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Research Article
Study of the Correlation Between Intestinal Health and Prevalence of Coccidiosis in Broiler Chickens of Brazilian Agribusinesses Between the Years 2015 and 2016

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Abstract

Objective: The objective of this study was to analyze the correlation between the lesions caused by *Eimeria* and the prevalence of coccidiosis and other alterations encountered in the gastrointestinal tract of broilers produced in Brazil from 2015-2016. **Materials and Methods:** Intestinal health assessments were conducted in 72 broiler integration businesses in Brazil, totaling 2,200 birds in two rearing phases: 1 (age 12-21 days) and 2 (age 22-40 days). Intestinal aspects, lesion scoring and oocyst counts of *Eimeria maxima* (*E. maxima*) were analyzed. **Results:** *E. acervulina* was the most prevalent species (mean of 13.5%) in both rearing phases, followed by *E. maxima* (5.6%) and *E. tenella* (2.2%). *E. maxima* was present in 30.4% of mucosal scrapings performed during phase 1, which represents a subclinical coccidiosis of 706.98% (7.07 times) in relation to clinical coccidiosis. In phase 2, *E. maxima* was found in mucosal scrapings of 34.3% of the birds, representing a subclinical coccidiosis of 497.11% (4.98 times) in relation to clinical coccidiosis. In the comparative analysis between the periods, subclinical coccidiosis struck 112.83% (1.13 times) more broilers in phase 2 in relation to stage 1. Subclinical coccidiosis struck a significant number of broilers in the Brazilian flocks and was correlated with various factors of intestinal health reduction. **Conclusion:** It was concluded that monitoring is of paramount importance to knowing the intestinal health status of poultry flocks because microscopic *E. maxima* is prevalent (32.3%) and correlated to factors that reduce intestinal health.

Key words: Broiler, coccidiosis, *Eimeria*, gastrointestinal tract, poultry farming

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The integrity of the gut is fundamental in birds as the mechanisms of digestion and absorption are inherent in the physiological metabolism of the gastrointestinal tract (GIT) and cannot be manipulated[1]. Numerous infectious and non-infectious agents can damage intestinal mucosa, as well as compromise digestion and absorption[2]. Among them, coccidia infections represent one of the most important enteric diseases. Coccidiosis is an infectious disease caused by a protozoan of the genus *Eimeria* (*Apicomplexa*: *Eimeriidae*) and is characterized by causing enteritis in various degrees in different segments of the intestinal tract, therefore decreasing performance parameters and increasing mortality[3]. Laboratory diagnosis of coccidiosis in poultry requires the identification of the species involved and quantification of the intensity of their infection by assessing the morphology, fecal oocyst count, location of the parasites in the gut, intensity of macroscopic lesions, prepatent period and oocyst sporulation time[4]. In the field, coccidiosis is diagnosed by assessing macroscopic lesions in the intestine of broilers. *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* are the species monitored on a regular basis[5,6]. A previous study showed that *E. acervulina* was the most prevalent (mean of 13.5%) species in Brazilian poultry operations, followed by *E. maxima* (6.75%) and *E. tenella* (4.35%)[7]. The purpose of the present study was to repeat and extend the evaluation and correlation of lesions caused by *Eimeria* with the other changes found in the gastrointestinal tract of broilers and the prevalence of coccidiosis in two production phases (phase 1: 12-21 days of age and phase 2: 22-40 days of age) in commercial poultry operations in Brazil between the years 2015 and 2016.

MATERIALS AND METHODS

Intestinal health monitoring was conducted in 72 broiler integration businesses in Brazil in the states of Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, São Paulo, Minas Gerai, Rio de Janeiro, Goiás, Distrito Federal, Alagoas, Pará and Paraíba during the period 2015-2016. The data were posted in the Intestinal Health Program (PSI) of Vetanco of Brazil in order to obtain the percentage of affected birds and their ratings according to lesion scores. The analyzed data were divided into two stages of production: The 1st phase from 12-21 days old and the 2nd phase from 22-40 days old. Hence, this was an observational study. The chickens evaluated received diets prepared by their respective integrated companies without any interference of the appraiser in the formulation and use of performance enhancers and anticoccidial drugs used at the time of evaluation.

The most common anticoccidial program is the dual system, which uses a drug in the first phase (1st-21st day of age) and another one in the second phase (22nd day until product withdrawal limit). This program reduces the possibility of parasite resistance, a fact that extends the time that the drug were efficacious in the field[8] and ensures more effective anticoccidial programs. To monitor the intestinal health of broilers, at least three birds/flock were evaluated. The birds were collected randomly inside the house at three different points (entry, middle and end).

