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Protective immunity in broiler chickens elicited by live commercial coccidia vaccines (LCV) against recent field isolates and vaccines

Abstract

Use of live commercial coccidia vaccines (LCV) have proven to be important for control of coccidiosis in broilers, however LCV from different companies can vary. The objectives of this study were to measure the level of protection provided by five LCV against homologous and heterologous challenges at 20, 26 and 33 days of age and measure the level of protozoa parasite infection for each isolate in coccidia naïve chickens via wet mount smears. A Randomized Complete Block design with six vaccine treatments (a non-vaccinated, Con and vaccines A-E) was used. The dependent variables were weight gain and microscopic parasitic scores (MS). Vaccination was performed via coarse spray (d0). Birds were challenged on 20, 26, and 33 d of age with 3-5 field isolates and a homologous antigen. Four to five birds were challenged for each field isolate and homologous antigen. MS were determined 6 d post challenge. The d 26-32 and d 33-39 average gains of the Con treated broilers were lower ($P \leq 0.05$) compared to the gain of broilers from the LCV treatments. The MS of the birds that were immunized with vaccines C, D, and E and challenged on d 20 with E. tenlla were 96, 83, and 92% numerically reduced, however this reduction was not significant. Similar results of MS were detected from the d 26 and 33 challenges. The average gain of broilers may be improved when LCV are used, however protection varies depending on Eimeria species challenged and the age of the broilers.

Keywords: broilers, coccidiosis, coccidia vaccines

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Introduction

Coccidiosis caused by the apicomplexan protozoan of genus *Eimeria* (E) is a common and costly diseases associated with poultry production across the world.[1,2] There are several species of chicken coccidia, i.e., *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox E. tenella*, *E. acervulina*, *E. hagani*, *E. mivati* and *E. brunetti*. Among the listed species, *E. maxima*, *E. tenella*, and *E. acervulina* are the most common species.[3-5] The spread of the disease is through fecal-oral route, and signs of *Eimeria* infection include edema of submucosa and hemorrhagic enteritis which is associated with thickening of the intestinal wall and mucin production. Damage caused by *Eimeria* to the gut epithelial cells results in reduced nutrient absorption, decreased performance, morbidity and mortality.[6-9] Use of anticoccidial drugs to control coccidiosis is a common practice in conventional broiler production, but their use has been associated with the development of drug resistance coccidia.[10-12] There is also increased pressure for the poultry industry to move away from the use of anticoccidial drugs due to legislative regulation and consumer demands for antibiotic free poultry products.[13-15]

Use of live vaccines have proven to be important for control of coccidiosis; however, vaccines from different companies have been shown to vary in terms of type of *Eimeria* species, total number of oocysts and attenuated status of the organism.[16,17] Application of *Eimeria* vaccines use spray cabinets and gels in most hatcheries in the United States. The birds become immune with fecal oral reingestion over time of vaccine derived parasites. A challenge associated with vaccines is that not all antigens provide enough immunity against heterologous species or different strains of the same species.[18-20]

The definition of a "good vaccine" may differ between stakeholders, and multiple parameters including mortality rate, body weight gain, feed conversion ratio, intestinal gross lesion and microscopic lesion scoring have been used to measure the efficacy of *Eimeria* vaccines. However, body weight gain is of primary significance in broiler production and microscopic lesion scoring have been shown to detect significant damage caused by varying oocyst dosages.[21,22] Variability in results from vaccines challenge studies makes it difficult to compare in part due to differences in experimental design and parameters evaluated. Therefore in this study, efficacy of commonly used live coccidia vaccines (LCV) against field isolates from commercially raised broiler chickens was evaluated.

Materials and methods

Husbandry

A 39 day trial was conducted at the University of Maryland Eastern Shore's (UMES) poultry research facility under the approval and guidelines of the University's Animal Care and Use Committee. Two thousand four hundred male broiler chicks and 2,400 female broiler chicks were obtained from a commercial hatchery on hatch day. Two hundred male chicks and two hundred female chicks were placed in one of 12 rooms in the UMES Environmental House so that each room were equally mixed with males and females. Each room contained used pine shaving bedding (approximately 7.62 cm depth) that was a minimum of six flocks old. Chicks were vaccinated for Newcastle Disease and Infectious Bronchitis in the hatchery (no field vaccination), and each room housed 400 birds (1 bird/0.305m2). Each room is 6.096 x 6.096 m and is equipped with commercial pan feeders, nipple drinkers, and thermostat controlled forced air heaters. A standard commercial non-medicated diet was provided. All feed and water were offered ad libitum.

