40 µg/mL) (SN) incubated overnight at 37ºC before being plated on Brilliant Green agar (CM 263, Oxoid) containing sodium nalidixate (100 µg/mL) and spectinomycin (100 µg/mL) (BGA NalSpc). In the absence of growth, new plating was performed from the incubated swab. Eggs collected during the experiment were dropped into sterile glass jars, the shell broken and contents mixed by agitation. Yolk from the ovaries was collected in a jar with SN broth. The jars were incubated at 37ºC overnight, and their contents were plated on BGA NalSpc and incubated at 37ºC. Samples from spleen and liver were homogenized in a pestle and mortar. The tissue homogenates and the caecal contents were mixed and diluted in phosphate-buffered saline, pH 7.4. The viable count of *Salmonella* Enteritidis $Nal^rSpc^r$ in the samples was estimated by plating aliquots of decimal dilutions on BGA NalSpc incubated overnight at 37ºC. When no *Salmonella* Enteritidis was found, the first dilution of the sample was added to an equal volume of double-strength SN broth, which was incubated at 37ºC overnight and plated on BG NalSpc agar.

## RESULTS

Table 1 shows the results concerning the presence of *Salmonella* Enteritidis in the spleen, liver and caecal contents two days after each challenge. The presence of *Salmonella* Enteritidis was similar in the liver and spleen among groups. It was only in the third challenge that *Salmonella* Enteritidis counting in the caecal contents differed between group $V_1$ and the Control Group ($p < 0.05$). *Salmonella* Enteritidis was not recovered from the ovaries. In the second trial, *Salmonella* Enteritidis was isolated from the caecal contents of two birds, one from the $V_1$ group and the other from the $V_2$ group. In the third trial, *Salmonella* Enteritidis $Nal^rSpc^r$ was found in the liver of one bird from the $V_2$ group, in the caecal contents of two birds from the $V_2$ group and in caecal contents of one bird from the CG group.

*Salmonella* Enteritidis fecal shedding data are in Table 2. Only in the first trial was the difference between $V_3$ group and the control group significant ($p < 0.05$).

The detection of *Salmonella* Enteritidis in eggs is shown in Table 3. In general, birds from the control group produced more contaminated eggs than birds from other groups, but the recovery means were variable. In the first challenge, the vaccine used in the laying hens from the $V_3$ group reduced the presence of *Salmonella* Enteritidis ($p < 0.05$) and in the last trial all vaccines reduced the presence of *Salmonella* Enteritidis in eggs ($p < 0.05$).

## DISCUSSION

Human foodborne salmonellosis has been strongly associated with eggs and egg products contaminated with *Salmonella* Enteritidis (3,26,28,35,38), although many efforts have been made for the control of *Salmonella* Enteritidis in laying hens to prevent egg contamination. Following oral infection, *Salmonella* Enteritidis colonizes the intestinal tract and may invade organs such as the liver, spleen, ovary and

**Table 1:** Means of viable count ($Log_{10}$) of *Salmonella* Enteritidis $Nal^r/Spec^r$ of three birds in spleen, liver and caecal contents two days after each challenge.

| Tissue | Challenge | Treatments | | | |
|---|---|---|---|---|---|
| | | $V_1$ | $V_2$ | $V_3$ | CG |
| Spleen | **1st** | 2.33 A | < 2.00 A | < 2.00 A | < 2.00 A |
| | 2nd | **< 2.00 A** | **< 2.00 A** | **< 2.00 A** | **< 2.00 A** |
| | **3rd** | < 2.00 A | < 2.00 A | < 2.00 A | 2.67 A |
| Liver | **1st** | 2.33 A | < 2.00 A | < 2.00 A | 2.30 A |
| | 2nd | **< 2.00 A** | **< 2.00 A** | **< 2.00 A** | **< 2.00 A** |
| | **3rd** | < 2.00 A | < 2.00 A | < 2.00 A | 2.33 A |
| **Cecal contents** | **1st** | 3.36 A | 2.33 A | < 2.00 A | 3.19 A |
| | **2nd** | 2.53 A | 2.59 A | 3.07 A | 3.67 A |
| | **3rd** | 2.67 B | 3.57 AB | 3.43 AB | 4.50 A |

Group of vaccinated (**$V_1$, $V_2$** and **$V_3$**) and unvaccinated (**CG**) hens; Means followed by different letters in the same line indicate significant differences by Tukey's test ($p < 0.05$).