In the intestinal analysis, the following lesions were observed: Presence of cell desquamation, excess fluid and mucus, ingestion of contaminated litter, thickening or thinning of the intestinal walls, Turkish towel appearance and necrotic enteritis. Injuries caused by *Eimeria acervulina, Eimeria maxima* and *Eimeria tenella* were scored according to their degree of intensity as specified by Johnson and Reid's method,[9] where the zero score indicates the absence of lesions and four indicates severe injury.

For microscopic assessments of *E. maxima*, the scraped intestinal mucosa technique for oocysts count was performed at the bowel portion next to Meckel's diverticulum. Costa and Paiva[5] described that the highest concentration of *E. maxima* is found in the jejunum and Meckel's diverticulum but can occur in the duodenum and at the end of the ileum. The content of the region was smeared on the microscope slide and a cover slip was placed over the content by pressing it gently. These slides were subjected to visual evaluation under the microscope at 100X magnification for oocyst counts at five different points (the four corners and center). The microscopic scores were ranked from 0-4, where 0 was the absence of oocysts, score 1 was 1-10 oocysts, score 2 was 11-20 oocysts, score 3 was 21-40 oocysts and score 4 was more than 41 oocysts[10].

Data were analyzed using the Pearson correlation coefficient SAS[11] with a 95% confidence interval.

RESULTS

According to the macroscopic analysis of lesions shown in Fig. 1, *Eimeria acervulina* was the most frequently observed species in both production phases. In phase 1, lesions related to *E. acervulina* were found in 8.8% of the necropsied broilers and were positively correlated (Table 1) with findings of cell desquamation, excess fluid, mucus, litter intake, thin intestine, feed passage, Turkish towel and duodenitis. In phase 2,

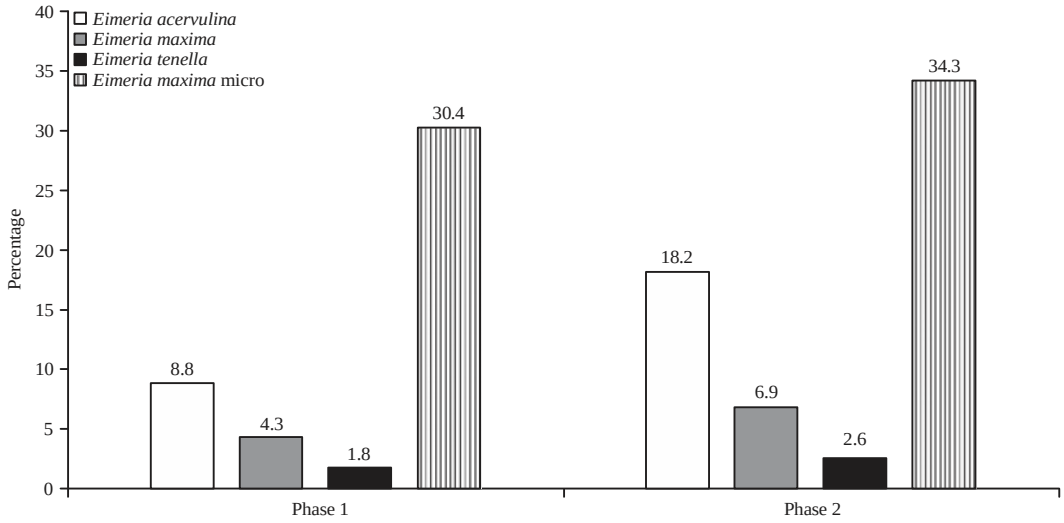

Fig. 1: Percentage of *Eimeria* in 1st and 2nd broiler production phases from 2015-2016

Table 1: Positive statistical correlations ($p \leq 0.05$) of broilers affected by the factors that decrease intestinal health in relation to *Eimeria* in 1st and 2nd phase from 2015-2016*

	Cell desquamation	Excess fluid	Excess mucus	Litter intake	Thin intestine	Feed passage	Altered intestinal tonus	Turkish towel	Duodenitis	*E. acervulina*	*E. maxima*
E. acervulina	Phase 1	Phase 1	Phase 1	Phase 1	Phase 1	Phase 1		Phase 1	Phase 1		
	0.66108	0.79805	0.58753	0.64774	0.73573	0.63448		0.55937	0.73671		
	0.0073	0.0004	0.0213	0.009	0.0018	0.0111		0.0302	0.0017		
	Phase 2	Phase 2		Phase 2		Phase 2					
	0.56266	0.45890		0.70789		0.56144					
	0.0121	0.0481		0.0007		0.0124					
E. maxima	Phase 2	Phase 2		Phase 2		Phase 2	Phase 2	Phase 2		Phase 2	
	0.80013	0.76361		0.67279		0.71580	0.59859	0.68981		0.46609	
	<0.0001	0.0001		0.0016		0.0006	0.0068	0.0011		0.0443	
E. tenella										Phase 2	Phase 1
										0.53216	0.60391
										0.0190	0.0171
E. maxima micro**		Phase 1							Phase 1	Phase 1	Phase 1
		0.70458							0.54294	0.71045	0.47294
		0.0034							0.0365	0.0030	0.0465
	Phase 2	Phase 2		Phase 2		Phase 2		Phase 2			Phase 2
	0.50908	0.51218		0.56810		0.58690		0.54621			0.52532
	0.0260	0.0250		0.0112		0.0083		0.0155			0.0209