All rooms have independent temperature and ventilation control. The ventilation was controlled by a ten-minute timer in each room. The minimum ventilation followed commercial recommendations[21] and was identical for all rooms. As minimum ventilation requirements increased with age, the time set on the timer fans in each room were set identical. Each room was also equipped with a backup thermostat fan which was set to run four degrees above the target temperature. In addition, a standard industry light program was followed.

Immunization

The following LCV experimental treatments (Table 2) were evaluated in this study: Vaccine A (contains live oocysts of *E. acervulina*, *E. maxima*, *E maxima* MFP, *E. mivati*, and *E. tenella*), Vaccine B (contains live oocysts of *E. acervulina*, *E. maxima* and *E. tenella*), Vaccine C (contains attenuated species of *E. maxima*, *E. acervulina*, and *E. tenella*), Vaccine D (contains *E. acervulina*, *E. brunetti*, *E. maxima, E. necatrix* and *E. tenella*) and Vaccine E (contains live oocysts of *E. acervulina*, *E. tenella* and *E. maxima*). The *Eimeria* species profile of each LCV are declared from each LCV manufacturer.

The six vaccine experimental treatments (Non-vaccinated control, Vaccine A, Vaccine B, Vaccine C, Vaccine D, and Vaccine E) were randomly assigned to rooms so that each treatment was replicated once within each block. Vaccination was performed via coarse spray with a spray cabinet following manufacturer's recommendations. Each LCV experimental treatment was replicated two times with 400 chicks/replicate. Each room was blocked based on room location and considered an experimental unit. Birds were allowed to stay in the rooms assigned throughout the immunization phase.

Control chickens were provided with an anticoccidial drug (amprolium at 0.012% dosage level via water) to minimize cross contamination. The drug was removed approximately 48 hours prior to each challenge days. Fresh fecal samples were collected on 7, 10, 14, 17, 21, 24 and 27 d of age to determine oocysts output and the point of cessation of oocysts shedding.

Challenge

Birds were challenged on 20, 26, and 33 d of age with 3-5 field isolates and a homologous antigen. Four to five birds were challenged for each field isolate and homologous antigen. The field isolates were from coccidia samples from clinical cases from pullets or broiler chickens. The descriptions of the field isolates and homologous antigens challenged for each LCV experimental treatment are provided in Tables 1 and 2.

Table 1 Antigen sources and dose levels per bird

Challenge Homologous[1]	20 days challenge/source (oocysts)	26 days challenge/source (oocysts)	33 days challenge/source (oocysts)
Vaccine A	26,000	50,000	100,000
Vaccine B	26,000	50,000	100,000
Vaccine C	26,000	50,000	100,000
Vaccine D	26,000	50,000	100,000
Vaccine E	26,000	50,000	100,000
Heterologous			
E. acervulina (broilers-NC)[2]	20,000	NA[3]	NA
E. maxima (broilers-DMV)[4]	10,000	15,000)	30,000
E. tenella (broilers-DMV)[4]	10,000	NA	25,000
E. brunetti (pullets-IA)[5]	NA	12,000	25,000
E. mivati (pullets -IA)[5]	NA	20,000	NA
E. mivati and *acervulina*	NA	NA	800,00[6]

[1]Vaccine A contains live oocysts of *E. acervulina*, *E. maxima*, *E maxima MFP*, *E. mivati*, and *E. tenella*, (Merck Animal Health), Vaccine B contains live oocysts of *E. acervulina, E. maxima* and *E. tenella*, (Huvepharma, Inc.), Vaccine C contains attenuated species of *E. maxima, E. acervulina, and E. tenella*, (Boehringer Ingelheim Animal Health), Vaccine D contains oocysts of *E. acervulina*, *E. brunetti*, *E. maxima, E. necatrix* and *E. tenella*, (Ceva Animal Health, LLC) and Vaccine E contains live oocysts of *E. acervulina*, *E. tenella* and *E. maxima*, (Huvepharma, Inc).

