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Evaluating Different Vaccine Routes Against Coccidiosis

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Abstract: The effect of coccidiosis vaccine route on animal development and the efficiency of different routes to protect birds against different *Eimeria* challenges were evaluated. 135 Isa Brown® birds were used in two experimental phases. In the first phase, the birds were distributed in three treatments: non-vaccinated, vaccinated against coccidiosis by water and vaccinated by spray. In the second phase, these birds were challenged separately with different eimeria species oocysts (*E. maximum*, *E. acervulina*, *E. tenella* and *E. necatrix*). Weight gain, relative weight of Fabricius bursa, degrees of lesions in the intestines and counting of oocysts in the coproparasitological examination were evaluated. As a result, in most cases, birds challenged with different eimeria species and vaccinated against coccidiosis, by water or spray, showed better physical conditions, mainly by: best weight gain and lower intestinal lesions scores, compared to the birds challenged and non-vaccinated. Another interesting result refers to the different vaccine routes, showing no significant difference regarding the effectiveness of the vaccine and the vaccine reaction in animals.

Key words: Coccidiosis, vaccination, administration routes, poultry

INTRODUCTION

Coccidiosis is an important avian disease with high economic impact on poultry production. Williams (1999) classified the total coccidiosis losses in 17.5% due to prophylaxis and 80% due to reduction on weight gain and feed conversion of animals. Coccidiostat drugs have been used in poultry diets for many years to control those losses. Nowadays there is a worldwide concern about the reduction of drugs on animal diet and the appearance of some resistance to these drugs due to wrong rotation management in field, leading to search for new technologies to coccidiosis control. The best alternative seems to be the vaccine. Joyner and Norton (1973) described that an early exposition to a small amount of these parasites induced immune protection against coccidiosis for all bird's life. One-day-old chicks are vaccinated by water or feed and the development of parasite cycle in intestine induces to a specific immunity against severe challenges (Vermeulen *et al.*, 2001). However, there is some concern about these vaccination routes. Eating competition is a very common behavior in poultry and it could result in an irregular flock vaccination so some birds could not be protected. Therefore, a spray route seems to be more effective for a regular flock vaccination because it covers all the birds and the peck behavior on their own feathers or on other birds can promote the ingestion of these parasites (Chapman *et al.*, 2002). Schetters *et al.* (1999) showed that 94% of birds were immunized against coccidiosis when the spray route was used.

The objective of this study was to evaluate the effect of coccidiosis vaccine route on animal development and the efficiency of these different routes to protect birds against different eimeria challenges.

MATERIALS AND METHODS

Experiment I: 135 Isa Brown® birds were distributed in a completely randomized experimental design with three treatment groups, with 45 replicates each, where: T1 = coccidiosis non-vaccinated birds, T2 = coccidiosis vaccinated birds by water route and T3 = coccidiosis vaccinated birds by spray route.

The birds initially received wing tag identification and were housed on floor pen and received feed and water *ad libitum*. The experimental diets were formulated according to the National Research Council (1994) recommendations for bird life stages (starter and grower). The birds of T2 and T3 groups were vaccinated against coccidiosis with a commercial vaccine at a dosage of 0.03 mL/bird, approximately 2,300 oocysts mix of *E. acervulina*, *E. maxima*, *E. mitis* and *E. tenella* per dose.

Birds were weighed every week to calculate Weight Gain (WG) and feces were collected directly from the cloacae and litter to oocyst count. At 28 days of age, four birds from each experimental group were euthanized by cervical dislocation for evaluation of intestinal coccidiosis damage on different intestinal portion (duodenum: from the pylorus to the distal duodenal loop; jejunum: from the distal duodenal loop to Meckel's diverticulum and ileum: between Meckel's diverticulum and the opening of the ceca) which were classified according to Johnson and Reid (1970) scheme. Each intestinal portion without content and Fabricius bursa were also weighed to calculate the percentage weight.

