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Effects of *in ovo* vaccination and anticoccidials on the distribution of *Eimeria* spp. in poultry litter and serum antibody titers against coccidia in broiler chickens raised on the used litters

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ABSTRACT

The present study reports the effects of various field anticoccidial programs on the distribution of *Eimeria* spp. in poultry litter and serum antibody titers against coccidia in broiler chickens raised on the used litters. The programs included *in ovo* vaccination and various medications with either chemicals, ionophores, or both. In general, serum samples from these chickens showed anticoccidial antibody titers when tested at days 7 and 14 post hatch with the peak response at day 43. Serum anticoccidial titers were highest in birds fed a non-medicated diet compared with those vaccinated or fed medicated diets. Total number of *Eimeria* oocysts and the composition of *Eimeria* spp. present in the litter samples from different treatment groups varied depending on the type of anticoccidial program. Oocyst counts in general ranged from 3.7×10^3 to 7.0×10^4 per g of litter. Importantly, both morphological and molecular typing studies revealed four major predominant *Eimeria* spp., *E. acervulina*, *E. maxima*, *E. praecox*, and *E. tenella* in the litter samples. Collectively, these results indicate that the field anticoccidial programs influenced the type and abundance of *Eimeria* spp. present in the litter samples and also modulated host immune response to *Eimeria*.

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1. Introduction

Avian coccidiosis is a major parasitic disease of industry concern worldwide. It is caused by at least seven distinct species of *Eimeria* apicomplexan protozoa that infect the intestinal mucosa with serious consequences. Afflicted birds exhibit various clinical manifestations, i.e., nutrients malabsorption, inefficient feed utilization, impaired body weight gain, and in severe cases, mortality. Additionally, pre-exposure to certain species of *Eimeria* has been strongly implicated in promoting necrotic enteritis (Williams et al., 2003) and gangrenous dermatitis (GD) (Li et al., 2010a,b). Although the preventive measures have been implemented, it is estimated that more than 50% of flocks shed *Eimeria* oocysts based on the epidemiological survey (Williams et al., 1996; Al-Natour et al., 2002; Haug et al., 2008; Nematollahi et al., 2009; Sun et al., 2009; Lee et al., 2010a). In the United States alone, the cost of anticoccidial medication has been assumed to be US $127 million annually (Chapman, 2009) based on the previous estimation in the UK (Williams, 1999).

Chickens infected with *Eimeria* spp. develop protective immunity against re-infection by the homologous parasite. Cell-mediated immunity, mediated mainly by antigen-specific and non-specific activation of T lymphocytes and macrophages, play an important role in protection against *Eimeria* spp. (Lillehoj and Trout, 1996). In addition to cell-mediated immunity, *Eimeria* infection also induced a specific humoral immune response (Song et al., 2001; Ding et al., 2004; Lillehoj et al., 2005; Lee et al., 2010b,c). Recent studies showed that *Eimeria*-specific antibody titers participated in the protection as well (Lee et al., 2010c; Wallach, 2010). The recombinant 3-1E or EtMIC2 proteins have been used to measure coccidia-specific serum antibodies in ELISA (Song et al., 2001; Ding et al., 2004; Lillehoj et al., 2005). The 3-1E gene was originally isolated from *E. acervulina* and known to be expressed by sporozoites and merozoites of *E. tenella*, *E. acervulina*, and *E. maxima*. EtMIC2 was originally cloned from *E. tenella* and shown to encode a microneme adhesion involved in parasitic motility and host cell invasion by the parasite.

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Although limited, the immunomodulatory effect of anticoccidial ionophores on immunity, especially humoral response, has been reported. For example, Munir et al. (2007) tested whether dietary salinomycin or monensin would affect antibody titers in Newcastle disease (ND) vaccinated chickens following challenge with pathogenic ND virus. For efficient poultry production, antibiotic growth promoters in combination with coccidiosis control programs, e.g., use of live coccidia vaccines or anticoccidial drugs classified as synthetic drugs or ionophores, are commonly practiced in poultry industry (Williams, 2005). It is expected that various field anticoccidial programs would affect the composition of *Eimeria* spp. as well as the host immunity against *Eimeria* spp. However, information with regard to the antibody response against *Eimeria* spp. in broiler chickens subjected to the various coccidiosis control programs is scarce. Thus, the current study was carried out to assess host serum antibody titers against coccidia parasites in broiler chickens. In this study, we used poultry litter as a bedding material obtained from a GD-endemic farm where at least ten broiler flocks had been grown. We have recently published the histological, immunological and molecular changes associated with GD infection in broiler birds (Li et al., 2010a,b) with the link between GD outbreak and pre-exposure to *Eimeria*. Thus, we also monitored the composition of *Eimeria* spp. present in litter samples and if any, histopathological changes in broiler chickens on the different anticoccidial programs.

