

# Pathogenicity and histopathological observations of commercial broiler chicks experimentally infected with isolates of *Eimeria tenella*, *E. acervulina* and *E. maxima*

## Patogenicidade e observações histopatológicas de frangos de corte infectados experimentalmente com isolados de *Eimeria tenella*, *E. acervulina* e *E. maxima*

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### Abstract

Coccidiosis is one of the most important causes of economic losses within the poultry industry. The objective of this study was to evaluate the pathogenicity of *E. tenella*, *E. acervulina*, and *E. maxima* strains in commercial broilers chicks. Thirty nine commercial one day old broiler chicks, unvaccinated against coccidiosis, were used during this experiment. At day 14, chickens of G1 (n=10), G2 (n=10) and G3 (n=10) were infected with  $2 \times 10^4$  sporulated oocysts of *E. tenella*, *E. acervulina*, and *E. maxima* respectively; G4 (n=9) served as the uninfected control group. All birds were sacrificed with 21 day old (seven days after infection). The prepatent period (PPP) for G1 and G3 was seven days, however, *E. acervulina* (G2) had a PPP of five days. No statistical differences were observed when the average weight gain (G1=182.7±63.4; G2=145.2±51.0; G3=183.3±56.8; and G4=211.5±89.0,  $p>0.10$ ) of the evaluated groups was compared. Average of lesion scores were determined G1 (1.3±0.48, scores 1(n=7) and 2(n=3)), G2 (0.4±0.52, scores 0(n=6), 1(n=4)), and G3 (1.1±0.99, scores 0(n=4), 1(n=1) and 2(n=5)). Chickens from the infected groups (G1, G2 and G4) did not demonstrate a lesion score above 2. The histopathological lesions induced by these strains were consistent with those described for infection by *Eimeria* spp.

**Keywords:** *Eimeria*, broilers, coccidiosis, pathogenicity

### Resumo

A coccidiose aviária é uma das principais causas de perdas econômicas na avicultura de corte. Considerando isto, o presente trabalho teve como objetivo avaliar a patogenicidade de cepas de *Eimeria tenella*, *E. acervulina* e *E. maxima* em aves de corte de uso comercial. Para tanto, 39 pintinhos tipo corte com um dia de idade, não vacinados para coccidiose, foram utilizados neste experimento. No 14º dia do

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experimento, os grupos foram infectadas com  $2 \times 10^4$  oocistos esporulados de *E. tenella* (G1, n=10), *E. acervulina* (G2, n=10) e *E. maxima* (G3, n=10). Um grupo com nove aves (G4) serviu como grupo controle não infectado. Todos os animais foram eutanasiados com 21 dias de idade (7 dias pós-infecção). O período pré-patente (PPP) nos grupos G1 e G3 foi de sete dias, quando excretaram 52.000 e 8.000 oocistos/g de fezes, respectivamente; no entanto, o grupo infectado com *E. acervulina* (G2) apresentou um PPP de 5 dias. Não foram verificadas diferenças estatísticas quanto ao ganho de peso vivo (G1=182.7±63.4; G2=145.2±51.0; G3=183.3±56.8; e G4=211.5±89.0,  $p>0.10$ ). Os escores de lesão foram determinados para cada grupo G1 (1.3±0.48, escores 1(n=7) e 2(n=3)), G2 (0.4±0.52, escores 0(n=6), 1(n=4)), e G3 (1.1±0.99, escores 0(n=4), 1(n=1) e 2(n=5)). Considerando todos os grupos infectados (G1, G2 e G4) nenhum mostrou escore maior que 2. As lesões histopatológicas induzidas por estas cepas foram compatíveis com aquelas descritas por infecção por *Eimeria* spp.

**Palavras-chave:** *Eimeria*, broilers, coccidiosis, pathogenicity

## Introduction

Coccidiosis is one of the most important causes of economic losses within the poultry industry (WILLIAMS et al., 1999). This disease is caused by *Eimeria* parasites, which infect epithelial cells of the intestines of birds. Clinically, coccidiosis is manifested by bloody diarrhea and listlessness (ALI TIPU et al., 2002); economic losses are primarily due to impaired feed conversion, depressed growth, lost of pigmentation, downgrading at processing, and mortality (McDOUGALD; REID, 1997).

