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Coccidiosis in commercial broilers in Brazil between 2012 and 2019: main species and degrees of injury

Coccidiosis en pollos de engorda comerciales en Brazil entre 2012 y 2019: especies principales y grados de daño

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ABSTRACT

The purpose of the present study was to determine the occurrence of coccidiosis in broilers, detect the most commonly occurring species of *Eimeria* spp, and parasitic burdens in industrial poultry in Brazil from 2012 to 2019. We studied 13,648 birds between 9–49 days of age at 82 companies in 13 Brazilian states. Birds were randomly selected (3–6 birds/aviary) and euthanized. Macroscopic lesions were analyzed, and the intestinal mucosa was scraped to count *E. maxima* oocysts. The classification of species was based on the size of the oocysts. We also quantified the infectious burden using a scoring system. Subclinical coccidiosis (*E. maxima* micro) was detected in an average of 34.8%, *E. acervulina* (16.1%), *E. maxima* (7.9%), and *E. tenella* (4.1%) were also identified with annual fluctuations. The count of oocysts was within score 1 (1–10 oocysts/bird) in 47.5–84.4% of the cases; followed by score 2 (11–20), 3 (21–40) and 4 (over 41 oocysts/bird). Subclinical coccidiosis is a cause for concern, with 3.5 out of every ten birds being infected. This is a factor responsible for the lower productive performance of broilers.

Keywords: Coccidiosis, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, prevalence.

RESUMEN

El propósito del presente estudio fue determinar la frecuencia de la coccidiosis en pollos de engorde, detectar las especies más comunes de *Eimeria* spp y cargas parasitarias en aves comerciales en Brasil de 2012 a 2019. Estudiamos 13,648 aves entre 9 y 49 días de edad en 82 empresas en 13 estados brasileños. Las aves se seleccionaron al azar (3 a 6 aves / aviario) y se sacrificaron por eutanasia. Se analizaron las lesiones macroscópicas y se raspó la mucosa intestinal para el conteo de ooquistes de *E. maxima*. La clasificación de especies se basó en el tamaño de los ooquistes. También cuantificamos la carga infecciosa mediante un sistema de puntuación. Se detectó coccidiosis subclínica (*E. maxima* micro) en un promedio de 34.8%. *E. acervulina* (16.1%), *E. maxima* (7.9%) y *E. tenella* (4.1%) también se identificaron con fluctuaciones anuales. El conteo de ooquistes estuvo dentro de la puntuación 1 (1 a 10 ooquistes / ave) en el 47.5 al 84.4% de los casos; seguido de una puntuación 2 (11 a 20), 3 (21 a 40) y 4 (más de 41 ooquistes / ave). La coccidiosis subclínica es motivo de preocupación, ya que 3.5 de cada diez aves estaban infectadas. Este es un factor responsable del menor rendimiento productivo de los pollos de engorde.

Palabras clave: Coccidiosis, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, prevalencia.

INTRODUCTION

In commercial and intensive poultry breeding, *Eimeria* spp. are a ubiquitous protozoan, widespread on six continents (Chapman *et al*., 2016; Clark *et al*., 2016). Seven species of *Eimeria* have been recognized (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*). They differ in pathogenicity (McDougald, 2008). *Eimeria* infection birds can reduce growth performance via the impaired intestinal function (Kim *et al*., 2017; Lu *et al*., 2019). More commonly, the birds remain asymptomatic until there is an infection by a large number of coccidia or some other aggravating pathology (Williams, 2005; Chapman *et al*., 2016; Gazoni *et al*., 2017). It was estimated in the early 2000s that the disease had an annual economic impact of approximately U.S. $3 billion, with losses to producers and the worldwide poultry industry (Dalloul and Lillehoj, 2006). In Romania in 2016, researchers found that total economic losses per 24 flocks of 18,000 chicks were about €37,948.2, with an average of €3,162.4 per flock. These losses were caused by mortality (34.8%) and poor feed conversion (65.2%) due to coccidiosis (Györke *et al*., 2016).

Traditionally, the diagnosis of farms occurs through the detection and enumeration of oocysts excreted in the feces, in addition to measuring bird dimensions. Post-mortem investigators evaluate the affected intestinal portion and the lesions (Long and Joyner, 1984). The specific diagnosis of *Eimeria* infections in broilers is fundamental to a better understanding of the disease's epidemiology and dynamics and is necessary for the prevention, surveillance, and effective control (Morris and Gasser, 2006; Gazoni *et al*., 2017).

Subclinical coccidiosis is commonly seen in poultry farms in Brazil, and accurate diagnosis is essential for tracing interventions, mainly because of problems with resistance to coccidiostats agents (Gazoni *et al*., 2020). Therefore, this study's objective was to determine the annual occurrence of *Eimeria* infection in industrial broilers from 2012 to 2019 in Brazil.