Experiment II: The same birds used on Experiment I were separated in new groups and were challenged with different *Eimeria* species. A factorial 3x4 experimental design was used, being each group vaccinated to coccidiosis by different routes (non-vaccinated group, vaccinated by water route and vaccinated by spray route) and challenged with *E. maxima*, *E. acervulina*, *E. tenella* or *E. necatrix*. This factorial totalized 12 treatments with 10 replicates each (Table 1). The birds were housed on floor pen and received feed and water *ad libitum*. The experimental diets were also formulated according to the National Research Council (1994) recommendations for bird life stages (grower).

All birds, according to experimental treatments (Table 1), at the 28th day were challenged through an oral inoculation of a solution with distillated water with 200,000 *E. acervulina* oocysts or 20,000 *E. maxima* oocysts or 30,000 *E. tenella* oocysts or 30,000 *E. necatrix* oocysts. These numbers were confirmed by counting on MacMaster chamber, according to Gordon e Whitlock modified method (Hoffmann, 1987). At 35 and 50 days of age, four birds from each experimental group were euthanized by cervical dislocation for evaluation of intestinal coccidiosis damage on different intestinal portions, as described before and which were classified according to Johnson and Reid (1970) scheme. Fabricius bursas and each intestinal portion without content were also weighed to calculate the percentage weight. Every week, birds were weighed to calculate Body Weight (BW) and feces directly from the cloacae and litter were collected to oocysts count. At 50 days of age, blood from ulnar vein was collected with heparin to evaluate complete blood cell count.

Body weight, organ relative weight and blood analysis values were submitted to ANOVA analysis ($p<0.05$) and when significant to Tukey test.

RESULTS

The results of Experiment I weigh gain are presented in Table 2. The control group at the 7th day showed weight gain significantly lower than water and spray vaccinated birds, in the other weeks there was not a significant difference in this parameter.

Table 3 shows the Experiment II weight gain results. At the 35th day, one week after the challenge, non-vaccinated (control group) chicks with *E. tenella* challenge presented significant lower weight gain. Chicks submitted to *E. tenella* challenge receiving water or spray vaccine presented significant higher weight gain than chicks non-vaccinated and submitted to the same challenge. At the 50th day non-vaccinated *E. maxima* challenge chicks presented significant lower weight gain than other groups.

Fabricius bursa weight values according to treatment at 28 and 35 day-old chicks are presented in Table 4.

Table 1: Birds distribution in experimental groups of Experiment II

Groups	Vaccination route (1st day)	28th day-old challenge
T1	Control (non-vaccinated)	*E. acervulina*
T2	Water	*E. acervulina*
T3	Spray	*E. acervulina*
T4	Control (non-vaccinated)	*E. maxima*
T5	Water	*E. maxima*
T6	Spray	*E. maxima*
T7	Control (non-vaccinated)	*E. tenella*
T8	Water	*E. tenella*
T9	Spray	*E. tenella*
T10	Control (non-vaccinated)	*E. necatrix*
T11	Water	*E. necatrix*
T12	Spray	*E. necatrix*

Table 2: Effect of coccidiosis vaccine administration route before challenge on chicks weight gain (g) from 1-28 days-old

Inoculation Route	1st day	7th day	14th day	21st day	28th day
Control (non-vaccinated)	30.3	53.2[b]	95.0	148.1	194.6
Water	30.1	58.4[a]	94.8	149.0	193.0
Spray	30.0	60.1[a]	96.5	152.1	193.5
P value	0.843	0.000	0.812	0.732	0.965

[a,b]Means followed by different letters in the same column differ by Tukey test (p<0,05).

Table 3: Effect of coccidiosis vaccine administration route after challenge on chicks weight gain (g) from 29-50 days-old

Vaccine	Challenge	35th day	50th day
Control (non-vaccinated)	*E. acervulina*	256[ab]	551[ab]
Water	*E. acervulina*	267[ab]	536[ab]
Spray	*E. acervulina*	264[ab]	542[ab]
Control (non-vaccinated)	*E. maxima*	237[ab]	440[c]
Water	*E. maxima*	246[ab]	521[ab]
Spray	*E. maxima*	226[ab]	532[ab]
Control (non-vaccinated)	*E. necatrix*	254[ab]	528[ab]
Water	*E. necatrix*	280[a]	524[ab]
Spray	*E. necatrix*	254[ab]	526[ab]
Control (non-vaccinated)	*E. tenella*	213[c]	449[ab]
Water	*E. tenella*	244[ab]	492[ab]
Spray	*E. tenella*	277[a]	569[a]
P value		0.053	0.013

[a,b,c]Means followed by different letters in the same column differ by Tukey test (p<0,05).