*Eimeria* drug resistance still remains an enormous obstacle, and *E. tenella*, *E. acervulina* and *E. maxima* are relatively tolerant to ionophores (LI et al., 2004). These three species are used as vaccine in the immunization with virulent, attenuated and ionophore tolerant strains (VERMEULEN; SCHAAP; SCHETTERS, 2001). To prevent drug resistant, new drugs and different methods of administration have been used, however, this have resulted in increased cost to the poultry industry (YOUN; NOH, 2001), while antibiotics in feed need to be replaced by other means to control infectious, such as vaccination improvement by better hygiene and use of feed supplements (VERMEULEN et al., 2001).

In this study the pathogenicity and histopathological observations of *E. tenella*, *E. acervulina*, and *E. maxima* strains were evaluated in commercial broilers chicks.

## Materials and Methods

### *Eimeria* strains

Oocysts of *E. tenella*, *E. acervulina*, and *E. maxima* were obtained from the Laboratory of Parasitology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina (DMVP/UEL). These strains were propagated in chickens and oocysts were preserved in 2% potassium dichromate solution to induce sporulation.

### Experimental design

Commercial one day old broiler chicks (Cobb), unvaccinated against coccidiosis, were used in this experiment. Thirty nine birds were divided into four groups (randomly allocated in separate battery cages); group 1 (G1), group 2 (G2), group 3 (G3) with 10 birds each, and group 4 (G4) with nine birds. The G1, G2, and G3 were the experimental groups, while G4 served as the control. All birds were maintained until the 10<sup>th</sup> day of the experiment with anticoccidial additives following the recommendations of the producer, after which, the birds were administered ration without anticoccidial additives until the end of experiment. Feed and water were administered *ad libitum*. At day 14 chickens of G1, G2, and G3 were infected with  $2 \times 10^4$  sporulated oocysts of *E. tenella*, *E. acervulina*, and *E. maxima* respectively, and G4 remained as uninfected group. All birds were sacrificed at day 21.

### Sampling, measurements and histopathological evaluation

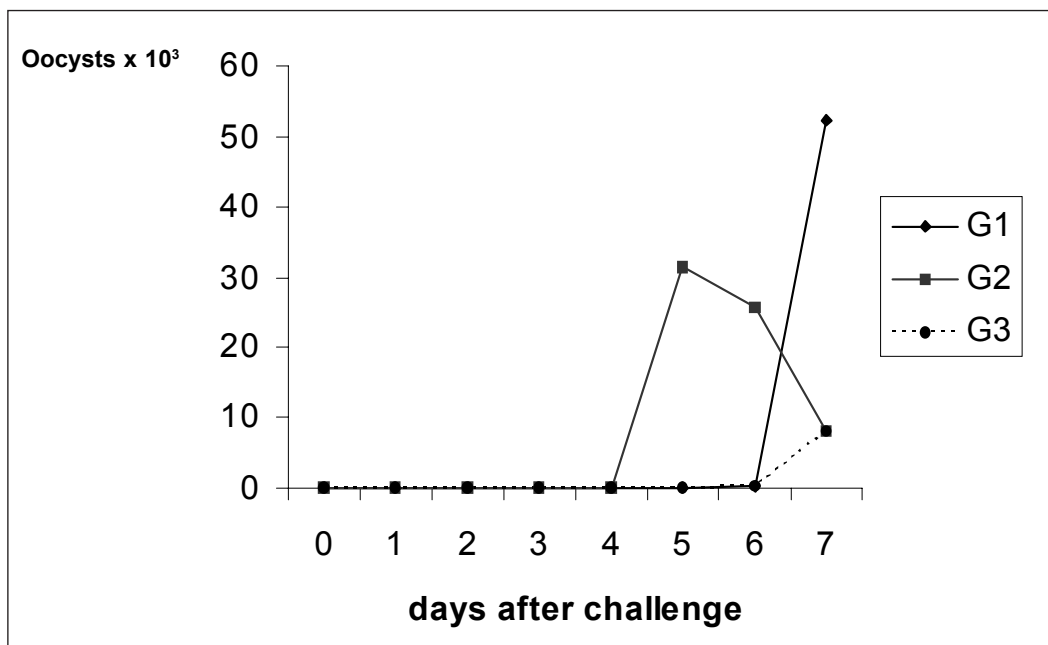
Body weight and rectal temperature were determined weekly. Bloody diarrhea was evaluated as previously described (YOUNG; NOH, 2001). Excreted oocysts were investigated with effect from day 16 to 21 (sacrifice). Blood samples were obtained at sacrifice and processed at the Veterinary Medical Hospital, Universidade Estadual do Centro Oeste (UNICENTRO). Lesion scores of the infected groups were assessed on day seven post-infection based on a previously described method (JOHNSON; REID, 1970). Intestinal sections (G1, cecum; G2, duodenum; and, G3, mid intestine) of all birds were collected at necropsy, fixed in 10% tamponated formalin solution, and routinely processed for histopathological evaluation (PROPHET et al., 1992). The histopathological lesions of each infection were described.