**Table 2:** Recovery of *Salmonella* Enteritidis Nal$^r$/Spec$^r$ from cloacal samples after each challenge of vaccinated (**$V_1$, $V_2$** and **$V_3$**) and unvaccinated (**CG**) birds.

| | 1st Challenge | | | | | | | | | | | | 2nd Challenge | | | | | | | | | | | | 3rd Challenge | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dpi | $V_1$ | | | $V_2$ | | | $V_3$ | | | CG | | | $V_1$ | | | $V_2$ | | | $V_3$ | | | CG | | | $V_1$ | | | $V_2$ | | | $V_3$ | | | CG | | |
| | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T | D | E | T |
| **2** | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| **10** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **16** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **20** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **∑** | **1$^{ab}$ /60** | | | **4$^a$ /60** | | | **0$^b$ /60** | | | **5$^a$ /60** | | | **0$^a$ /60** | | | **2$^a$ /60** | | | **1$^a$ /60** | | | **2$^a$ /60** | | | **0$^a$ /60** | | | **3$^a$ /60** | | | **0$^a$ /60** | | | **0$^a$ /60** | | |

**Dpi**: days after the challenge; **D:** positive result in first swab; **E:** positive result in second plating; **T:** Total = D + E; **Σ** = total of positive cloacal swabs in 60 observations; ab= Means followed by different letters in the line for each challenge indicate significant differences by Chi-Square's test ($p < 0.05$).

**Table 3:** Recovery of *Salmonella* Enteritidis Nal$^r$/Spec$^r$ from egg samples after challenge of vaccinated (**$V_1$, $V_2$** and **$V_3$**) and unvaccinated (**CG**) birds.

| Dpi | 1st Challenge | | | | 2nd Challenge | | | | 3rd Challenge | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $V_1$ | $V_2$ | $V_3$ | CG | $V_1$ | $V_2$ | $V_3$ | CG | $V_1$ | $V_2$ | $V_3$ | CG |
| **1** | 1 | 5 | 1 | 5 | 3 | 3 | 0 | 3 | 2 | 1 | 1 | 4 |
| **2** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| **3** | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| **4** | 0 | 1 | 0 | 0 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 1 |
| **5** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **6** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **8** | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **9** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **10** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **11** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **12** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **14** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **15** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **16** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Pos.*** | 3$^{ab}$/ 123 | 7$^{ab}$/ 136 | 2$^b$/126 | 8$^a$/122 | 5$^a$ /125 | 5$^a$ /126 | 4$^a$ /121 | 8$^a$ /124 | 2$^b$ /145 | 2$^b$ /149 | 1$^b$ /145 | 8$^a$ /133 |

**Dpi=** days after infection; **Pos.*** = Number of positive samples/ total eggs examined; ab = Means followed by different letters in the line for each challenge indicate significant differences by Chi-Square's test ($p < 0.05$).

heart (10,20). In this study, quite a few *Salmonella* Enteritidis organisms were found in the liver and spleen (Table 1) of birds from all the groups during the experiment, with no difference between vaccinated and unvaccinated birds ($p > 0.05$). These findings might have been expected since Berchieri Jr. *et al*. (12) demonstrated that *Salmonella* Enteritidis did not persist longer in mature birds. It was also showed that 20-40 weeks old laying hens are naturally more resistant to *Salmonella* Enteritidis infection (25). In contrast, the beneficial effect of *Salmonella* Enteritidis inactivated oil-emulsion vaccines in preventing organ colonization by *Salmonella* Enteritidis was demonstrated by Nakamura *et al*. (33) and Gast *et al.*(21).

In the present study, *Salmonella* Enteritidis fecal shedding (Table 2) did differ between the $V_3$ group and the control group in the first challenge ($p < 0.05$). Depending on the composition, the inactivated vaccine can decrease of *Salmonella* Enteritidis fecal shedding (29). A study conducted by Barbour *et al*. (5), comparing six inactivated *Salmonella* Enteritidis vaccines, showed a variable decrease in the *Salmonella* Enteritidis fecal shedding. These authors suggested that several factors regarding the type and composition of the adjuvant, strain of *Salmonella* Enteritidis and inactivation method, could be responsible for this variation, and could explain the results depicted in Table 2. Unfortunately, not all information on the commercial vaccines used was available.

