The efficacy of a poultry commercial anticoccidial vaccine in experimental challenge with *Eimeria* field isolates

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Abstract:

BACKGROUND: The control of coccidiosis in poultry industry is dominated by prophylactic chemotherapy; however, drug resistance is a serious problem. Alternative control methods such as vaccination have been accepted as a practical method for controlling coccidiosis in chickens. Considering the immunological variation of *Eimeria* strains, the efficacy of live coccidiosis vaccines may be compromised. **OBJECTIVES:** To evaluate the efficacy of a commercial anticoccidial vaccine in poultry, vaccination was followed by experimental challenge with 3 *Eimeria* field isolates. **METHODS:** The efficacy of Livacox® Q anticoccidial vaccine was evaluated on male broiler chicks, reared in battery cages. Different factors including weight gain, FCR, OPG (oocysts per gram of feces) and intestinal lesion scores were assessed. **RESULTS:** Vaccinated challenged groups (VC) gained less weight than the un-vaccinated un-challenged (UVUC) birds ($p \leq 0.05$). Fourteen days post-challenge, the weight gain of VC groups challenged with isolate 2 differed significantly from its un-vaccinated challenged (UVC) counterpart; however, there were no significant differences in weight gain of groups challenged by isolates 1 and 3 with their respective UVC groups. Lesion score and FCR were significantly improved in VC groups comparing with their associated UVC groups ($p \leq 0.05$). Lesion score and FCR were significantly improved in VC groups comparing with their UVC counterparts ($p \leq 0.05$). **CONCLUSIONS:** The present study suggests that the use of live anticoccidial vaccine has the potential for improving live weight gains and FCR; nonetheless, immunity to local *Eimeria* species should be evaluated separately and in trial designs it should be more approximate to the actual field condition.

Introduction

Avian coccidiosis, caused by the substantial replication of seven species of coccidian parasites belonging to the genus *Eimeria*, is a major parasitic disease within the intensively reared poultry (Shirley & Bedrnik 1997; Allen & Fetterer 2002). Coccidiosis is considered as one of the commonest pernicious diseases of poultry and costs the world's commercial chicken producers at least US$ 1.5 billion every year (Yadav & Gupta 2001). The intensive use of anticoccidial drugs which has led to the development of resistance and the public concern of chemical

residues in poultry products and pollution of the environment has stimulated research for alternative control methods such as applying a vaccine early in life or development of new drugs (Barriga 1994; Chapman 1997; Li et al., 2004; De Pablos et al., 2010; Yim et al., 2010). The first commercial anticoccidial vaccine, CocciVac®, which is a live vaccine comprising several wild-type strains of *E. tenella* (*E. t*) oocysts, was introduced to the US market in 1952 (Shirley & Bedrnik 1997). Attenuated vaccines are produced mainly by either passaging through embryonated eggs, such as *E. t* in Livacox® vaccines, or by selection for precocity, such as the other species of Livacox vaccines and the Paracox® vaccines (Rami & Lillehoj 2006). Live vaccines comprising attenuated or virulent oocysts of various *Eimeria* species have offered a practical alternative to anticoccidial drugs for the sustainable control of coccidiosis in chickens, and in fact several such vaccines have been commercially available in the world market. However, *Eimeria* sp. induces solid immunity to homologous challenge and immune variation, as documented in *Eimeria maxima* (*E. ma*), may provide the basis for the lack of cross protective immunity among geographically isolated strains (Williams 1998; Chapman et al., 2002; Allen et al., 2005; McDonald & Shirley 2009). The degree of heterologous protection by a given vaccine may be addressed by obtaining local samples from where the vaccine is intended for use, and carrying out cross protection studies with the candidate vaccine lines. The aim of this study was to assess the efficacy of a commercial live attenuated vaccine available and widely used in poultry industry in Iran in terms of weight gain, feed conversion ratio, oocyst per gram of feces, and lesion score in experimentally infected chickens with three representative local mixed *Eimeria* field isolates.

Materials and Methods

Calculation of infectious dose. Three mixed isolates with a known biopathogenecity (Arabkhazaeli et al., 2011) were used as domestic poultry *Eimeria* isolates briefly containing 12% *E. acervulina* (*E. a*), 16% *E. brunetti* (*E. b*), 44% *E. ma*, 12% *E. mitis* (*E. mi*), 12% *E. t* and 4% *E. necatrix* (*E. n*) in isolate 1, 24% *E. a*, 6% *E. b*, 34% *E. ma*, 16% *E.* mi, 18% *E. t* and 2% *E. n* in isolate 2 and for isolate 3 containing 40% *E. a*, 15% *E. b*, 25% *E. ma*, 8% *E . mi*, 6% *E. t* and 6% *E. n*.