2. Materials and methods

2.1. Experimental design

Broiler chickens (280 total, Ross breed; $n = 35$/group) were placed on the used litter at the University of Delaware Research Farm (Georgetown, DE). All broiler chicks used in this study received vaccinations including Marek's disease (turkey herpes virus (HVT), SB-1 and Rispen) *in ovo* at 18 days of incubation, and ND and infectious bronchitis (IB) via coarse spray after hatch. Used litter was obtained from a commercial broiler house of a farm with endemic GD where at least 10 broiler flocks had been grown. Used litter was subjected to natural composting process. Used litter was transported to the poultry diseases research facility at the Lasher Poultry Diagnostic Laboratory of the University of Delaware Extension Service, well homogenized and then evenly distributed in each of eight floor pens. Each pen had seven nipples and one tube feeder, and was 140 cm × 150 cm wide. The population density was set at 0.06 m^2/bird at 1 week, 0.07 m^2/bird at 2 week, 0.09 m^2/bird at 3 week, 0.11 m^2/bird at 4 week, and 0.15 m^2/bird at 5 week and kept throughout the experiment. Temperature and ventilation was controlled in the window-less environmentally control experimental facility.

2.2. Treatments

The experimental design and the antimicrobial concentrations used in diets are shown in Table 1. The basal diet was a mash type consisting of corn, soybean meal, poultry and animal by-product, and distiller's dried grains soluble. The coccidiosis control programs used in this study were either *in ovo* vaccination or in-feed medication with coccidiostats (chemicals or antibiotic ionophores) and selected based on the commonly practiced methods in poultry industry at the DELMAVA area in United States. Initially, all 18-d-old fertilized and developing eggs were vaccinated in *ovo* with Marek's disease (turkey herpes virus (HVT), SB-1 and Rispen) while for the vaccination group (CVAC), the eggs were vaccinated with Marek's disease plus live *Eimeria* Inovocox vaccine (Pfizer, Durham, NC). Live *Eimeria* Inovocox® vaccine is consisting of live oocysts of *E. acervulina*, *E. tenella* and two strains of *E. maxima*. All vaccination using the Pfizer Embrex INOVOJECT Egg Injection system (Embrex, Pfizer, Durham, NC) was practiced at the hatchery (Mountaire Farms, Inc., Millsboro, DE). The CVAC group was provided the diets with virginiamycin (20 g/ton) from hatch to day 18, bacitracine methylene disalicylate (BMD) (50 g/ton) and roxarsone (3-nitro) (45 g/ton) between days 19 and 35, and virginiamycin (20 g/ton) between days 36 and 43. The coccidiostat programs used in this study include diclazuril (Clinacox™; CLIN) (1 g/ton), monensin (Coban®; COBN) (100 g/ton), decoquinate (Deccox®; DECX) (27 g/ton), nicarbazin + narasin (Maxiban®; MAXI) (82 g/ton), salinomycin (Bio-Cox®; SAL) (60 g/ton), and a combination (NCB/SAL) of nicarbazin (Nicarb®) (113 g/ton) from hatch to day 18, and SAL (60 g/ton) at days 19 to 35. Along with the indicated coccidiostat (Table 1), all coccidiostat groups received BMD and 3-nitro from hatch to day 35 and virginiamycin (20 g/ton) at days 36 to 43. The unmedicated control group (NONE) received no medicated base diet. At day 36, all anticoccidial agents were removed from the diet. Feed and water were given *ad libitum* basis. All protocols were approved by the Institutional Animal Care and Use Committees of the Beltsville Agricultural Research Center and the University of Delaware.

2.3. Sample collections

Blood was sampled from 5 or 6 birds per group at days 7, 14, 25, 34, and 43. Once sampled, the birds were removed from the

Table 1
Experimental design and concentration of the antimicrobials added into broiler chicken diets.

	Anticoccidial programs[a,b]							
	NONE	CVAC	DECX	CLIN	MAXI	COBN	NCB/SAL[c]	SAL
Vaccination	–	+	–	–	–	–	–	–
Days 0–18								
Coccidiostat	0	0	27	1	82	100	113	60
Bacitracin methylene disalicylate	0	0	50	50	50	50	50	50
3-Nitro	0	0	45	45	45	45	45	45
Virginiamycin	0	20	0	0	0	0	0	0
Days 19–35								
Coccidiostat	0	0	27	1	82	100	60	60
Bacitracin methylene disalicylate	0	50	50	50	50	50	50	50
3-Nitro	0	45	45	45	45	45	45	45
Days 36–43								
Virginiamycin	0	20	20	20	20	20	20	20

[a] NONE, non-medicated control; CVAC, Pfizer inovocox *in ovo* vaccination; CLIN, diclazuril as Clinacox™; COBN, monensin as Coban®; DECX, decoquinate as Deccox®; MAXI, nicarbazin + narasin as Maxiban®; NCB/SAL; nicarbazin/salinomycin; SAL, salinomycin.
[b] Values are g/ton.
[c] NCB/SAL indicates addition of nicarbazin during 0–18 days and switch to salinomycin during 19–35 days.