



Effect of Infectious Bursal Disease (Ibd) Vaccines on Infection of *Salmonella Heidelberg* in Broiler Chickens

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ABSTRACT

Salmonella Heidelberg (SH) has represented a great concern to the Brazilian poultry industry in the last years. It is known that immunosuppression in poultry is a contributing factor to increase *Salmonella* faecal shedding and to disturb control programmes. Not only infectious bursal disease (IBD) virus but also some live vaccines have been reported to induce immunosuppression. In the present study we assessed the effects of two live vaccines against IBD on SH-infected broiler chicks. At 7 days of age, birds of three groups (vaccinated with recombinant HVT-IBD vector, with immune complex-IBD vaccine and unvaccinated) were orally challenged with 1×10^8 CFU of SH. A group of hatchmates remained unvaccinated/unchallenged to serve as negative controls. Caecal colonization and systemic invasion were evaluated by bacterial enumeration at 1, 3, 5, 7 and 14 days post-infection (Dpi) and SH faecal shedding assessed by cloacal swabs at 3, 7, 10 and 14 Dpi. The counts of SH in caecal contents were higher in birds vaccinated with immune complex-IBD than in those that received the HVT-IBD vector vaccine at 5, 7 and 14 dpi ($p < 0.01$). There were no statistical differences in bacterial counts in liver and spleen among birds of different groups. Cloacal swabs also indicated that the birds vaccinated with immune complex-IBD shed more SH than those vaccinated with HVT-IBD vector or those unvaccinated ($p < 0.01$). The results of the present study suggested that the immunosuppressive effect of the immune complex-IBD vaccine helped to increase the SH-faecal shedding in the infected birds.

INTRODUCTION

Annually, millions of cases of human foodborne diseases worldwide are caused by *Salmonella* (WHO, 2018). *Salmonella* serotype Heidelberg (SH) is amongst the most prevalent serotypes isolated from human and non-human sources (CDC, 2018). The majority of the foodborne infections caused by SH has been associated with poultry meat (Etter *et al.*, 2019). In Brazil, SH represented about 56% of all *Salmonella* isolates recovered from broiler carcass in 2017 (Brasil, 2018).

In order to reduce the levels of contamination of poultry products, actions need to be taken at the whole poultry production chain (Gast, 2013). In this context, factors that favor horizontal or vertical transmission of *Salmonella* at farm level are detrimental to any control programme (Koutsoumanis *et al.*, 2019).

The immune responses of poultry to *Salmonella* are crucial to intestinal and systemic clearance (Wigley, 2014). The intestinal immunity, including secretory immunoglobulin A (IgA) is important against *Salmonella* that colonizes the intestine. While cell mediated immunity plays a role in controlling mainly systemic infection (Withanage *et al.*, 2005). Effects of immunosuppression caused by bursectomy, infection bursal disease (IBD) virus or some IBD vaccines on immune responses to



Salmonella serotypes Typhimurium (ST) and Enteritidis (SE) have been demonstrated (Corrier *et al.*, 1991; Arnold & Holt, 1995; Phillips *et al.*, 1995; Bautista *et al.*, 2004; Arafat *et al.*, 2017). However, this was not yet investigated during infection by SH. In the present study we assessed the effects of two IBD vaccines on caecal colonization, systemic invasion and faecal excretion of SH in broiler chicks.

MATERIAL AND METHODS

The experiment was carried out at the facilities of the Avian Diseases Laboratory of the Department of Preventive Veterinary Medicine of the Federal University of Minas Gerais (UFMG).

Bacteria

A spontaneous nalidixic acid resistant strain of *Salmonella enterica* subsp *enterica* serotype Heidelberg (SH Nal^r) was used. This strain was provided by Professor Angelo Berchieri Junior from the State University of São Paulo, Jaboticabal campus. It was previously isolated from a broiler flock from the Brazilian South region.

Broiler chicks

One hundred and twenty-one day-old broiler chicks were purchased from a commercial hatchery. The birds were not vaccinated against Marek's disease at the hatchery. On arrival, samples of faeces in the transport cardboard boxes were collected and processed to assure the birds were free of *Salmonella* spp. (Zancan *et al.* 2000).

Experimental design

The chicks were divided in four groups (A, B, C and D) and housed in acclimatised rooms. On day one, the chicks from group A were vaccinated (0.2 mL/chick subcutaneously) with a recombinant turkey herpesvirus (HVT) expressing the VP2 gene of IBD virus (HVT-IBD). Meanwhile, the birds of group B were vaccinated (0.2 mL/chick) with a live vaccine with virus coated with anti-IBD antibodies (immune complex-IBD). The birds of group C and D did not receive any IBD and HVT vaccine. On day 7, the chicks of groups A, B and C were orally challenged with 1×10^8 CFU of SH Nal^r. The birds in group D were kept as negative control. This experiment was approved by the institutional ethical committee (Protocol 345/2018; approved on 25 February 2019).