Based on observations during propagation, the challenge dose of the three selected farm isolates were estimated as following: for isolates 1 and 2, originating from Mazandaran province, 300000 sporulated oocyst per bird and for isolate 3, from Hamedan province, 250000 sporulated oocyst per bird.

Animals and husbandry. Two hundred and forty male one-day-old Ross308 broiler chicks were assigned by a randomized procedure to 8 groups of approximately equalized initial weights. Each group contained 30 chicks, comprising of three replicates of 10 and kept in battery cages. The birds were leg tagged so that individual data could be recorded. They were provided with a diet based on corn and soybean meal, which has been formulated to meet or exceed all required nutrients for the birds (NRC, 1994), and food and water were provided ad-libitum throughout the experimental period.

Vaccination and Challenge inocula. One hundred and twenty birds were orally inoculated with Livacox Q® (Biopharm, Research Institute of Biopharmacy and Veterinary Drugs, Czech Republic) at 3rd day of age, according to the manufacturer's recommendation. The control group received PBS orally. The infectious dose was given orally on the 14th day of age (10 days post-vaccination) to one hundred and eighty birds. A group of 30 chicks was allocated as uninfected unvaccinated negative control (Table 1).

Evaluation of the vaccine efficacy. Data regarding weight gain (WG), feed intake (FI), lesion score (LS), oocysts index (OI), and mortality were recorded in a 7-day period after inoculation of the infectious dose. Feed conversion ratio (FCR) was calculated (Daugschies et al., 1998; Conway et al., 2007; Arabkhazaeli et al., 2011).

On the 7th day post-inoculation, 9 birds from each group were selected for post-mortem examination and intestinal lesion score for a mixed infection, according to Conway and McKenzie (2007). Faecal examination was conducted daily up to 10 days post-challenge, and number of oocyst per gram of droppings was calculated by using the McMaster counting technique (Ryley et al., 1976).

Statistical analysis. All data were subjected to

ANOVA and two way t-test to see whether the differences between groups are significant. Differences among means were considered significant at $p<0.05$.

Results

There were significant differences in weight gain between unvaccinated-challenged (UVC) and unvaccinated- unchallenged (UVUC) groups for each isolates. These results confirm that the challenge dose was sufficient. The results are summarized in table 1. The vaccinated unchallenged (VUC) birds' weight gain, lesion score, and FCR were not significantly different ($p\leq0.05$) from those of the control group (UVUC). Expectedly vaccinated challenged (VC) groups gained less weight than the UVUC birds ($p\leq0.05$). Seven days post-challenge weight gain of the VC groups challenged with isolates 1 and 2 were significantly more than their respective unvaccinated challenged (UVC) counterparts. In VC groups challenged with isolate 3, weight gain was not significant caompared to the related UVC group ($p\leq0.05$). Fourteen days post-challenge, the weight gain of VC groups challenged with isolates 1 and 3 did not differ significantly from their UVC counterparts. However, there was significant difference in weight gain of groups challenged by isolate 2 ($p\leq0.05$).

FCR were significantly improved in VC groups comparing with their respective UVC groups ($p<0.05$). The best FCR was calculated for UVUC group which was not significantly different from the FCR of the VUC group ($p\leq0.05$).

The VUC group showed the lowest lesion score and VC groups had lower lesion scores comparing to their related UVC cgroups ($p\leq0.05$).

OPG results were inconclusive (Fig. 1). Although numerically the UVC groups had higher OPG, there were no significant differences among the groups ($p\leq0.05$).

No mortality was observed during the experiment. Our results about growth factors showed a coordination with apparent clinical sings in three UVC groups challenged with three different isolates. Accordingly, clinical signs as morbidity, emaciation, and diarroeha were more severe in UVC challenged with isolate 3.

Table 1. Effect of immunization† and subsequent‡ challenge with local *Eimeria* field isolates on the performance of birds kept in battery cages. A VC1, VC2 & VC3: Vaccinated and Challenged by isolate 1, 2 & 3, respectively; UVC1, UVC2 & UVC3: Unvaccinated and Challenged by isolate 1, 2 & 3, respectively; UVUC: Unvaccinated and Un-Challenged; FCR: Food conversion Ratio (Mean food consumption/Mean weight); SEM: Standard Error of Means; a-h: Means followed by different letters are significantly different ($p<0.05$). B Mean weight gain (7 days post-challenge). C Mean weight gain (14 days post-challenge). † Male broiler chicks orally inoculated with Livacox Q® at 3rd day of age. ‡ The infectious dose (3.0×10^5 oocyst/bird for isolates 1 &2 and 2.5×10^5 oocyst/bird for isolate 3) was given orally to the challenged birds on 14th day of age.