There was no significant difference between Fabricius bursa percentage weight when compared to body weight among treatments at the 28th day. Although after challenge (35 day-old chicks) there was significant difference, spray vaccinated chicks presented higher bursa relative weight than water vaccinated and non-vaccinated chicks.

There was a significant difference among challenge treatments for Fabricius bursa relative weight, where chicks receiving spray vaccine challenged with *E. maxima* and non-vaccinated chicks challenged with *E. tenella* showed higher bursa relative weight than chicks non-vaccinated and challenged with *E. acervulina* or water vaccinated and challenged with *E. tenella*. For better comprehension of these values, Tukey test was applied to the interaction, as showed in Table 4 and 5. Chicks challenged with *E. maxima* presented lower

Fabricius bursa relative weight than other challenges. On the other hand, spray vaccinated chicks presented higher bursa relative weight than water vaccinated and non-vaccinated chicks in 35 day-old chicks. There was no significant difference for Fabricius bursa of 50 day-old chicks among experimental groups.

There was no significant difference between the portions of small intestine and ceca of 35 and 50 day-old chicks when analyzed the interaction of vaccine and challenge. According to Table 6 vaccinated 28 day-old (before challenge) chicks presented mild lesions in all intestinal segments, except water vaccinated chicks which did not show any lesion in ileum and non-vaccinated group which presented only mild lesion in duodenum.

Table 7 shows the intestinal lesions from 35 and 50 day-old chicks at necropsy. In most cases, control group (non-vaccinated) presented for all challenges (*E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix*) more severe intestinal lesions when compared to water and spray vaccinated groups. The most severe lesions were founded in control group challenged with E. *tenella*, were 100% of birds presented score four ceca lesions. Generally, intestinal lesions in 50 day-old chicks were less intense than those founded in 35 day-old chicks. The exceptions that presented more intense lesions at 50th day were: control group (non-vaccinated) challenged with *E. acervulina* in duodenum; spray vaccinated and challenged with *E. maxima* group in ileum; control group challenged with *E. tenella* in jejunum and ileum and control group submitted to *E. necatrix* challenge in jejunum and ileum.

The number of oocysts founded in feces and litter before challenge in non-vaccinated groups was lower than in vaccinated ones. Although, after challenge, was noticed that non-vaccinated challenged chicks presented more oocysts in feces than water or spray vaccinated at 29-35 days and for *E. tenella* at 36 to 42 days (Table 8).

The blood analysis of 50 day-old chicks did not show significant difference in vaccination route versus challenge interaction, although it was observed an influence of challenge in total plasmatic protein and absolute band neutrophil number. Chicks challenged with *E. acervulina* presented the lowest total plasmatic protein. *E. maxima* presented lower number of band neutrophils than *E. necatrix*. On the other hand, as shown in table 9, non-vaccinated chicks presented lower number of eosinophils than vaccinated ones (any route).

DISCUSSION

There was no significant difference in weight gain until the 28th day among treatments, except at the 7th day, when control group had less weight gain, showing that before challenge, water or spray vaccinated chicks against coccidiosis did not present difference in weight gain from non-vaccinated ones.

Table 4: Effect of vaccine administration route in Fabricius bursa percentage weight in 28 day-old chicks, before *Eimeria* sp. challenge, and in 35 day-old chicks, after *Eimeria* sp. challenge

Inoculation route	Before challenge	After challenge
Control (non-vaccinated)	0.57	0.575^{b}
Water vaccine	0.67	0.520^{b}
Spray vaccine	0.61	0.649^{a}
P value	0.828	0.043

^{a,b}Means followed by different letters in the same column differ by Tukey test (P<0.05).