Bacteriology

At 1, 3, 5, 7 and 14 days post-infection (Dpi), five birds from each infected group were euthanized and

samples of the spleen, liver and caecal content were collected for bacterial enumeration. Bacterial shedding in faeces was also monitored by cloacal swabs twice a week. All bacteriological procedures followed the methodology described by Berchieri *et al.* (2001). Briefly, the enumeration of SH Nal^r in the samples was estimated by plating aliquots of decimal dilutions onto brilliant green agar (BGA) (Oxoid, US) plates, containing 100 µg / mL of nalidixic acid (Sigma-Aldrich, US). The first dilution of each sample was added to an equal volume of double-strength selenite broth (Oxoid, US) and incubated. The plates and selenite enrichment cultures were also incubated for 24 hours at 37°C. Cloacal swabs were plated on BGA and further incubated in selenite broth. Those samples for which no bacteria grew on BGA were re-streaked onto new BGA plates from the enriched cultures.

Statistical analysis

Statistical differences amongst mean counts of SH Nal^r recovered from caecal contents, livers and spleens were determined using Tukey's test. Data on faecal shedding obtained by cloacal swabs were compared by Chi-Square's test. Statistical analyses were performed using GraphPad Prism version 8.0.1 (GraphPad Software, US).

RESULTS

Examination of the liver, spleen and caecal content of the birds of uninfected control group (D) indicated that they kept SH-free over the experiment.

The results of SH enumeration in livers, spleens and caecal contents of the birds belonging to groups A (HVT-IBD vector), B (immune complex-IBD) and C (unvaccinated) are shown in table 1. There were no significant differences among the counts in livers and spleens at 1, 3, 5, 7 and 14 Dpi ($p > 0.05$). At 1 and 3 Dpi, SH counts in caecal contents were also similar ($p > 0.05$). However, at 5 Dpi, birds of group B showed higher counts in caecal contents than those of group A. At 7 Dpi SH counts in caeca of birds of group B were higher than in birds of groups A and C (also in figure 1). At 14 Dpi the amounts of SH in caeca of birds of group B were still higher than in birds of group A ($p < 0.05$).

SH shedding was also monitored by cloacal swabs of the birds and the results are displayed in table 2. The total number of positive cloacal swabs in the birds of group B was also higher than in the birds of group C ($p < 0.01$). If only the direct plating of the swabs is considered, the



Table 1 – Mean counts of *Salmonella* Heidelberg (SH Nal^r) of five birds in spleen, liver and caecal contents at 1, 3, 5, 7 and 14 days post-infection (Dpi). Values are expressed as means ± standard deviation of bacterial counts (log₁₀ CFU/g).

| Tissue | Dpi | Groups | | |
|---------------|-----|---------------------|------------------------|------------------|
| | | A (HVT-IBD vector) | B (immune complex-IBD) | C (Unvaccinated) |
| Spleen | 1 | 0.40 A ± 0.89 | 1.82 A ± 2.58 | 1.74 A ± 2.90 |
| | 3 | 1.06 A ± 1.52 | 2.36 A ± 2.45 | 0.40 A ± 0.89 |
| | 5 | 0.50 A ± 1.00 | 1.32 A ± 2.04 | 0.80 A ± 1.10 |
| | 7 | 0.0 A ± 0.0 | 0.0 A ± 0.0 | 0.80 A ± 1.10 |
| | 14 | 0.40 A ± 0.89 | 0.40 A ± 0.89 | 0.80 A ± 1.10 |
| Liver | 1 | 0.0 A ± 0.0 | 1.16 A ± 1.70 | 0.80 A ± 1.10 |
| | 3 | 0.66 A ± 1.48 | 1.36 A ± 2.10 | 0.0 A ± 0.0 |
| | 5 | 0.0 A ± 0.0 | 0.80 A ± 1.10 | 0.0 A ± 0.0 |
| | 7 | 0.0 A ± 0.0 | 0.40 A ± 0.89 | 0.0 A ± 0.0 |
| | 14 | 0.0 A ± 0.0 | 0.0 A ± 0.0 | 0.0 A ± 0.0 |
| Cecal content | 1 | 6.68 A ± 0.63 | 6.04 A ± 0.62 | 6.19 A ± 0.56 |
| | 3 | 5.48 A ± 2.31 | 6.61 A ± 0.64 | 5.81 A ± 0.84 |
| | 5 | 3.77 A ± 3.46 | 6.71 B* ± 0.38 | 4.25 AB ± 1.34 |
| | 7 | 1.72 A ± 2.86 | 7.17 C** ± 0.28 | 4.06 B* ± 2.33 |
| | 14 | 0.0 A ± 0.0 | 3.04 B** ± 1.44 | 1.60 AB ± 0.89 |

Group A: Birds vaccinated with HVT-IBD vector vaccine in the first day of life and challenged with SH at 7 days. Group B: Birds vaccinated with immune complex-IBD in the first day of life and challenged with SH at 7 days. Group C: Birds were not vaccinated with any IBD vaccine and challenged with SH at 7 days. Dpi: Days post-infection. Means followed by different letters in the same row indicate significant differences by Tukey's test (at $p < 0.05^*$; or $p < 0.01^{**}$).