### Statistical analysis

Statistical evaluation was done using ANOVA and the t-Student test to establishment differences between the evaluated parameters. A P value of  $\leq 0.05$  was considered as significant.

### Results

Bloody diarrhea was observed only in the G1 bird with effect from the 6<sup>th</sup> day post-infection (DPI). Oocysts observed in feces demonstrated different kinetics of shedding (Figure 1). G1 and G3 birds excreted higher quantities of oocysts at 7<sup>th</sup> DPI, 52,000 and 8,000/ g of feces, respectively; while, G2 birds excreted higher oocysts at 5<sup>th</sup> DPI (31,000/ g of feces).



**Figure 1.** Oocysts shedding (grams of feces) in commercial broilers infected with *Eimeria tenella* (G1), *E. acervulina* (G2) and *E. maxima* (G3). Uninfected group (G4) did not eliminate oocysts.

Pathogenicity of strains is shown in Table 1. There were no statistical differences when the average of weight gain (obtained from one to 21 day old) of each group was compared (G1=182.7±63.4; G2=145.2±51.0; G3=183.3±56.8, and G4=211.5±89.0, p>0.10). Chickens from infected groups demonstrated lesion scores above 2 (G1=1.3±0.48; G2=0.4±0.52, and G3=1.1±0.99). There were no statistical differences between monocytes

(G1=30.4±63.4, G2=21.1±51.0, G3=18.4±56.8, G4=15.4±89), lymphocytes (G1=59.5±16.9, G2=64.4±10.3, G3=64.5±15.2, G4=61.4±16.5), and eosinophiles (G1=4.38±4.5, G2=9.8±7.4, G3=6.4±3.2, G4=9.8±7.08) counts. However, G1 birds showed lower number of heterophyles than G4 ((G1=1.25±2.05, G2=4.2±7.63, G3=11.6±12.8, G4=13.3±11.8, p<0.05). The uninfected group (G4) did not shed oocyst during the entire experiment.

**Table 1.** Pathogenic parameters observed in commercial broilers infected with *E. tenella* (G1), *E. acervulina* (G2) and *E. maxima* (G3). G4 uninfected group at sacrifice.

N	Leukocytes (%)											
	Lesion Score <sup>1</sup>		Weight gain <sup>2</sup>	Monocytes <sup>2</sup>	Lymphocytes <sup>2</sup>	Eosinophyles <sup>2</sup>	Heterophyles <sup>1</sup>					
	0	1	2	3	4	Mean±SD						
G1	10	0	7	3	0	0	1.3±0.48	182.7±63.4	30.4±16.1	59.5±16.9	4.38±4.5	1.25±2.05
G2	10	6	4	0	0	0	0.4±0.52	145.2±51.0	21.1±8.18	64.4±10.3	9.8±7.4	4.2±7.63
G3	10	4	1	5	0	0	1.1±0.99	183.3±56.8	18.4±6.07	64.5±15.2	6.4±3.2	11.6±12.8
G4	9	0	0	0	0	0	NC	211.5±89.0	15.4±15.7	61.4±16.5	9.8±7.08	13.3±11.8

<sup>1</sup>p<0.05 <sup>2</sup>p>0.05

The major tabulated histopathological findings (Table 2) were the presence of different stages of parasite (10/10), villous atrophy (7/10), and severe inflammation (7/10) in G1; villous atrophy (10/10),

severe inflammation (9/10), and mononuclear inflammatory infiltrate (7/10) in G2; and villous atrophy (6/10), severe inflammation (6/10) and mononuclear inflammatory infiltrate (5/10) in G3.