[2]Strains obtain from commercial broiler farms in North Carolina.

[3]Not applicable, birds were not challenged with this heterologous strain on the designated challenge day.

[4]Strains obtain from commercial broiler farms in Delmarva region.

[5]Strains obtain from commercial pullet farms in Iowa.

[6]40,000 oocysts of *E. mivati* and 40,000 oocysts of *E. acervulina*.

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Table 2 Vaccine treatments of broiler chickens that were challenged at 20, 26, and 33 days of age with various field isolates and a homologous antigen

Vaccine Treatment Group[1] (n=2)	Challenge Day	Field Isolates	Homologous antigen
Vaccine A	20[2]	*E. acervulina, E. maxima, and E. tenella*	Vaccine A
Vaccine B	20	*E. acervulina, E. maxima, and E. tenella*	Vaccine B
Vaccine C	20	*E. acervulina, E. maxima, and E. tenella*	Vaccine C
Vaccine D	20	*E. acervulina, E. maxima, and E. tenella*	Vaccine D
Vaccine E	20	*E. acervulina, E. maxima, and E. tenella*	Vaccine E
Control (no vaccine)	20	*E. acervulina, E. maxima, and E. tenella*	Vaccines A, B, C, D, and E
Vaccine A	26[3]	*E. mivati, E. brunetti, and E. maxima*	Vaccine A
Vaccine B	26	*E. mivati, , E. brunetti, and E. maxima*	Vaccine B
Vaccine E	26	*E. mivati, E. brunetti, and E. maxima*	Vaccine E
Vaccine C	26	*E. mivati, E. brunetti, and E. maxima*	Vaccine C
Vaccine D	26	*E. mivati, E. brunetti, and E. maxima*	Vaccine D
Control (no vaccine)	26	*E. mivati, E. brunetti, and E. maxima*	Vaccines A, B, C, D, and E
Vaccine A	33[4]	*E. mivati and acervulina, E. maxima, E. brunetti and E. tenella*	Vaccine A
Vaccine B	33	*E. mivati and acervulina, E. maxima, E. brunetti and E. tenella*	Vaccine B
Vaccine E	33	*E. mivati and acervulina, E. maxima, E. brunetti and E. tenella*	Vaccine E
Vaccine C	33	*E. mivati and acervulina, E. maxima, E. brunetti and E. tenella*	Vaccine C
Vaccine D	33	*E. mivati and acervulina, E. maxima E. brunetti and E. tenella*	Vaccine D
Control (no vaccine)	33	*E. mivati and acervulina, E. maxima, E. brunetti and E. tenella*	Vaccines A, B, C, D, and E

[1]Two replicates (400 birds/replicate)/ LCV experimental treatment.

[2]Five birds were challenged for each field isolate and each homologous antigen (20 birds/LCV replicate and 40 birds/control replicate)

[3]Four birds were challenged for each field isolate and each homologous antigen (16 birds/LCV replicate and 32 birds/control replicate)

[4]Four birds were challenged for each field isolate and each homologous antigen (20 birds/LCV replicate and 36 birds/control replicate)

On the day of challenge each bird was given 1 ml of one of the field (heterologous strains) or homologous antigen challenge strains via gavage. Each bird was given approximately 10,000-100,000 sporulated oocysts. The dose level was determined by the species of coccidia (Table 1). The challenged birds were housed separately from the other immunized birds.

Evaluation

The efficacy of the vaccines used in this study were evaluated based on microscopic scoring and body weight gain of the challenged birds.

1. Coccidia Microscopic Parasitic Scoring (MS): Between 132 to 144 hr post-challenge, wet mount smears were prepared for all intestines from all challenged birds from each room to determine the level of parasitism using a 0 to 4 system.[24] The lesion scorer was blinded to the LCV treatment and challenge antigen group being scored.

2. Bird weights: Birds were group weighed (4-5 birds/*Eimeria* challenge) on each challenge day and 132-144 hr post challenge to determine average bird gain during the challenge period. Five birds/*Eimeria* challenge were weighed on d 20 and d 26. Four birds/*Eimeria* challenge were weighed on d 26 and d 32 and d 33 and 39.