Group	Treatment A	mean weight gain ± (g) (mean±SEM)		FCR	Lesion score
		7B	14C		
1	VC1	268.7 ± 7.6^{b}	719.0 ± 17.4^{bc}	1.8^{bc}	1^{b}
2	VC2	246.0 ± 9.7^{bc}	758.4 ± 46.8^{ab}	1.9^{d}	0.7^{b}
3	VC3	151.9 ± 8.9^{e}	610.3 ± 25.7^{d}	2.5^{f}	1.7^{c}
4	VUC	340.0 ± 7.9^{a}	789.3 ± 24.5^{ab}	1.7^{ab}	0.2^{a}
5	UVC1	237.0 ± 9.8^{c}	648.5 ± 25.5^{cd}	2.0^{e}	1.8^{c}
6	UVC2	205.6 ± 8.0^{d}	669.0 ± 27.1^{cd}	2.0^{e}	1.5^{c}
7	UVC3	145.3 ± 8.9^{e}	612.0 ± 20.2^{d}	2.7^{g}	2.3^{d}
8	UVUC	349.6 ± 10.9^{a}	816.2 ± 28.5^{a}	1.6^{a}	0^{a}

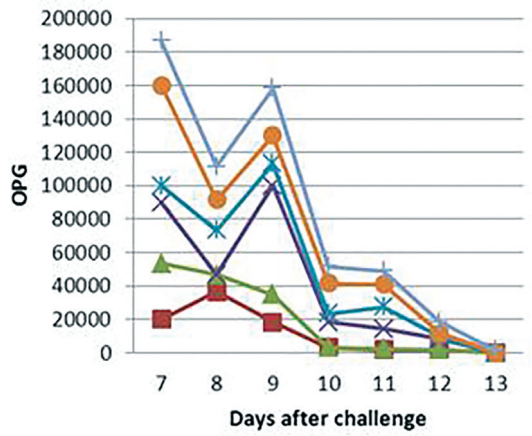

Figure 1. Effect of immunization and subsequent challenge with local *Eimeria* field isolates on oocyst per gram of feces (OPG) of birds vaccinated with Livacox®. VC1, VC2 & VC3: Vaccinated and Challenged by isolate 1, 2 & 3, respectively; UVC1, UVC2 & UVC3: Unvaccinated and Challenged by isolate 1, 2 & 3, respectively. UVC3 UVC2 UVC1 VC3 VC2 VC1

Discussion

Many laboratory and experimental studies have demonstrated that a live multivalent vaccine induces a strong immunity to challenge with virulent

homologous or heterologous *Eimeria* strains in chickens (Martin et al., 1997; Li et al., 2004). Immune-variation in five strains of *E. ma* taken from different geographical areas of North America indicated that vaccination with a given suspension of oocysts may not be effective in protecting against field strains in different geographical locations (Danforth 1998). To assess the degree of heterologous protection by a vaccine line, researchers should test local samples in cross protection studies (Chapman et al., 2005).

In this research, the efficacy of a live multivalent anticoccidial vaccine was tested on performance of broilers (male Ross308) reared in battery cages in response to challenge with three local *Eimeria* field isolates. The present study suggests that the use of live attenuated anticoccidial vaccines has the partial potential to relatively improve live weight gains and FCR; although none of the immunized birds had significantly higher average weight gains than the UVUC group for the entire experiment, VC group challenged by isolate 2 had a significant higher weight gain than the analogous UVC group and comparable weight gain to the UVUC group showing that the vaccinated birds were immune to the virulent challenge (Table 1).

On the subject of criteria for evaluating vaccine efficacy, it has been previously reported that unlike lesion score, the criterion of weight gain during seven days following challenge with virulent coccidia strains and the numerical results of FCR calculations can provide definitive evidence for the degree of the bird immunity (Williams & Catchpole 2000). Whilst the absence of lesions following virulent challenge of a vaccinated bird may be taken as evidence for protection against coccidiosis in parasitological terms, the presence of lesions does not necessarily indicate a lack of protection (Williams & Catchpole 2000). As seen with our results, the lesion scores of all VC groups were significantly different from their related UVC counterparts which may be inferred as protection efficacy of the vaccination; however, weight gains of the unvaccinated groups challenged with isolates 1 and 3, 14 days post-challenge shows compromised vaccine efficacy.

Based on oocyst count, no conclusion could be inferred. Although the absolute numbers varied among the groups, the pattern of oocyst production remained consistent overall. Oocyst production is affected by various factors including the inherent potential of each species to reproduce; the 'crowding' factor; competition with other species of coccidia or other infectious agents; nutrition of the host and genetic differences in strains of parasites (Fayer 1980; Chapman et al., 2002), hence oocyst counting alone for assessing immunity in chicks, whilst proving to be a sensitive method of detecting maternally transferred antibodies, is inappropriate for demonstrating protection against clinical coccidiosis (Williams & Catchpole 2000).

