



EFFICACY OF ANTICOCCIDIAL VACCINATION OF CHICKENS VIA DIFFERENT ROUTES: A COMPARATIVE STUDY

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Summary

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Use of anticoccidial vaccines as an alternative for anticoccidial drugs is increasing worldwide and thus, selecting the most effective method of administration of anticoccidial vaccine is important. For this purpose, 70 day-old broiler chickens were divided into 7 equal groups: 1) vaccinated (drinking water at 3 days old); 2) vaccinated (eye drop at day-old), 3) vaccinated (spray onto birds at day-old), 4) vaccinated (spray into feed at day-old); 5) treated by diclazuril (1 ppm 2 days before challenge and continued to the end of the experiment); 6) untreated infected controls and 7) untreated non-infected controls. Birds were challenged with *E. tenella* at 17 days of age (except for group 7). The evaluation criteria were body weight, feed conversion ratio (FCR), blood in faeces, survival rate, lesion scores, oocyst output per 1 g faeces and histopathological lesions. All groups had better performance in comparison with group 6. Administration of vaccine by drinking water and spray into feed had a slight positive effect on body weight and FCR at the end of the experiment. Maximum and minimum faecal oocyst excretions were observed in birds vaccinated by spray onto birds and spray into feed, respectively. Lesion scores in groups vaccinated by drinking water and spray into feed were lower than those of groups 3, 5 and 6. The severity of the histopathological lesions in group vaccinated by spray onto birds was higher as compared to the other vaccinated groups. In conclusion, it seemed that anticoccidial vaccination of broiler chickens via drinking water and spray into feed were equally more effective than vaccination via spray onto birds and eye drop.

Key words: anticoccidial drug, anticoccidial vaccine, broiler chicken, route of administration

INTRODUCTION

Coccidiosis, an intestinal disease, is one of the most important diseases of poultry worldwide provoked by protozoan parasites

of genus *Eimeria* in chickens. Clinical and subclinical infections cause impaired feed conversion and since feed costs com-

prise about 70% of the costs of producing broiler chickens, the economic impact of coccidiosis is considerable. Such costs are especially relevant to the poultry industry, where intensive housing of birds favours the spread of coccidiosis. Worldwide, the annual costs inflicted by coccidiosis to commercial poultry industry have been estimated at € 2 billion, stressing the urgent need for more efficient strategies to control the parasite (Shirley *et al.*, 2004; Dalloul & Lillehoj, 2006). According to news of the poultry site an estimated \$90 million is spent in the US, and over \$3 billion spent worldwide, for coccidiosis prevention annually (Anonymous, 2013).

Since 1939 when it has been discovered that sulfonamides could cure coccidiosis in chickens (Levine, 1939), many ionophorous and chemical anticoccidial feed additives have been used. Unfortunately, with the widespread and uncontrolled use of anticoccidial drugs, the efficacy of many anticoccidials has been reduced by drug resistance (Chapman, 1997; Peek & Landman, 2003). The increased occurrence of resistance against all anticoccidial drugs has left the poultry industry with a renewed challenge for coccidiosis prevention and control and propelled the search for other strategies. Although various alternative methods like prebiotic (Elmusharaf *et al.*, 2007), probiotic (Lee *et al.*, 2007), phytotherapy (Chandrakesan *et al.*, 2009) and subunit vaccine (Dalloul & Lillehoj, 2006) have been also used during the past decennia for the treatment of coccidiosis, vaccination is still of major importance.

Across the globe, vaccinating broilers for coccidiosis has become a safer, sensible and cost-effective means of controlling this costly disease in poultry. However, there is some concern about the vaccination routes. Vaccination failure occurs

when following vaccine administration the chickens do not develop adequate protection and are susceptible to a field disease outbreak. Knowing the proper route of administration is important. Vaccination by water is less consistent because the oocysts tend to settle down and the distribution is not uniform. So vaccination via drinking water is superseded by spray vaccines onto birds – a route that seems to be more effective for a regular flock vaccination because it covers all the birds and the peck behaviour on their own feathers or on other birds can promote the ingestion of these parasites (Chapman *et al.*, 2002). Spraying vaccines directly into feed is another route, a procedure considered to result in a more uniform exposure to vaccinal oocysts (Chapman, 2000). The eye drop method is an individual route. It is believed that oocysts pass through the lacrimal duct into the nasal cavity and then reach the intestinal tract via the oropharynx. This route of administration relies upon the initiative of the individual chicken to receive a full dose of vaccine (Chapman & Cherry, 1997).