Statistical analysis

A Randomized Complete Block (RCB) design was used with six LCV experimental treatments and two replicates/treatment. Block was considered a random factor, and vaccine and challenge treatments were fixed effects. Significant differences among treatments means were determined using Tukey's-HSD test with a 5% level of probability. The dependent variables that were measured were body weight gain and MS. All data were subjected to analysis of variance (ANOVA) as a RCB, using the general linear model procedure of STATISTIX® 10 software (Analytical Software, Tallahassee, FL, USA).

Results

The effect of LCV treatments on the average gain of birds are provided in Table 3. The weight gains of all of the challenged birds/LCV treatments were averaged to determine the effect of vaccination on bird gain. No differences ($P > 0.05$) were detected in the d 20 to 26 average gain of broilers between the treatments (Table 3). However differences were detected in the d 26-32 and d 33-39 average gain of

broilers (Table 3). The average gain of the non-vaccinated broilers from d 26-32 and d 33-39 was lower ($P \leq 0.05$) compared to the gain of broilers from the vaccinated treatments. No differences were detected in the d 26-32 weight gain of birds between the vaccinated treatments. The d 33-39 weight gains of birds vaccinated with vaccine treatments A, B, C, and D were not significantly different from each other (Table 3). The effect of the LCV treatments that were challenged with *Eimeria* species are presented in Tables 4 and 5. No significant differences in the d 20-26 and 26-32 weight gain were detected between any of the treatments (Table 4). However, the d 33-39 weight gain of birds that were immunized with vaccine B and challenged with the homologous antigen was higher ($P \leq 0.05$) compared to the d 33-39 weight gain of the control birds that were challenged with the homologous antigen (713.5 g and 266.7 g, respectively). No significant reductions in the MS of immunized experimental birds challenged on d 20 with *E. acervulina*, *E. maxima*, *E. tenella*, and the homologous antigens were detected when compared to the MS of the control birds challenged on d 20 with *E. acervulina*, *E. maxima*, *E. tenella* and their respective homologous antigens. The MS of the birds from the control vaccine treatment that were challenged with *E. tenella* at 20 d of age was 2.4. The MS of the birds that were immunized with vaccines C, D, and E and challenged with *E. tenella* were reduced numerically by 96, 83, and 92%, respectively, however this reduction was not significant (Table 5).

Table 3 The average gain of broiler chicks[1] from 20-26, 26-32 and 33-39 d by vaccination treatment[2]

Vaccine3 (n=2)	Average gain D20-264 (g)	Average gain D26-325 (g)	Average gain D33-396 (g)
Control	311.04	400.96^{B}	452.19^{C}
Vaccine A	265.95	499.64^{A}	611.76^{AB}
Vaccine B	280.84	486.7^{A}	669.46^{A}
Vaccine C	309.68	502.32^{A}	577.01^{AB}
Vaccine D	250.32	472.21^{AB}	608.56^{AB}
Vaccine E	311.45	513.57^{A}	560.46^{B}
P value (SD)	0.19 (63.8)	< 0.01 (76.5)	< 0.01 (106.4)

[1]Birds were group weighed (4 or 5 birds) prior to each challenge (d 20, 26 and 33). Six d post-challenge, birds were group weighed to determine weight gain during the challenge period. The weight gains of all the challenged birds/LCV treatments were averaged to determine the effect of vaccination on bird gain.

[2]Vaccination was performed via coarse spray with a spray cabinet following manufacturer's recommendations.

[3]Manufactured recommended dose was administered for each vaccine

[4]Five birds were challenged for each field isolate and each homologous antigen (20 birds/LCV replicate and

40 birds/control replicate)

[5]Four birds were challenged for each field isolate and each homologous antigen (16 birds/LCV replicate and 32 birds/control replicate)

[6]Four birds were challenged for each field isolate and each homologous antigen (20 birds/LCV replicate and 36 birds/control replicate)

[A-C]Means within the same column with no common superscript differ significantly ($P \leq 0.05$) according to Tukey's HSD all pairwise comparison test.