*1st phase (12-21 days of age), 2nd phase (22-40 days of age), **Microscopic assessment of *E. maxima* in the intestinal mucosa

E. acervulina occurred in 18.2% of birds with its occurrence positively correlated with findings of cell desquamation, excess fluid, litter intake and feed passage.

E. maxima was the second most frequently observed species in affected birds. In phase 1, this coccidia was present in 4.3% of the birds. There was an increase in the prevalence of lesions caused by this species in phase 2, where it struck 6.9% of the birds. Lesions from *E. maxima* were positively correlated with cell desquamation, excess fluid, litter intake, feed passage, altered intestinal tonus, Turkish towel and *E. acervulina*.

E. tenella had the lowest prevalence in phase 1 and phase 2 (1.8 and 2.6%, respectively). There was a positive correlation between the occurrence of lesions caused by *E. tenella* in phase 1 with *E. maxima* and the occurrence of macroscopic lesions of *E. acervulina* in phase 2.

Under microscopic evaluation, *E. maxima* was present in 30.4% of the mucosal scrapings evaluated in phase 1, which represented a subclinical coccidiosis of 706.98% or 7.07 times in relation to clinical coccidiosis (macroscopic lesions of *E. maxima* according to Johnson and Reid).[9] There was a positive correlation between the occurrence of *E. maxima*

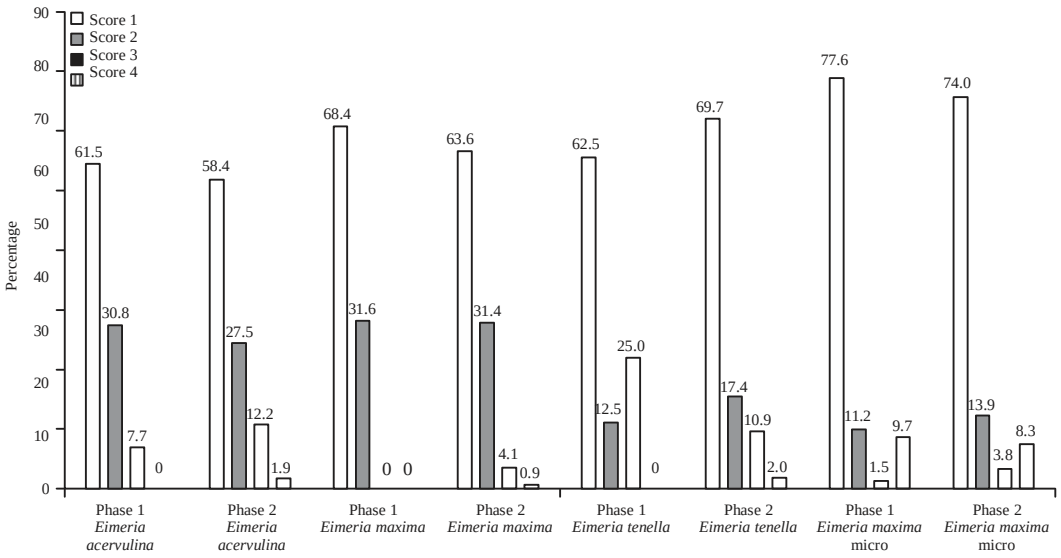

Fig. 2: Percentage of *Eimeria* scores in 1st and 2nd broiler production phases from 2015-2016

micro with findings of excess fluid, duodenitis and the occurrence of macroscopic lesions of *E. acervulina* and *E. maxima.* In phase 2, there was a significant increase in the occurrence of *E. maxima* micro, where 34.3% of the assessed mucosal scrapings showed oocysts of *E. maxima.* This prevalence represented a subclinical coccidiosis of 497.11% or 4.98 times compared to clinical coccidiosis. There was a positive correlation between the occurrence of *E. maxima* micro with cell desquamation, excess fluid, litter intake, feed passage, Turkish towel and macroscopic lesions of *E. maxima.* The data showed an increase in subclinical coccidiosis occurrences along the birds' lifespan in that there was an increase of 112.84% or 1.13 times in the number of affected birds in phase 2 compared to phase 1.