Table 5: Fabricius bursa percentage weight compared to body weight of 35 and 50 day-old chicks according to treatment, after *Eimeria* sp challenge. BW = body weight

		Fabricius bursa (% BW)	
Vaccine	Challenge	35 day-old	50 day-old
Control (non-vaccinated)	*E. acervulina*	0.42^{b}	0.59
Water	*E. acervulina*	0.50^{ab}	0.65
Spray	*E. acervulina*	0.56^{ab}	0.68
Control (non-vaccinated)	*E. maxima*	0.58^{ab}	0.59
Water	*E. maxima*	0.60^{ab}	0.57
Spray	*E. maxima*	0.78^{a}	0.63
Control (non-vaccinated)	*E. necatrix*	0.50^{ab}	0.75
Water	*E. necatrix*	0.55^{ab}	0.72
Spray	*E. necatrix*	0.63^{ab}	0.68
Control (non-vaccinated)	*E. tenella*	0.79^{a}	0.57
Water	*E. tenella*	0.40^{b}	0.57
Spray	*E. tenella*	0.62^{ab}	0.56
P value		0.023	0.850

^{a,b}Means followed by different letters in the same column differ by Tukey test (p<0,05).

Experiment II results showed that one week after challenge, 35 day-old non-vaccinated chicks challenged with *E. tenella* presented the lowest weight gain. It was also noticed that weight gain from water or spray vaccinated chicks submitted to *E. tenella* challenge presented significant higher weight gain than non-vaccinated ones, showing a protector effect of the vaccine (water or spray) against weight loss caused by the infection. 50 day-old non-vaccinated chicks challenged with *E. maxima* presented lower weight gain than other groups. According to Kawazoe (2000) and Coccidiose (2000) *E. maxima* infections characteristically cause hemorrhagic enteritis in the small intestines middle region, being associated with severe weight loss. It is important that water or spray vaccinated groups challenged with this eimerias species also presented the best weight gain, demonstrating the vaccine efficiency.

Before challenge there was no difference in Fabricius bursa percentage weight in experimental groups. After challenge, in the vaccine x challenge interaction, the intestines relative weight analysis had no significant difference between experimental groups. Although for Fabricius bursa analysis at 35 day-old chicks was noticed that spray vaccinated *E. maxima* challenged and non-vaccinated *E. tenella* challenged chicks presented higher relative bursa weight than non-vaccinated *E. acervulina* challenged or water vaccinated *E. tenella*

Table 6: Percentage of chicks presenting the maximum coccidiosis lesion score in different intestinal segments at 28th day

Vaccine	Duodenum [max.score (%)]	Jejunum [max.score (%)]	Ileum [max.score (%)]	Cecum [max.score (%)]
Control (non-vaccinated)	+(50)	-	-	-
Water	++(25)	+(75)	-	+(25)
Spray	+(75)	+(75)	+(25)	++(25)

Table 7: Percentage of chicks presenting maximum coccidiosis lesion score according to intestinal segment at the 35th and 50th day [max. score (%)]

		Duodenum		Jejunum		Ileum		Cecum	
Vaccine	Challenge	35th	50th	35th	50th	35th	50th	35th	50th
Control (non-vaccinated)	*E. acervulina*	++(75)	+++(75)	+++(50)	+++(25)	+++(50)	++(25)	++(50)	-
Water	*E. acervulina*	++(50)	++(25)	++(25)	++(50)	+(25)	-	-	-
Spray	*E. acervulina*	+(50)	+(75)	+(50)	+(50)	+(25)	-	-	-
Control (non-vaccinated)	*E. maxima*	++(50)	+(75)	++(50)	+(25)	+(25)	-	++(50)	-
Water	*E. maxima*	+(75)	-	+(75)	+(50)	+(25)	+(25)	-	-
Spray	*E. maxima*	+(25)	+(25)	+(50)	-	-	+(25)	+(25)	-
Control (non-vaccinated)	*E. necatrix*	++(25)	++(25)	++(25)	+++(25)	+(100)	++(25)	++(25)	-
Water	*E. necatrix*	+(75)	++(25)	+(75)	+(25)	+(75)	-	-	-
Spray	*E. necatrix*	+(75)	+(75)	+(50)	+(25)	+(50)	+(25)	-	-
Control (non-vaccinated)	*E. tenella*	+(100)	+(75)	-	++(25)	-	++(25)	++++(100)	++(100)
Water	*E. tenella*	+(25)	+(25)	-	-	-	-	-	-
Spray	*E. tenella*	+(25)	+(25)	-	-	-	-	+(25)	-