MATERIALS AND METHODS

Animals and data collection

Monitoring of coccidiosis was carried out in 82 companies in the states of Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, São Paulo, Minas Gerais, Rio de Janeiro, Goiás, Distrito Federal, Alagoas, Para, Paraiba and Pernambuco from 2012 and 2019, using 13,648 broilers. Brazil is a continental country with a very particular climatic variation for each state, because of this, it was not raised in consideration of climatic information. The data were released by the Intestinal Health Program (PSI) of Vetanco do Brasil, in order to obtain the percentage of affected birds and their degree of classification by injury scores in collaboration with researchers from the State University of Santa Catarina (UDESC). The birds were from the Cobb 500 and Aviagen lines and were 9–49 days old. They were provided feed prepared by their respective companies, without any interference from the evaluator in terms of

formulation or use of performance enhancers and anticoccidial agents. The most commonly used anticoccidial program is the dual system, where an active ingredient is used in the first phase (1st to 21st day of age), and another is used in the second phase (22nd day to 3 days before slaughter), the broilers do not receive vaccines programs coccidiosis, in order to reduce the possibility of the appearance of antimicrobial resistance.

Sample collection

We monitored the intestinal health of broilers using 3–6 birds per flock (aviary). Birds were randomly selected from three different points in the aviary (entrance, middle, and bottom). These birds were euthanized by cervical dislocation, followed by dissection for sample collection and visual evaluation.

Macroscopic tissue evaluation

In the gastrointestinal tract, the presence of lesions caused by *E. acervulina*, *E. maxima*, and *E. tenella* was observed, for example figure 1. When present, lesions were classified according to their degree of intensity, according to the methodology of Johnson and Reid (1970), where the score 0 indicates the absence of injury, and score 4 indicates severe damage.

Lesions *E. acervulina* Lesions *E. maxima* Lesions *E. tenella*

Figure 1. Presence of lesions caused by *E. acervulina*, *E. maxima*, and *E. tenella*.

Mucosa scraping technique and oocyst count

To evaluate *E. maxima* micro, the intestinal mucosa scraping technique was used to count oocysts, performed in the intestinal portion surrounding Meckel's diverticulum. The fecal content was deposited on a slide, covered by a coverslip, and visualized in five fields (extremities and center) for counting oocysts under optical microscopy (100x). Four scores were used for the classification, according to Vetanco do Brasil (2011), where score 0 represented absence of oocysts; score 1 represented 1–10 oocysts; score 2 represented 11–20 oocysts; score 3 represented 21–40 oocysts, and score 4 meant more than 41 oocysts, for example figure 2.

Score 1 represented 1–10 oocysts

Score 2 represented 11–20 oocysts

Score 3 represented 21–40 oocysts

Score 4 meant more than 41 oocysts

Figure 2. Four scores were used for the classification for *E. maxima* micro.

RESULTS

The annual occurrence showed significant differences between species; while one was high, the others were smaller in the year in question. Subclinical coccidiosis had the most increased occurrence (average of 34.8%) during the evaluated period (2012–2019), revealing greater frequency and dissemination in the Brazilian poultry industry. For macroscopic lesions attributed to *E. acervulina*, *E. maxima*, and *E. tenella*, the average occurrence was 16.1%, 7.9%, and 4.1%, respectively. We also observed that none of the *Eimeria* species showed linear behavior; instead, they demonstrated fluctuations across years: sometimes, there was an increase. Sometimes, there was a decrease in occurrence (Table 1).

Considering each species across periods, we observed that, in 2012, the occurrences of *E. maxima* (22.2%) and *E. tenella* (10.0%) were higher, and they were lower in the other years. *E. acervulina* had a significant increase in 2016, reaching 30.5% of occurrence, with a gradual decrease after that date (9.7% in 2019). *E. maxima micro* showed almost incremental growth until 2017 (from 28.8 to 45.5%), remaining with a high annual percentage in the subsequent years (38.0–42.6%). These results are shown in Table 1.

Table 1. *Eimeria* species and percentage of eimeriosis in broilers between 2012 to 2019.

Species	Annual occurrence (%)								
	2012	2013	2014	2015	2016	2017	2018	2019	Average
E. acervulina	9.3	18.7	13.6	13.5	30.5	16.6	17.1	9.7	16.1
E. maxima	22.2	7.8	4.8	5.9	8.1	4.4	4.9	5.4	7.9
E. tenella	10.0	5.0	4.1	2.0	4.5	2.3	1.7	3.1	4.1
E. maxima, micro	28.8	29.1	25.8	33.5	34.7	45.5	38.0	42.6	34.8

In terms of lesion scores, in practically every year and for all species, the oocyst count was within score 1, followed by scores 2, 3, and 4 (this was rarely higher than 3, and when it occurred, it was observed for *E. maxima micro*). Score 4 was infrequent, often resulting in a null value. The percentage between species and between years did not follow a pattern. Instead it varied for both species and year (Table 2).

Table 2. Percentage of coccidiosis scores (*Eimeria*) obtained between 2012 and 2019 in broilers from 9 to 49 days in Brazilian agribusiness.