Figure 2 demonstrates that level 1 of the macroscopic lesion score was the most commonly observed in both phases of production (1 and 2) for all evaluated species of *Eimeria*, while level 4, which is the most severe lesion score according to Johnson and Reid[9] was present in a very small number of birds in the macroscopic evaluation. However, regarding micro scores for *E. maxima*, the score of level 4 was present in a significant number of birds (9.7 and 8.3% in phases 1 and 2, respectively). This represents a large number of oocysts present in the intestinal mucosa. Once eliminated in the bedding, these oocysts promote poultry recontamination and can lead to a higher chance of infection in other birds in the flock. Having large numbers of oocysts in the bedding leads to a greater chance of coccidiosis occurrence in its clinical form.

DISCUSSION

The prevalence of *E. acervulina* (13.5%) is in agreement with our previous findings[7], which described this species as the most prevalent species of *Eimeria* in Brazilian poultry commercial operations. However, in a similar study, Do Amaral and Otutumi[6] described an average prevalence of only 8.21% for *E. acervulina*.

Gazoni[12] reported an average prevalence of 5.12% for *E. maxima.* This number increased to 6.75% by Gazoni *et al.*[7], although these percentages are lower than the 8.52% reported by Do Amaral and Otutumi[6].

The average occurrence of *E. tenella* reported by Gazoni[12] was 11.47%, whereas Do Amaral and Otutumi[6] claimed that *E. tenella* presented lesions in 2.42% of the birds. The result described by Gazoni[12] for *E. tenella* is well above the values reported in this study (mean 2.2%) and by Do Amaral and Otutumi.[6] These differences can be explained by the different general disturbances in the gut as disbacterioses, viruses and mycotoxicoses in each evaluation.

Due to a lack of maturity of their immune system, young birds are the most susceptible to clinical infections that often occur in asymptomatic adult birds, who spread the parasite. However, severe outbreaks can occur in adult birds as demonstrated in this study, in which there was a higher percentage of birds affected by the described species of *Eimeria* in the second phase. The route of infection is fecal-oral and the sporulated oocyst is the infective form. For the oocysts to sporulate, moderate temperatures (between 18 and 30°C), relative humidity greater than 70% and well-

oxygenated conditions are required. The average time of sporulation is approximately 17 h for *E. acervulina*, 18 h for *E. tenella* and 30 h for *E. maxima*[4].

In contrast with our previous findings which showed that the micro scores of *E. maxima* had a prevalence of 23.8%[7], the scores reported here had an average prevalence of 32.35%. This represents an increase of 135.71% in the last two years. According to Ito *et al*.[2], intestinal scraping is the most suitable method for subclinical coccidiosis detection.

Teeter[13] evaluated the impact of coccidiosis challenge on the poultry growth curve. In Teeter's work[13], it was observed that for each point of increase in microscopic evaluation of coccidiosis lesions, daily body weight dropped 1.5% during the challenge period of 6 days. Therefore, it is important to know the degree of lesion to quantify the performance loss in the birds. It is estimated that during the coccidia asexual and sexual phases, each ingested oocyst destroys 2,048 enterocytes[14]. Hence, the economic importance of coccidiosis goes far beyond mortality and is mainly related to subclinical losses of asymptomatic animals, which maintain environmental contamination through oocyst elimination in their feces. Birds that did not exhibit clinical disease can also become uneconomical due to malabsorption syndrome[1]. Therefore, it is important to monitor the gut health of the birds by performing necropsies and mucosal scrapings to provide an early diagnosis of coccidiosis. Then, situations where anticoccidial programs are inefficient can be detected and preventive or therapeutic measures can be taken, providing coccidiocide drugs via drinking water[14].

CONCLUSION

During the monitored period of two years (2015 and 2016), the macroscopic evaluation of lesions related to coccidiosis demonstrated that *E. acervulina* is still the most prevalent species in both phases of production analyzed (phase 1 from 12-21 days old and phase 2 from 22-40 days old), affecting on average 13.5% of broilers.

The occurrence of lesions caused by *Eimeria* positively correlated with several factors that decrease the intestinal health in two production stages, including cell desquamation, excess fluid, mucus, litter intake, thin intestine, feed passage, altered intestinal tonus, Turkish towel and duodenitis.

The lesion score level 1 was the most frequent score in both the 1st and the 2nd production phases. The subclinical coccidiosis, detected by mucosal scrapings microscopy, was observed in most of the chickens evaluated (mean 32.3%) and is one of the probable factors causing a reduction in the growth performance of broiler flocks in Brazil.

The analysis of necropsy findings and microscopy of the two evaluation phases demonstrated that the second phase (22-40 days of age) presented more severe lesion scores (scores 3 and 4). Hence, we have the greatest productive performance loss of broiler flocks in this stage.

It is vital that professionals in the poultry sector perform the intestinal tract evaluations mainly in the second phase (22-40 days of age) and if necessary, they may intervene in order to maintain the productive performance of broilers.

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