Table 4 The Day 20-26, 26-32 and 33-39 weight gain (WG)[1])of broiler chicks vaccinated with a live cocci vaccine at 0 days of age and challenged with field isolates of cocci and their respective homologous antigen (vaccine treatment) at 20, 26 and 33 days of age

Vaccine Treatment (n=2)		Vaccine Treatment (n=2)		Vaccine Treatment (n=2)	
Control		**Control**		**Control**	
Challenge[2]	WG D20-26(g)	Challenge[3]	WG D26-32(g)	Challenge[4]	WG D33-39 (g)
E. acervulina[5]	214	*E. mivati*[9]	409.13	*E. mivati/acervulina*[11]	500.75^{AB}
E. maxima[6]	344	*E. maxima*[6]	432.75	*E. maxima*[6]	505.27^{AB}
E. tenella[7]	330	*E. brunetti*[10]	433.75	*E. brunetti*[10]	482.25^{AB}
Vaccine A[8]	313	Vaccine A[8]	437.5	*E. tenella*[7]	510.87^{AB}
Vaccine B[8]	369	Vaccine B[8]	372.6	Vaccine A[8]	514.37^{AB}
Vaccine C[8]	327	Vaccine C[8]	472.38	Vaccine B[8]	266.7^{B}
Vaccine D[8]	276	Vaccine D[8]	312.88	Vaccine C[8]	466^{AB}
Vaccine E[8]	315	Vaccine E[8]	355	Vaccine D[8]	443.77^{AB}
				Vaccine E[8]	471.37^{AB}
Vaccine Treatment (n=2)		**Vaccine Treatment (n=2)**		**Vaccine Treatment (n=2)**	

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Table Continued

Control		**Control**		**Control**	
Vaccine A		**Vaccine A**		**Vaccine A**	
E. acervulina	272	*E. mivati*	507.75	*E. mivati/acervulina*	619.37^{A}
E. maxima	198	*E. maxima*	574.88	*E. maxima*	604.5^{AB}
E. tenella	284	*E. brunetti*	488	*E. brunetti*	620.12^{A}
Vaccine A	307	Vaccine A	486.5	*E. tenella*	648.75^{A}
				Vaccine A	579.25^{AB}
Vaccine B		**Vaccine B**		**Vaccine B**	
E. acervulina	259	*E. mivati*	534	*E. mivati/acervulina*	659.5^{A}
E. maxima	295	*E. maxima*	512.1	*E. maxima*	565.75^{AB}
E. tenella	278	*E. brunetti*	438.6	*E. brunetti*	687.75^{A}
Vaccine B	310	Vaccine B	507.1	*E. tenella*	713.62^{A}
				Vaccine B	713.5^{A}
Vaccine C		**Vaccine C**		**Vaccine C**	
E. acervulina	273	*E. mivati*	490.1	*E. mivati/acervulina*	525.5^{AB}
E. maxima	311	*E. maxima*	566.4	*E. maxima*	611^{AB}
E. tenella	326	*E. brunetti*	561.3	*E. brunetti*	588.37^{AB}
Vaccine C	312	Vaccine C	5520	*E. tenella*	605.87^{AB}
				Vaccine C	638.62^{A}
Vaccine D		**Vaccine D**		**Vaccine D**	
E. acervulina	206	*E. mivati*	474.4	*E. mivati/acervulina*	672.62^{A}
E. maxima	245	*E. maxima*	479.4	*E. maxima*	573.62^{AB}
E. tenella	271	*E. brunetti*	436.1	*E. brunetti*	619.62^{A}
Vaccine D	223	Vaccine D	477	*E. tenella*	596^{AB}
				Vaccine D	602.12^{AB}
Vaccine E		**Vaccine E**		**Vaccine E**	
E. acervulina	272	*E. mivati*	489	*E. mivati/acervulina*	609^{AB}
E. maxima	368	*E. maxima*	574.1	*E. maxima*	531^{AB}
E. tenella	291	*E. brunetti*	523	*E. brunetti*	555.37^{AB}
Vaccine E	273	Vaccine E	504.4	*E. tenella*	639.37^{A}
				Vaccine E	618.62^{A}
P value (SD)	0.55 (63.8)	P value (SD)	0.051 (82.2)	P value (SD)	0.01 (105.77)

[1]Birds were group weighed (4 or 5 birds) prior to each challenge (d 20, 26 and 33). Six d post-challenge, birds were group weighed to determine weight gain during the challenge period.