Table 8: Weekly oocysts countering founded in feces and litter after challenge. Oocysts number/x10^{-3}

Vaccine	Challenge	29-35 days	36-42 days
Control (non-vaccinated)	*E. acervulina*	69	1
Water	*E. acervulina*	43	30
Spray	*E. acervulina*	18	5
Control (non-vaccinated)	*E. maxima*	79	0,8
Water	*E. maxima*	75	0,9
Spray	*E. maxima*	12	5
Control (non-vaccinated)	*E. necatrix*	104	7
Water	*E. necatrix*	80	8
Spray	*E. necatrix*	40	10
Control (non-vaccinated)	*E. tenella*	3	4
Water	*E. tenella*	0	0
Spray	*E. tenella*	0	0

Table 9: Absolute eosinophil of 50 day-old chicks after challenge

Vaccine	Eosinophil (%)
Control (non-vaccinated)	412.3b
Water	1291.3a
Spray	1130.4a
P value	0.05

a,b Means followed by different letters in the same column differ by Tukey test (p<0,05).

challenged chicks. Those values were submitted to Tukey test, where *E. maxima* presented the lowest relative bursa weight and spray vaccinated chicks presented higher relative bursa weight than water vaccinated and non-vaccinated chicks. They could present a more intense bursa stimulation due to spray vaccine, although this requires more studies in this subject such as lymphocyte number presented in the organ and local/humoral antibody production.

At the 28th day necropsy, before challenge, vaccinated chicks presented mild lesion scores compared to non-vaccinated ones, which is pertinent because the intestinal parasite cycle must occur to induce specific immunity in vaccinated chicks (Joyner and Norton, 1973). In general, it does not seem to have a difference in necropsy if the vaccine is either by spray or water.

At the 35th day necropsy, after challenge, it was observed that in all eimeria challenges *(E. acervulina, E. maxima, E. tenella* and *E. necatrix*), the control group presented more severe intestinal lesions than vaccinated ones, other authors found the same results (Schetters *et al.*, 1999) showing that the vaccine could induce immunity, protecting chicks against future infection. The most severe lesions were found in control group challenged with *E. tenella*. These results agree with the lower weight gain showed by this group and did not occur in water and spray vaccinated *E. tenella* challenged chicks. According to Kawazoe (2000) severe weight loss found in severe *E. tenella* infection was due to the reduction of feed and water consumption, higher energy use and water loss as a result of diarrhea.

In the first four weeks, oocysts in litter and animal feces were low for vaccinated chicks and negative for non-vaccinated ones. This result was similar to those showed by Williams (1998) who, four weeks after water vaccination, verified a maximum oocyst number and the low oocyst number in litter was probably due to a low production in immunized chicks.

Right after challenge an oocyst number increase was noticed in all eimeria species and this number was also higher in non-vaccinated chicks.

Blood analysis showed that chicks challenged with *E. tenella* had total plasmatic proteins higher than the ones challenged with *E. acervulina*, this could occur because of a more intense vaccine reaction caused by *E. tenella* challenge, which promotes a high production of immunological response factors, which are proteins. Band neutrophil are young defense cells and it is possible to speculate that results showing a higher number of these cells in *E. necatrix* challenge were related to a late inflammatory response in infected chicks, which was also related to higher lesion score in

50 day-old chicks challenged with this eimeria species. Vaccinated chicks presented higher eosinophils levels than non-vaccinated ones, this could be due to an early parasitological stimulation induced by coccidiosis vaccine.

Conclusion: Avian coccidiosis immunization may cause vaccinal reactions in animal's body with a mild inflammatory reaction in intestines. But the administration route has no influence in that parameter and there is no difference between water and spray routes. However, regarding using the vaccine against coccidiosis or not, it can be affirmed that when the birds are challenged with different eimeria species, the ones vaccinated have less severe degree of intestinal lesions and perform better than those birds challenged and non-vaccinated. In this context, it is important and necessary the vaccination against coccidiosis in animals challenged by those protozoa in the field, resulting good animal performance and ensuring good returns in poultry production.

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