Regarding the lack of the data about efficacy of administration of anticoccidial vaccines via different routes, the purpose of the present study was to evaluate and compare the efficacy of drinking water, eye drop, spray onto birds and into feed administration routes in broiler chickens experimentally infected with *E. tenella* on the basis of performance, blood in faeces, lesion scoring, oocyst output and microscopic findings.

MATERIALS AND METHODS

Experimental design

Seventy day-old broiler chickens (Cobb 500) of both sexes were randomly divided into 7 groups (10 birds/group) and housed

in pens of identical size with a single tray per group to collect faecal material. During the study feed and water were provided *ad libitum* and the amount of consumed feed was recorded for each group. All birds were fed a standard commercial diet based on corn and soybean meal and formulated without any anticoccidial medication. Strict sanitation practices were maintained in the house before and during the course of the experiment.

The groups were as follow: 1) vaccinated via drinking water at 3 days old, 2) vaccinated via eye drop at day-old, 3) vaccinated via spray onto birds at day-old, 4) vaccinated via spray into feed at day-old, 5) treated by diclazuril 6) untreated infected chickens (positive control) and 7) untreated non-infected (negative control).

For bird vaccination, quadrivalent live attenuated coccidiosis vaccine – Livacox[®] Q (manufactured by FATRO, ITALY that comprised four *Eimeria* species (*E. tenella*, *E. acervulina*, *E. maxima* and *E. necatrix*) was used. Ten doses of Livacox[®] Q (0.1 mL of Livacox[®] Q = 10 doses) were reconstituted in 10 mL and 0.5 mL of sterile distilled water for drinking water and eye drop vaccination, respectively. For spray into feed and onto bird vaccination, 20 doses of Livacox[®] Q were reconstituted in 4 mL of sterile distilled water. Diclazuril (Clinacox[®], Jamedat Afagh Pharmaceutical Company, Iran) was used in-feed in the dose recommended by the manufacturers (1 ppm) 2 days before challenge and continued to the end of the experiment.

Preparation of oocysts and infection of birds

Coccidial oocysts of *E. tenella* were obtained from the caeca of naturally infected chicks from poultry farms in northern Iran. Identification of the *Eimeria* was

made on the basis of morphology as described by Thienpont *et al.* (1979) and the site of lesions. *E. tenella* oocysts were propagated in 3 healthy chickens. Eight days post inoculation (DPI), birds were euthanised, caeca contents were obtained and after purification, the oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator (2–4 °C) until use. The methods of preparation of oocysts were described by Davies *et al.* (1963). *E. tenella* was washed three times with phosphate buffered saline. Each bird was challenged with 1.5×10^4 oocysts of *E. tenella* at 17 days of age.

Evaluation of the efficacy of vaccination

The efficacy of different routes of vaccine administration was evaluated on the basis of body weight gain, feed conversion ratio (FCR), blood in faeces, survival rate, lesion scores, oocyst output per gram faeces and histopathological evaluation.

Body weights of all birds were individually measured at 6, 9 and 12 DPI. At the end of the experiment (12 DPI) their FCR were calculated. Bloody diarrhoea was observed from fifth to ninth day after challenge. The extent of blood in faeces was evaluated from (–) no blood in faeces to (+++) severe bloody diarrhoea according to the method suggested by Youn *et al.* (1993). To determine caeca lesion score, five chickens from each group were randomly chosen at day 8 after *Eimeria* infection and lesion scoring was performed according to the 5-point scale of Conway & McKenzie (2007). A score of "0" was given for intestines without any gross lesions; +1: few petechiae in the caecal wall with the presence of normal contents with/without slight amount of blood; +2: petechiae which are apparent on the serosal surface, Thickened caecal

wall and bloody contents; +3”: more severe bleeding with clotting appearing and marked thickening of caecal wall with caecal core; +4: severe bleeding, much thickened caecal wall and distension of caecum packed with caseous cores.