[2]Five birds were challenged for each field isolate and each homologous antigen (20 birds/replicate for each vaccine treatment and 40 birds/replicate for control treatment)

[3]Four birds were challenged for each field isolate and each homologous antigen (16 birds/replicate for each vaccinated treatment and 32 birds/replicate for control treatment)

[4]Four birds were challenged for each field isolate and each homologous antigen (20 birds/replicate for each vaccinated treatment and 36 birds/replicate for control treatment)

[5]Each bird was challenged with 20,000 oocysts.

[6]Each bird was challenged with 10,000, 15,000 and 30,000 oocysts on d 20, 26 and 33, respectively.

[7]Each bird was challenged with 26,000 and 25,000 oocysts on d 20 and 33, respectively.

[8]Each bird was challenged with 26,000, 50,000 and 100,000 oocysts on d 20, 26 and 33, respectively.

[9]Each bird was challenged with 20,000 oocysts on d 26.

[10]Each bird was challenged with 12,000 and 25,000 oocysts on d 26 and 33, respectively.

[11]Each bird was challenged with 40,000 oocysts of *E. mivati* and 40,000 oocysts of *E. acervulina* on d 33.

[A-B]Means within the same column with no common superscript differ significantly ($P \leq 0.05$) according to Tukey's HSD all pairwise comparison test.

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Table 5 The Day 20-26, 26-32 and 33-39 intestinal microscopic parasitic score (MS[1]) of broiler chicks vaccinated with a live cocci vaccine at 0 days of age and challenged with field isolates of cocci and their respective homologous antigen (vaccine treatment) at 20, 26 and 33 days of age

Vaccine Treatment (n=2)		**Vaccine Treatment (n=2)**		**Vaccine Treatment (n=2)**	
Challenge[2]	MS D20-26	Challenge[3]	MS D26-32	Challenge[4]	MS D33-39
E. acervulina[5]	1.5^{AB}	*E. mivati*[9]	1.25^{BCD}	*E. mivati/acervulina*[11]	1.5^{ABC}
E. maxima[6]	0.60^{AB}	*E. maxima*[6]	1.5^{BCD}	*E. maxima*[6]	2.00^{ABC}
E. tenella[7]	2.4^{AB}	*E. brunetti*[10]	0.75^{CD}	*E. brunetti*[10]	2.13^{ABC}
Vaccine A[8]	3.2^{AB}	Vaccine A[8]	3^{ABC}	*E. tenella*[7]	2.88^{ABC}
Vaccine B[8]	0.80^{AB}	Vaccine B[8]	0.75^{CD}	Vaccine A[8]	2.25^{ABC}
Vaccine C[8]	1.6^{AB}	Vaccine C[8]	3.38^{AB}	Vaccine B[8]	2.88^{ABC}
Vaccine D[8]	3.1^{AB}	Vaccine D[8]	5.25^{A}	Vaccine C[8]	2.00^{ABC}
Vaccine E[8]	3.6^{A}	Vaccine E[8]	5.38^{A}	Vaccine D[8]	1.75^{ABC}
				Vaccine E[8]	2.00^{ABC}
Vaccine A		**Vaccine A**		**Vaccine A**	
E. acervulina	0.70^{AB}	*E. mivati*	0.13^{D}	*E. mivati/acervulina*	0.75^{ABC}
E. maxima	0.20^{B}	*E. maxima*	0.25^{D}	*E. maxima*	1.50^{ABC}
E. tenella	0.70^{AB}	*E. brunetti*	0.25^{D}	*E. brunetti*	0.63^{ABC}
Vaccine A	0.90^{AB}	Vaccine A	1.13^{BCD}	*E. tenella*	0.13^{C}
				Vaccine A	0.25^{BC}
Vaccine B		**Vaccine B**		**Vaccine B**	
E. acervulina	1.2^{AB}	*E. mivati*	0.88^{BCD}	*E. mivati/acervulina*	0.88^{ABC}
E. maxima	1.2^{AB}	*E. maxima*	0.75^{CD}	*E. maxima*	3.25^{AB}
E. tenella	2.5^{AB}	*E. brunetti*	0.75^{CD}	*E. brunetti*	1.50^{ABC}
Vaccine B	1.9^{AB}	Vaccine B	0.38^{D}	*E. tenella*	0.25^{BC}
				Vaccine B	1.38^{ABC}
Vaccine C		**Vaccine C**		**Vaccine C**	
E. acervulina	0.50^{AB}	*E. mivati*	1B^{CD}	*E. mivati/acervulina*	1.38^{ABC}
E. maxima	1.5^{AB}	*E. maxima*	0.5^{CD}	*E. maxima*	1.88^{ABC}
E. tenella	0.1^{ABC}	*E. brunetti*	0.38^{D}	*E. brunetti*	0.38^{ABC}
Vaccine C	1.5^{AB}	Vaccine C	0.98^{BCD}	*E. tenella*	1.88^{ABC}
				Vaccine C	3.38^{A}
Vaccine D		**Vaccine D**		**Vaccine D**	
E. acervulina	1.4^{AB}	*E. mivati*	0.38^{D}	*E. mivati/acervulina*	0.5^{ABC}
E. maxima	0.60^{AB}	*E. maxima*	0.5^{CD}	*E. maxima*	1.75^{ABC}
E. tenella	0.40^{AB}	*E. brunetti*	0.25^{D}	*E. brunetti*	0.38^{ABC}
Vaccine D	1.80^{AB}	Vaccine D	2.13^{BCD}	*E. tenella*	0.38^{ABC}
				Vaccine D	2.55^{ABC}