It is proposed that cell-mediated immunity is more important for tissue clearance of *Salmonella* Enteritidis, while humoral response seems to be responsible for the reduction of intestinal colonization (6,7,24,32,37). One of the criteria for an ideal vaccine is the promotion of bird protection against mucosal and systemic infection by effective stimulation of both immune responses (37). Some authors showed that the *Salmonella* Enteritidis inactivated vaccines induce only a good humoral immune response, which reduces the intestinal colonization by *Salmonella* Enteritidis (4,6,14,18,24,32). This might be in the reason for the decrease of *Salmonella* Enteritidis in bird feces (caecal *Salmonella* Enteritidis counting and cloacal swabs) of the $V_1$ and $V_3$ groups (Tables 1 and 2) in the present work.

There was a positive effect of the vaccination of birds in the $V_3$ group in the first challenge and in all the groups of vaccinated birds in the third challenge (Table 3). These results are in agreement with previous works in which the presence of *Salmonella* Enteritidis in eggs laid was reduced by a vaccination program using an inactivated vaccine (21, 30 e 39). About 68.2% of the outbreaks of human foodborne salmonellosis caused by *Salmonella* Enteritidis is related to egg and food containing raw eggs (31), despite the fact that one out of 20,000 eggs laid is supposed to be contaminated by *Salmonella* Enteritidis (19). Therefore, any reduction is very welcome. In addition, inactivated vaccines may induce enough passive immunity to protect the progeny (27).

In the first and second challenges, it was possible to observe some correlation between *Salmonella* Enteritidis in feces and in eggs. Similar results were observed by Gast & Beard (20) and Woodward *et al.* (39). According to Barrow & Lovell (10) eggs are mainly contaminated with *Salmonella* Enteritidis by contact with fecal material in the cloacae, although transovarian contamination also occurs.

Intrinsic factors in the eggshell and in the albumen may interrupt the multiplication of *Salmonella*. At low temperatures, *Salmonella* can be kept viable in the yolk (2) and can multiply in 72hs, at 15ºC (1), and in hot weather these factors become less active. Thus, the best way to prevent human foodborne salmonellosis is by taking precautions during the bird's lifetime. It is known that *Salmonella* Enteritidis may persist in vaccinated flocks of laying hens (15). But in view of the results obtained in this work, a vaccination program to control the presence of *Salmonella* Enteritidis in laying hens can be adopted as an additional tool to minimize the presence of *Salmonella* Enteritidis in eggs, in association with general practices of hygiene and disinfection in poultry husbandry.

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## RESUMO

### "Controle de *Salmonella enterica* sorovar Enteritidis em poedeiras comerciais com a utilização de vacinas inativadas"

*Salmonella* Enteritidis é um dos agentes responsáveis por toxinfecção alimentar em humanos e tem sido associada a alimentos de origem avícola. Entre os métodos disponíveis para o seu controle está a vacinação de poedeiras e matrizes com vacinas inativadas (bacterinas). Os resultados a respeito da proteção das bacterinas contra *Salmonella* Enteritidis em aves são variados. Face à inexistência de dados referentes ao uso dessas vacinas no Brasil, realizou-se o presente trabalho. Foram utilizadas 400 pintinhas de uma linhagem de postura leve. Na 8º semana de idade, as aves foram divididas em quatro grupos ($V_1$, $V_2$, $V_3$ e CG). Três diferentes bacterinas comerciais foram administradas às aves do $V_1$, $V_2$ e $V_3$ em duas doses, na 8º e 16º semanas de vida; as do CG não receberam vacina. Treze aves por grupo foram infectadas com *Salmonella* Enteritidis nas 20º, 25º e 31º semanas. No 2º dia após cada desafio foram sacrificadas três aves por grupo, para contagem de *Salmonella* Enteritidis em fígado, baço, conteúdo cecal e pesquisa do microrganismo no ovário. Suabes de cloaca foram realizados dois dias pós-infecção (dpi) e duas vezes por semana. Todos os ovos foram examinados. Após a ausência de *Salmonella* Enteritidis em quatro suabes de cloaca consecutivos, esse microrganismo foi pesquisado em fígado, conteúdo cecal e ovário. Não houve diferença da contagem de *Salmonella* Enteritidis nos órgãos. O conteúdo cecal das aves do $V_1$ teve menos *Salmonella* que as do CG. As aves do $V_3$ excretaram menos *Salmonella* em fezes e ovos. Conforme os resultados observados no $V_3$, é possível reduzir excreção de *Salmonella* Enteritidis com o uso de bacterinas; contudo, deve ser utilizado de forma complementar a boas práticas de manejo, limpeza e desinfecção.

**Palavras-chave:** *Salmonella* Enteritidis, vacinas oleosas inativadas, controle, poedeiras

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