Livacox® Q is a quadrivalent live attenuated coccidiosis vaccine containing the economically important *Eimeria* species, namely: *Eimeria acervulina*, *E. ma*, *E. tenella* and *E. n* all of which were identified in domestic isolates used in this study (Arabkhazaeli et al., 2011). According to the manufacturer, the vaccine is applicable to layer and breeder chickens raised both on litter and in cages; however, birds must have access to the droppings as a reservoir of attenuated coccidian oocysts as booster antigen. Apparently, birds reared in cages with wire-mesh floors have limited exposure to fecal material, little opportunity for auto-reinfection and do not develop full immunity (Chapman et al., 2005). Despite this fact, based on FCR, the vaccine prevented adverse effects of challenge with the three tested isolates; however, based on weight gain, it was not fully effective against isolates 1 and 3 which may be due to immune variation of vaccine strains and challenge isolates. Since immunized chickens were challenged with multiple *Eimeria* species as a mixture, it was impossible to determine which one or more of the species, included in the challenge dose, adversely affected vaccine efficiency. Such experiences provide good reason for assessing immunity to each *Eimeria* species separately (Williams & Catchpole 2000).

Drug resistance and a relatively short life span (40-45 days) in raising broiler chickens has forced certain withdrawal periods for the safety of consumers. Such concerns have made vaccination an applicable practice in poultry industry. However, the degree of heterologous protection by a given vaccine should be evaluated in a given geographical region. The present study suggests that the use of live attenuated anticoccidial vaccine has the potential to

partially improve live weight gains and FCR; nevertheless, immunity to local *Eimeria* species should be evaluated separately and in trial design more approximate to the actual field condition.

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ارزیابی اثربخشی واکسن زنده تجاری ضدکوکسیدیا در کوکسیدیوز تجربی ایجاد شده توسط جدایه‌های بومی ایمریای ماکیان

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چکیده

زمینه مطالعه: امروزه درکنار ترکیبات ضدکوکسیدیا، واکسیناسیون نیز به عنوان یکی از روش‌های مهم درکنترل کوکسیدیوز در صنعت پرورش طیورکاربرد دارد. اثربخشی واکسن‌های ضدکوکسیدیا ممکن است تحت تأثیر اختلافات ایمنی‌شناسی سویه‌های ایمریا قرارگیرد. **هدف:** در این طرح اثربخشی یکی از واکسن‌های ضدکوکسیدیایی، با انجام واکسیناسیون وسپس چالش تجربی با ۳ جدایه بومی مزرعه‌ای ایمریامورد ارزیابی قرار گرفت. **روش کار:** جوجه‌های گوشتی نر در سه روزگی، توسط واکسن لیواکوکس Q واکسینه‌شده و اثربخشی واکسن پس از چالش با سه جدایه بومی ایمریا، بر اساس افزایش وزن، ضریب تبدیل غذایی، تعداد اُاُسیست در گرم مدفوع و جراحات روده‌ای مورد ارزیابی قرار گرفت. **نتایج:** گروه‌های واکسینه شده‌ی چالش شده در مقایسه با پرندگان واکسینه نشده و چالش نشده، افزایش وزن کمتری داشتند ($p\leq ۰/۰۵$). چهارده روز پس از چالش، وزن پرندگان گروه‌های واکسینه شده ی چالش یافته با جدایه های ۱ و ۳، در مقایسه با پرندگان گروه‌های واکسینه نشده چالش یافته مربوطه، اختلاف معنی‌داری نشان ندادند در حالی‌که در مورد جدایه ۲ این اختلاف معنی‌دار بود. ضایعات روده‌ای وضریب تبدیل غذایی در تمام گروه‌های واکسینه‌شده ی چالش یافته به صورت معنی‌داری بهتر از گروه‌های واکسینه نشده چالش یافته بود ($p\leq ۰/۰۵$). تعداد اُاُسیست در هر گرم مدفوع فاقد هرگونه اختلاف معنی‌دار در میان تمامی گروه ها بود. **نتیجه‌گیری نهایی:** نتایج نشان می‌دهند که استفاده از واکسن زنده تخفیف حدت یافته، قابلیت مقابله با اثرات نامطلوب ناشی از کوکسیدیوز را از طریق بهبود افزایش وزن، ضریب تبدیل غذایی و کاهش جراحات روده‌ای دارد ولی برای به حداکثر رساندن توان ایمنی‌زایی آن باید با انجام آزمایش‌های تکمیلی، در شرایط مشابه با شرایط واقعی پرورش در مزرعه ونیز با استفاده از هریک از گونه‌های بومی ایمریا به صورت جداگانه، ارزیابی دقیق‌تری از میزان همپوشانی ایمنی‌زایی واکسن در مقابله با سویه‌های بومی داخلی به دست آورد.

واژه‌های کلیدی: واکسن ضدکوکسیدیا، کوکسیدوز، اثربخشی، ایمریای ماکیان

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