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Table Continued

Vaccine E		**Vaccine E**		**Vaccine E**	
E. acervulina	0.50^{AB}	*E. mivati*	0.5^{CD}	*E. mivati/acervulina*	0.75^{ABC}
Vaccine Treatment (n=2)		**Vaccine Treatment (n=2)**		**Vaccine Treatment (n=2)**	
E. maxima	0.60^{AB}	*E. maxima*	0.13^{D}	*E. maxima*	1.25^{ABC}
E. tenella	0.20^{B}	*E. brunetti*	0^{D}	*E. brunetti*	0.63^{ABC}
Vaccine E	1.8^{AB}	Vaccine E	1.13^{BCD}	*E. tenella*	1.13^{ABC}
				Vaccine E	1.25^{ABC}
P value (SD)	<0.01 (1.11)	P value (SD)	< 0.01 (1.48)	P value (SD)	0.01 (1.05)

[1]MS were determine six d post-challenge.

[2]Five birds were challenged for each field isolate and each homologous antigen (20 birds/replicate for each vaccine treatment and 40 birds/replicate for control treatment)

[3]Four birds were challenged for each field isolate and each homologous antigen (16 birds/replicate for each vaccinated treatment and 32 birds/replicate for control treatment)

[4]Four birds were challenged for each field isolate and each homologous antigen (20 birds/replicate for each vaccinated treatment and 36 birds/replicate for control treatment)

[5]Each bird was challenged with 20,000 oocysts.

[6]Each bird was challenged with 10,000, 15,000 and 30,000 oocysts on d 20, 26 and 33, respectively.

[7]Each bird was challenged with 26,000 and 25,000 oocysts on d 20 and 33, respectively.

[8]Each bird was challenged with 26,000, 50,000 and 100,000 oocysts on d 20, 26 and 33, respectively.

[9]Each bird was challenged with 20,000 oocysts on d 26.

[10]Each bird was challenged with 12,000 and 25,000 oocysts on d 26 and 33, respectively.

[11]Each bird was challenged with 40,000 oocysts of E. mivati and 40,000 oocysts of E. acervulina on d 33.

[A-D]Means within the same column with no common superscript differ significantly ($P \leq 0.05$) according to Tukey's all pairwise comparison test.

Similar results of MS were detected from the d 26 and 33 challenges. Although not significant, an 83% and 91% reduction of the MS of birds challenged on d 26 with *E. maxima* and immunized with vaccines A and E, respectively compared to the MS of control treatment birds challenged with *E. maxima*. Immunizations with vaccines A, B and D tended also to reduce the MS of d 33 *E. tenella* challenged birds with compared to the MS of the control birds challenged with *E. tenella*.