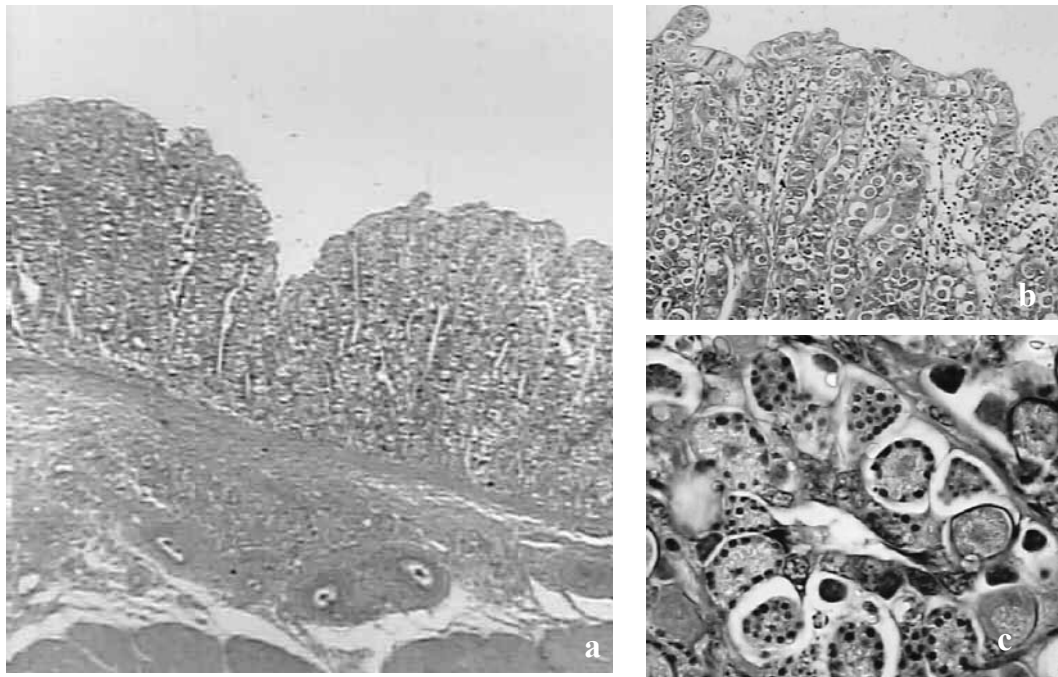
**Table 2.** Principal histopathological findings in chickens infected with 2 x 10<sup>4</sup>sporulated

Histopathological lesions	T*		
	G1	G2	G3
Presence of different phases of intralesional parasite	10	2	1
Villous atrophy	7	10	6
Severe Inflammatory process	7	9	6
Mononuclear infiltrate	5	7	5

T\*, number of birds with the described lesion

Microscopically, lesions induced by *E. tenella* (G1) were characterized principally by severe villous atrophy, marked proliferation of epithelial cells of intestinal crypts, dilation and necrosis of submucosal

glands, multifocal areas of severe inflammatory infiltrate, foci of discrete hemorrhage associated with various intralésionais forms of the parasite (Figure 2).