Oocysts were counted by using McMaster technique as described by Long *et al.* (1979) for determination of oocyst output per gram faeces (OPG). After medication, the samples for OPG counting were taken from five random spots from the litter of each group on 6, 7, 8 and 9 DPI.

For histopathological evaluation, at the end of the experiment (12 DPI), three birds from each group were euthanised humanely by cervical dislocation and 2 cm tissue pieces from caeca were collected and fixed in 10% buffered formalin solution. Multiple transverse slices of the caeca were embedded in paraffin wax. Five µm sections were made and stained with haematoxylin-eosin (Gridley, 1960).

Statistical analysis

Body weight gain, OPG counts and lesion scores were presented as mean ± SD. Data analysis was carried out by using one-way ANOVA and *post hoc* Tukey's multiple comparison test (SPSS 11.5 for Win-

dows). Differences were considered significant at $P < 0.05$.

RESULTS

Performance

No significant differences among groups were observed with respect to the weight gain (527 g) prior to infection. Results related to different routes of anticoccidial vaccine administration on the body weight gain and feed conversion ratio are shown in Table 1. The body weight gain was significantly higher in all treated groups in comparison with positive control ($P < 0.05$) and there was no significant difference among the treated infected groups. At the end of the experiment (12 DPI), birds vaccinated via drinking water and spray into feed had insignificantly better body weight ($P > 0.05$) in comparison with treated groups. The highest FCR among treated groups was observed in the diclazuril group (1.61). Ten percent (10%) mortality was observed in positive control group.

Lesion and faeces scoring

No lesions were found in birds from the negative control group. Lesion scores of

Table 1. Body weight (mean±standard deviation) and final feed conversion ratio (by the 12th day post inoculation) in the different groups

Groups		Body weight, g			Final FCR
		Days post inoculation			
		6	9	12	
1	Drinking water	950±6 ^a	1180±8 ^a	1320±8 ^{ac}	1.51
2	Eye drop	945±6 ^a	1120±9 ^{ab}	1260±8 ^a	1.56
3	Spray onto bird	975±5 ^a	1090±11 ^b	1250±10 ^a	1.59
4	Spray into feed	960±5 ^a	1190±8 ^a	1315±8 ^{ac}	1.52
5	Diclazuril	945±6 ^a	1080±9 ^b	1240±9 ^a	1.61
6	Positive control	900±8 ^b	985±12 ^c	1170±16 ^b	1.71
7	Negative control	980±5 ^a	1210±6 ^a	1380±5 ^c	1.45

^{a, b, c} Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 2. Blood in faeces, survival rate (%) and lesion scoring (mean $\pm$ SD) in different groups

Groups		Blood in faeces*				Lesion score	Survival rate (%)
		Days post inoculation					
		6	7	8	9		
1	Drinking water	–	+	+	–	1.6±0.5 ^a	100
2	Eye drop	++	++	++	–	2.2±1.0 ^{ac}	100
3	Spray onto bird	++	++	++	–	2.6±0.5 ^{ad}	100
4	Spray into feed	–	+	–	–	1.6±0.8 ^a	100
5	Diclazuril	++	++	++	+	3.0±1.0 ^{ac}	100
6	Positive control	+++	+++	+++	+	3.2±0.8 ^{bcd}	90
7	Negative control	–	–	–	–	0	100

^{a,b,c,d,e,f} Means in the same column with different superscripts differ significantly ($P < 0.05$); * (–) Normal faecal contents with no blood; (+) presence of some blood in the faecal content; (++) bloody droppings; (+++) severe bloody diarrhoea.