Discussion

Coccocidial vaccines are considered viable means to control the disease.[25] However, according to Blake et al.,[26] results of the vaccination can be influenced by variation in pathogenicity and immunogenicity of *Eimeria* species. Use of strain or isolate from the vaccine as a challenge has been suggested as a means to ensure antigenic and species homogeneity. In addition, use of antigenic distinct strains can be used to assess cross protection.[27] In this study, the MS of the birds that were immunized with vaccines C, D, and E and challenged with *E. tenlla* were 96, 83, and 92% numerically lower. Additionally, vaccines A and E numerically reduced (83% and 91%, respectively) the MS of birds challenged on d 26 with *E. maxima* compared to the MS of the control treatment birds challenged with *E. maxima*. Immunizations with vaccines A, B and D also tended to reduce the MS of d 33 *E. tenella* challenged birds with compared to the MS of the control birds challenged with *E. tenella*. The data suggests that vaccinated birds may have developed protection against secondary infection of coccidiosis based on reduction of MS when compared to the control (Table 5). However, the level of cross protection against various *Eimeria* species was highly variable. In contrast, Nollet et al.[28] reported no difference in the overall MS of chicks that were immunized on d 0 for coccidiosis and challenged at 15 d of age with *Eimeria* species compared to the MS of chicks that were not immunized and challenged on d 15.

There are multiple parameters used to measure pathogenicity of *Eimeria* species infections in broiler chickens. Some of these include weight gains, intestinal lesion scores and changes in serum alkaline phosphatase activity.[29] Over the years, gross lesion score (GLS) describe by Johnson and Reid,[30] have been used to assess effectiveness of *Eimeria* treatment including vaccines, however this method has been reported to have a wide range of pathologic variation[21] and chickens inoculated with same dose of oocysts have been reported to have a large variation in GLS.[29] In contrast MS is reported to be a more objective method to detect mucosal damage caused by varying oocyst dosage.[22] Idris et al.,[22] suggests that MS can detect the presence of coccidiosis infection in broilers that may be overlooked by using GLS alone. The authors reported that *E. acervulina* infections produce more obvious GLS. However, *E. maxima* is a more difficult species to perform GL scoring because the level of infections does not always correlate with the severity of the gross lesion scores.[30] While presence and or absence of lesions can be used evaluate the efficacy of the vaccines, lesion scores should be correlated with other criteria i.e. performance (weight gain and feed efficiency).[31]

Coccidian challenge has been reported to have a significant impact on intestinal integrity of broiler chickens which in turn affect nutrient digestibility and absorption. Day 0 vaccinated chicks and challenged at 26 and 33 d of age had higher weight gains compared to the non-vaccinated challenged birds. Crouch et al.,[32] reported similar results

with vaccinated birds. It was reported, chicks that were vaccinated via spray cabinet at 1 d of age and challenged at 28 d of age with *Eimeria* species had higher weight gains compared to the weight gains of non-vaccinated challenged birds.[32] The vaccinated administered chicks saw no reduction in weight gain due to the coccidiosis challenge. Similar results were reported when birds were inoculated with *E. tenella* and challenged at 25 d of age with the homologous oocysts,[33] moreover a challenge with *Eimeria* maxima was shown to result in reduced villi height in the jejunum and reduced expression of nutrient transporters located in the brush border of the intestinal epithelial cells.[34] Hence higher weight gain in our experiment for the vaccinated birds during challenge can be explained in part due to secondary immunity provided by vaccines during secondary exposure to *Eimeria* species.[35]

Conclusion

Immunizing birds using LCV at d 0 resulted in improved weight gain of birds challenged with *Eimeria* species at 26 and 33 d of age. Although immunizing birds with LCVs did not significantly reduce the MS of challenged birds compared to the controls, the MS tended to be reduced numerically.

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None.

Conflicts of interest

The authors have read the journal's guidelines and have the following competing interests: the co-author S. Fitz-Coy is an employee of Merck Animal Health which provided funding for the study. The other authors have no competing interests.

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