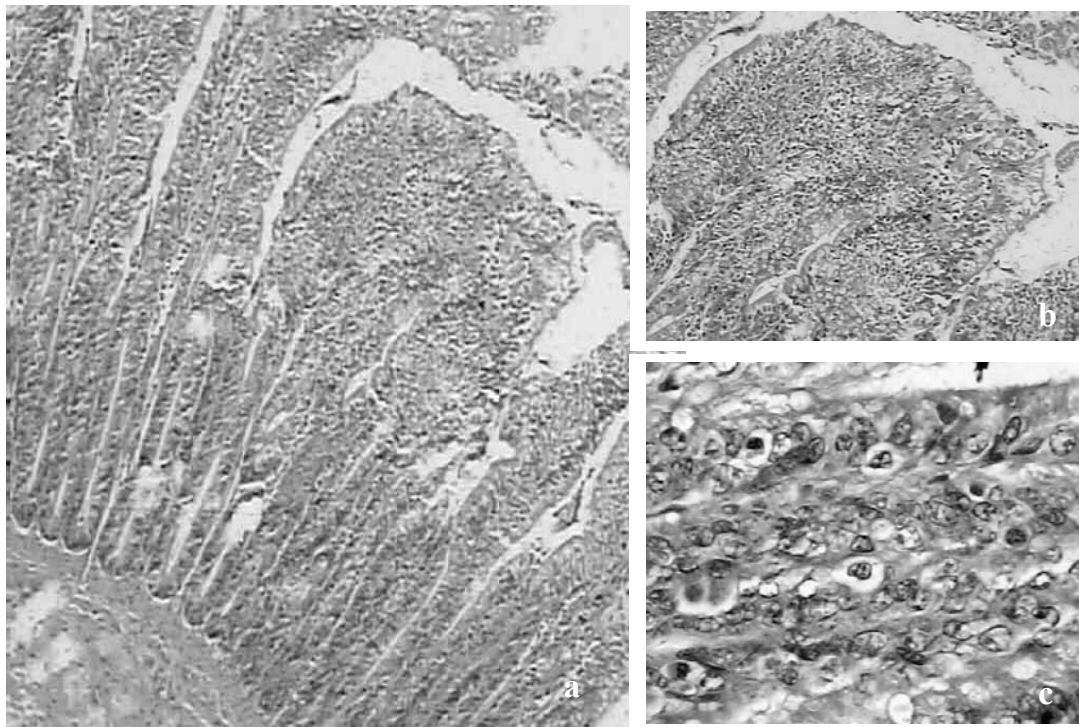


**Figure 2.** Histopathological observations at 7 days after infection with *Eimeria tenella* in commercial broiler chickens. (a) Panoramic vision showing inflammatory process and mononuclear infiltrate (100X), (b, 40X) and (c, 1000X) presence of different phases of parasite.

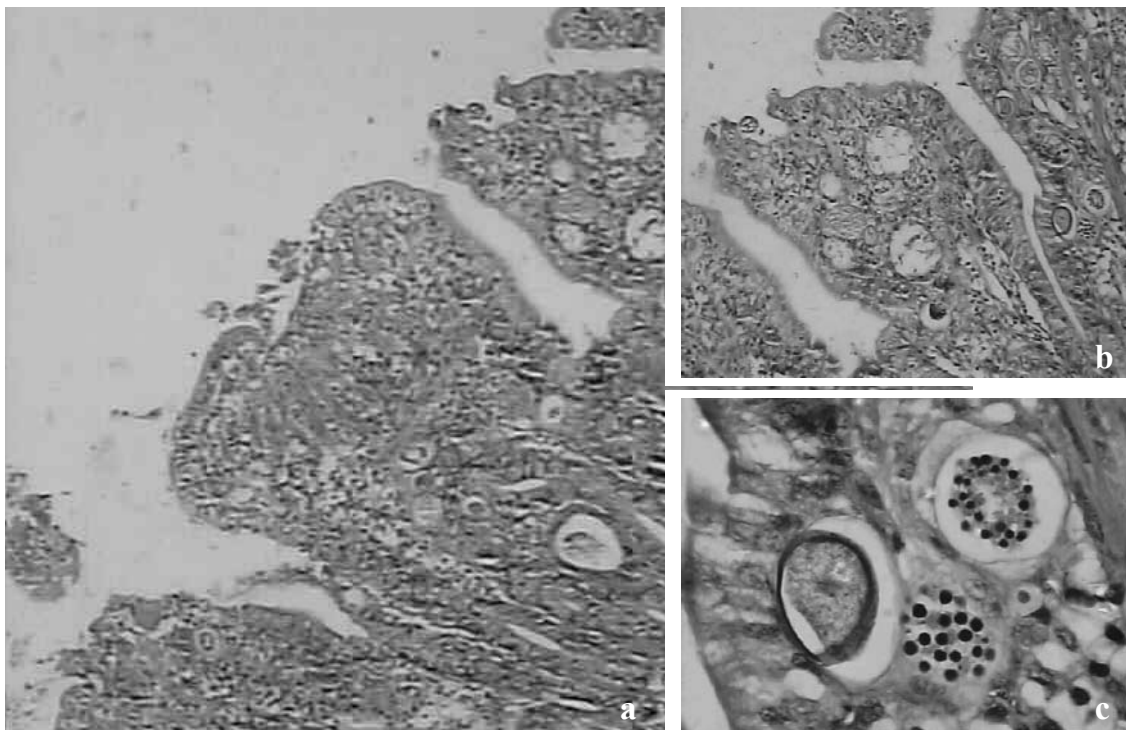
In some cases, parasites in various stages of development were transmurally located throughout the mucosa, with necrosis being more severe where there were massive accumulations of schizonts with merozoites. *E. acervulina* induced lesions (G2) demonstrated moderate villous atrophy and fusion of villi, discrete hemorrhage, marked proliferation of epithelial cells of crypts, foci of intense mononuclear infiltrate at the submucosa membrane, multifocal and discrete interstitial edema at the submucosa e