Table 2 – Recovery of *Salmonella* Heidelberg (SH Nal^r) of 80 birds of each group by cloacal swabs taken at 3, 7, 10 and 14 days post-infection (Dpi).

| Dpi | Groups | | | | | | | | |
|---------------------|--------------------|----|----|------------------------|----|----|------------------|----|----|
| | A (HVT-IBD vector) | | | B (immune complex-IBD) | | | C (Unvaccinated) | | |
| | D | E | T | D | E | T | D | E | T |
| 3 | 18 | 10 | 28 | 29 | 1 | 30 | 21 | 6 | 27 |
| 7 | 6 | 10 | 16 | 11 | 8 | 19 | 6 | 7 | 13 |
| 10 | 3 | 12 | 15 | 5 | 14 | 19 | 1 | 14 | 15 |
| 14 | 1 | 12 | 13 | 5 | 7 | 12 | 0 | 8 | 8 |
| Total | 25A | 39 | 64 | 44B** | 27 | 71 | 25A | 31 | 56 |
| Total of positives: | 80% AB | | | 88.75% B** | | | 70% A | | |

Group A: Birds vaccinated with HVT-IBD vector vaccine in the first day of life and challenged with SH at 7 days. Group B: Birds vaccinated with immune complex-IBD in the first day of life and challenged with SH at 7 days. Group C: Birds were not vaccinated with any IBD vaccine and challenged with SH at 7 days. Dpi: Days post-infection; D: positive result after direct plating of swab; E: positive result after enrichment; T: Total = D + E. Means followed by different letters in the row indicate significant differences by Chi-Square's test ($p < 0.01$).

number of positives (44) in group B would be higher than in groups A (25) and C (25) ($p < 0.01$).

DISCUSSION

There are several tools (probiotics, vaccines, organic acids, etc.) available to control *Salmonella* in poultry farming (Vandeplas *et al.*, 2010; Schneitz *et al.*, 2016). However, they will have good effects only if applied together with biosecurity measures and the environmental challenge is not too high (Barrow, 2000; Gast, 2013). Therefore, immunosuppressive agents that favour *Salmonella* shedding and consequently the environmental contamination may affect the effectiveness of control programmes.

Studies have indicated that not only infectious bursal disease virus, but also some live IBD vaccines can reduce B lymphocytes populations (Avakian *et al.*, 2001) and consequently affect the immune responses to other pathogens, including *Salmonella* (Arafat *et al.*, 2017). Camilotti *et al.*, (2016) described severe atrophy

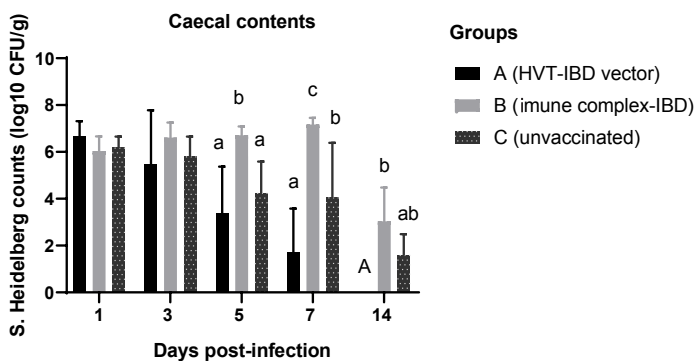


Figure 1 – *Salmonella* Heidelberg (SH) counts in caecal contents at 1, 3, 5, 7 and 14 days post-infection (Dpi). Group A: Birds vaccinated with HVT-IBD vector vaccine in the first day of life. Group B: Birds vaccinated with immune complex-IBD in the first day of life. Group C: Birds were not vaccinated with any IBD vaccine. All birds were challenged with SH at 7 days. Different letters on the plots mean there was statistical significance by Tukey's test among groups by Dpi.



of the Bursa of Fabricius (BF) in birds vaccinated with an immune complex-IBD vaccine, whereas birds that received HVT-IBD vector vaccine showed preserved BF tissue.

It is proposed that cell-mediated immunity is important for tissue clearance of invasive *Salmonella* in poultry, while IgA responses seem to be key to the intestinal clearance (Withanage *et al.*, 2005). A study of Desmidt *et al.* (1998) with *Salmonella* Enteritidis (SE)-infected bursectomized chickens showed increased faecal excretion and higher caecal counts, while having normal SE-counts in internal organs, indicating a protective effect of IgA against intestinal colonization. Similar results were observed in the present study, in which birds vaccinated with an immune complex-IBD vaccine had more *Salmonella* Heidelberg (SH) in the intestine than those vaccinated with HVT-IBD vector and no differences were observed in spleen and liver over the experiment. Apparently, only humoral responses were compromised in the birds vaccinated with immune complex-IBD. Arafat *et al.* (2017) also reported that broiler chicks vaccinated with a live IBD vaccine excreted more SE than the unvaccinated birds and correlated this finding with lower levels of intestinal IgA.

In the present study, birds vaccinated with an immune-complex IBD vaccine showed lower ability to clear intestinal SH.

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