Table 3. Oocyst excretions ($\times 1000$) from chickens in different groups during days post inoculation by *E. tenella*. Data are presented as mean $\pm$ SD

Groups	Days post inoculation			
	6	7	8	9
1 Drinking water	21.7 $\pm$ 3.2 ^{ac}	37.7 $\pm$ 7.2 ^{acd}	0.7 $\pm$ 0.5 ^a	0 ^a
2 Eye drop	180.0 $\pm$ 57.7 ^b	72.3 $\pm$ 16.3 ^{ad}	0.7 $\pm$ 0.5 ^a	0 ^a
3 Spray onto bird	163.6 $\pm$ 69 ^b	164.3 $\pm$ 32.4 ^{bd}	6.0 $\pm$ 1.0 ^a	0.7 $\pm$ 0.5 ^a
4 Spray into feed	3.7 $\pm$ 0.5 ^{ac}	20.7 $\pm$ 14.1 ^{ac}	0.7 $\pm$ 0.5 ^a	0 ^a
5 Diclazuril	243.0 $\pm$ 77.8 ^b	78.7 $\pm$ 27.1 ^d	6.3 $\pm$ 0.5 ^a	0.7 $\pm$ 0.5 ^a
6 Positive control	290.0 $\pm$ 19.0 ^b	165.0 $\pm$ 17.3 ^b	61.0 $\pm$ 13.4 ^b	3.7 $\pm$ 0.5 ^b
7 Negative control	0 ^c	0 ^c	0 ^a	0 ^a

^{a,b,c,d} Means in the same column with different superscripts differ significantly ($P < 0.05$).

the positive control group were significantly higher than those of groups 1 and 4. Although birds in group 5 had the highest and birds in group 1 and 4 – the lowest lesion score, there were no significant difference among treated groups (Table 2). Bloody diarrhoea was observed in almost all groups (except non-infected controls) from the fifth to ninth day after challenge with *E. tenella*. The extent of bloody diarrhoea in the groups vaccinated by drinking water and spray into feed was less severe than that of other groups.

Oocyst output

Oocyst outputs of the treated groups were significantly lower than that of the positive control group on days 7 (except for group 3), 8 and 9 post challenge. On the 6th DPI, the OPG count in positive control group was significantly higher only than groups 1 and 4. On 6th and 7th DPI, birds in group 4 had the lowest OPG count although not significantly different from group 1 on day 6 and from group 1 and 2 on day 7. There was no significant difference in the OPG among the groups treated with diclazuril and vaccinated by spray onto birds and eye drop routes (Table 3).