muscular membranes associated with various intralésionais forms of the parasite within epithelial cells (Figure 3).

Lesions induced by *E. maxima* (G3) were characterized by discrete villous atrophy, proliferation of epithelial cells, cystic dilation of the submucosa, discrete hemorrhage and dilation of submucosal glands associated with intralésionais examples of parasite in various stages of development (Figure 4).



**Figure 3.** Histopathological observations at 7 days after infection with *Eimeria acervulina* in commercial broiler chickens. (a) Panoramic vision showing inflammatory process and mononuclear infiltrate (100X), (b, 40X) and (c, 1000X) presence of different phases of parasite.



**Figure 4.** Histopathological observations at 7 days after infection with *Eimeria maxima* in commercial broiler chickens. (a) Panoramic vision showing inflammatory process and mononuclear infiltrate and presence of parasites in the border of villi (100X). (b) (200X) and (c) Presence of different phases of parasite (1000X).

## Discussion

There are differences among *Eimeria* isolates around the world. The antigenic variation observed in *E. acervulina* and *E. maxima* isolates has been described in samples collected from different geographical regions (MARTIN et al., 1997; DANFORTH, 1998; KAWAZOE et al., 2005). Consequently, vaccines could not confer immunity against coccidiosis. Thus, the regional *Eimeria* isolate evaluation are very important to verify their pathogenicity, and the possible utilization in the production of attenuated vaccines.

The prepatent period observed during this study for the *E. tenella*, *E. acervulina*, and *E. maxima* isolates varied from 5 to 7 days; similar results were described (JEURISSEN et al., 1996; YOUNG; NOH, 2001; GABRIEL et al., 2006). Various studies using early growing strains of this parasite (MONTES et al., 1998; KAWAZOE; MANARINI, 2001; KAWAZOE et al., 2005) were aimed at the determination of the prepatent period from the parent strain.

During this study equal challenges of  $2 \times 10^4$  sporulated oocysts were used in all experimental groups. However, this concentration was not sufficient to promote lesions with scores above 2. Similar results were previously described using a challenge of  $10^4$  sporulated oocysts of *E. maxima* and *E. acervulina* (CONWAY et al., 1993). However, these authors observed lesion scores of 3 and 4 in 75% of the chickens challenged by *E. tenella*

No significant statistical differences were observed between the average weight gains of birds from G1, G2, and G3 when compared with G4 birds (control group), although G4 birds demonstrated the most elevated weight gain during this study (Table 1). This similarity could be related to the short period of experimental infection. Additionally, the adapted lines used during this study provoked significant increases in large mononuclear white blood cells (monocytes) in chickens; this increase corresponds to periods of severe tissue destruction (KOGUT;

GORE; LONG, 1984). However, during this study, the white blood cell counts were not significantly different when the infected groups were compared with the control group (Table 1), except by heterophyle counts between G1 and G4.

The principal histopathological lesions observed in these birds at 7 DPI were consistent with those associated with infection induced by *Eimeria* spp. (McDOUGALD; REID, 1997). During this study, *E. tenella* induced-lesions were very severe; those induced by *E. acervulina* were moderate; while *E. maxima* lesions were discrete.

This study is the beginning of investigations that are being realized to evaluate the pathogenicity of *Eimeria* isolates from chickens from northern Paraná state, southern Brazil. Therefore, the determination of the level of pathogenicity of these locally produced *Eimeria* isolates will be of great importance for future studies, since we are presently evaluating the effects of live and dead vaccines to protect chickens against oocyst production.

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