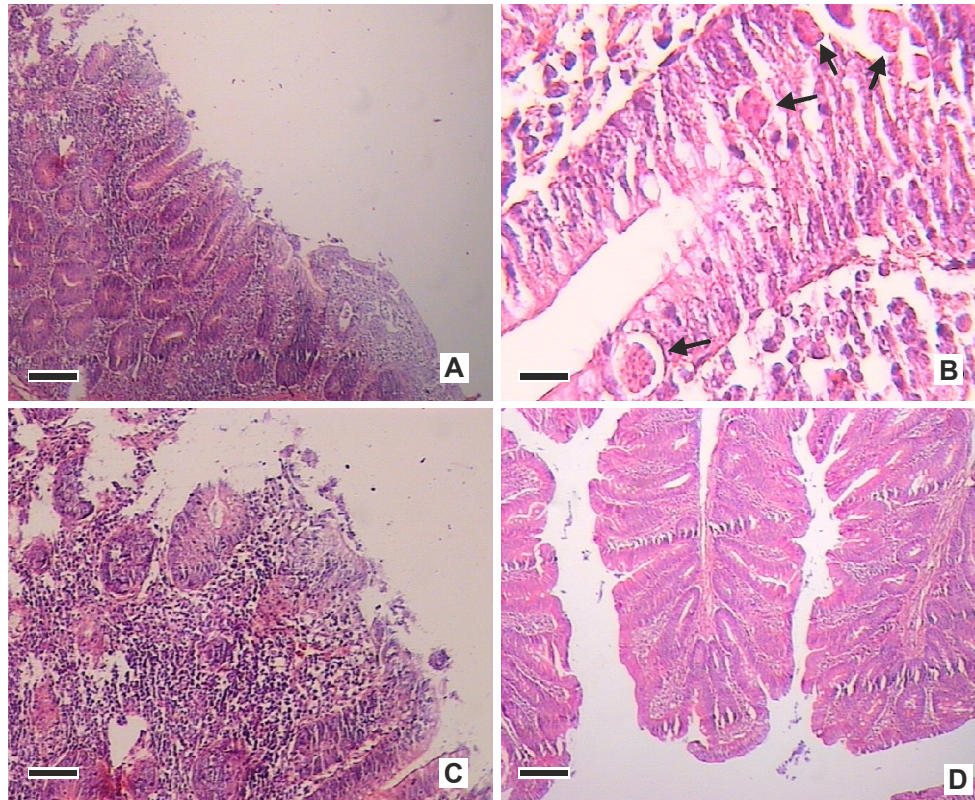


Fig. 1. **A.** Crypt hyperplasia and villus atrophy in group 3 (spray onto bird) (H & E, bar=125 μ m); **B.** different stages of oocyst development (arrows) in group 2 (eye drop) (H & E, bar=25 μ m); **C.** extensive damage to mucosa and mononuclear inflammatory cell infiltration to mucosa in group 6 (positive controls) (H & E, bar=50 μ m); **D.** normal villi in group 7 (negative controls) (H & E, bar=125 μ m).

Histopathological findings

Vaccinated groups had milder lesions in comparison with birds that received diclazuril. Vaccination by means of spray onto birds was found to be less effective than other methods of vaccination in reducing the severity of lesions. On 12 DPI, in groups 1, 2 and 4 lesions included intracellular developmental stages of parasitic in the epithelial cells and internal glands. In group 3, in addition to the above find-

ings, villus atrophy and mononuclear inflammatory cell infiltration and mucosal necrosis were seen. In group 5, lesions were more severe than those observed in groups 1, 2, 3 and 4, and these birds revealed parasitic necrotic enteritis and crypt dilatation. In group 6, lesions included crypt hyperplasia, villus atrophy, extensive damage to mucosa and mucosal necrosis. The gametogenous stages and oocysts appeared in large numbers than in other groups (Fig. 1).

DISCUSSION

Various attempts to vaccinate domesticated birds against coccidiosis have been reported since the early 1950's (Edgar, 1956; Shirley & Long, 1990). As is well known, live anticoccidial vaccines have proved to be an effective alternative for the anticoccidial drugs for the control of chicken coccidiosis but the efficacy of a vaccine is considerably influenced by the efficiency of the method of administration. Numerous studies have been conducted on the effectiveness of anticoccidial vaccination in broilers but a study which compares these four routes of vaccination is lacking.

Our findings showed that the use of live vaccine could reduce clinical coccidiosis in broilers and achieve a production performance slightly superior to that using anticoccidial drugs and it was also previously described by Williams (2002). Groups which were vaccinated had lower oocyst output, macroscopic lesion score, and milder microscopic lesion in comparison with birds that received diclazuril. Although OPG is not a reliable means for assessment of vaccine efficacy because of oocyst shedding by vaccinated chickens and inability to differentiate species of *Eimeria* oocysts shed by vaccinated chickens, our results showed that the administration route has an important role in success of vaccination in which the birds vaccinated via drinking water and spray into feed have performed better than birds vaccinated via eye drop and spray onto birds after challenge with *Eimeria tenella*. Moreover, groups vaccinated via drinking water and spray into feed showed the lowest lesion score and had milder microscopic lesions in comparison with birds in other vaccinated groups.

In contrast to the findings of the present study, Saad *et al.* (2009) showed no

significant difference regarding the effectiveness of the vaccine and the vaccine reaction. In their study however, birds vaccinated by water and spray onto birds methods showed better physical condition, mainly by best weight gain and lower intestinal lesion scores, compared to the birds challenged and non-vaccinated.

Danforth (1998) compared four methods of vaccinating day-old chicks with ImmucoxTM (Vetech Laboratories, Guelph, Canada) in battery trials; these were crop gavage, gel delivery, spray cabinet and slurry delivery. All methods of immunisation resulted in a significant protective immune response to challenge with a homologous infection, but the author concluded that the gel delivery system was the most effective.

In summary, the results obtained from the present study demonstrate that the use of the anticoccidial vaccine could control clinical coccidiosis in broilers and achieve production performance superior to that using anticoccidial drugs after challenge. Also anticoccidial vaccination of broilers via drinking water and spray into feed were equally more effective than vaccination via spray onto birds and eye drop.

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