Year	Species	Score (%)			
		1	2	3	4
2012	*E. acervulina*	48.7	39.5	11.8	0.0
	E. maxima	56.1	35.6	7.2	1.1
	E. tenella	79.1	17.2	1.8	1.8
	E. maxima, micro	73.0	9.8	9.4	7.9
2013	*E. acervulina*	57.0	32.9	9.0	1.1
	E. maxima	69.1	24.1	5.8	1.0
	E. tenella	84.4	12.3	3.3	0.0
	E. maxima, micro	58.8	14.4	7.8	19.0
2014	*E. acervulina*	65.9	26.5	7.6	0.0
	E. maxima	64.7	25.2	5.9	4.2
	E. tenella	76.5	19.6	3.9	0.0
	E. maxima, micro	65.3	14.6	4.2	15.9
2015	*E. acervulina*	62.2	27.4	8.9	1.5
	E. maxima	65.8	30.7	3.5	0.0
	E. tenella	69.2	17.9	12.8	0.0
	E. maxima, micro	76.2	12.8	2.5	8.6
2016	*E. acervulina*	51.0	28.4	18.6	2.0
	E. maxima	59.3	33.3	3.7	3.7
	E. tenella	66.7	13.3	13.3	6.7
	E. maxima, micro	70.7	14.7	7.8	6.9
2017	*E. acervulina*	81.1	13.8	5.1	0.0
	E. maxima	79.5	15.1	5.5	0.0
	E. tenella	57.9	31.6	10.5	0.0
	E. maxima, micro	71.5	10.3	1.9	16.3
2018	*E. acervulina*	53.6	41.2	5.2	0.0
	E. maxima	66.7	28.3	5.0	0.0
	E. tenella	76.2	23.8	0.0	0.0
	E. maxima, micro	84.2	6.4	2.3	7.0
2019	*E. acervulina*	75.8	18.7	5.5	0.0
	E. maxima	66.7	29.4	3.9	0.0
	E. tenella	65.5	34.5	0.0	0.0
	E. maxima, micro	47.5	22.5	2.5	27.5

DISCUSSION

We reported the occurrence of coccidiosis in the Brazilian poultry industry for over seven years. Studies reporting epidemiological characteristics for coccidiosis in Brazil are rare. It is important to pay attention to the species found in the territory; our findings agree with those of other authors who frequently reported *E. acervulina*, *E. maxima* and *E. tenella* (Moraes *et al.,* 2015; Chapman *et al.,* 2016; Kim *et al.,* 2017); these species, in addition to being reported most commonly, are the ones that commonly develop resistance to synthetic drugs (Shivaramaiah *et al.*, 2014).

All species of *Eimeria* are widespread on the six continents. However, there may be a regional division in the genetic diversity and population structure of species (Prakashbabu *et al.,* 2017); or even, as described by Clark *et al*. (2016), there may be genetic variants between the southern and northern hemispheres, representing a risk to food security and animal welfare if it spreads to previously absent areas. This variation is believed to be attributable to the use of anticoccidial drugs and vaccines (Prakashbabu *et al.,* 2017).

A study regarding the prevalence of coccidiosis in Santa Catarina (B.R.) using PCR showed that 96% of the farms were positive for *Eimeria*, with seven species identified: *E. maxima* (63.7%) and *E. acervulina* (63.3%), *E. tenella* (54.6%), *E. mitis* (38.6%), *E. praecox* (25.1%), *E. necatrix* (24.3%) and E*. brunetti* (13.1%), with an average of 2.96 species per farm (Moraes *et al*., 2015). Another study in a small region of the state of Tocantins reported the occurrence of coccidiosis in all farms, with the presence of *E. maxima*, *E. acervulina*, *E. mitis*, and *E. tenella* (Toledo *et al*., 2011). The high preponderance of an *Eimeria* species may indicate its resistance to the drugs usually exposed, as is the case of *E. tenella* studied in Nigeria (Ojimelukwe *et al.*, 2018) *E. maxima micro* and *E. acervulina* in the present study and the others reported in Brazil. The diagnosis is important and necessary to outline strategies, as according to Teeter *et al.* (2008), it was observed that for each point of increase in the microscopic evaluation of coccidiosis lesions, daily weight gain decreased 1.5% of the body weight (g) during the six-day challenge period. Therefore, it is essential to know the degree of intestinal damage to quantify the birds' performance.

The rapid intestinal replication cycle (4 to 6 days) and the oral/fecal route make coccidiosis a severe problem of intensive breeding, caused by recurrent infections and resistance to coccidiostats agents (Shivaramaiah *et al.,* 2014). Without effective control, the number of parasites can increase to the point of clinical coccidiosis. To avoid resistance, drug rotation, and vaccination programs are carried out (Lan *et al*., 2017). The rotation of coccidiostats agents can help clarify the significant oscillations of occurrence between *Eimeria* species, as verified in this study.

Vaccination is the most interesting prophylactic measure; however, it is used only in broiler breeders (Abdul Rasheed and Matsler, 2020) and laying hens (Chapman *et al.,* 2014). Commercial *in ovo* vaccination for coccidiosis in broilers has become widely accepted in the U.S. poultry industry; however, its effects on performance have yet to be studied (Sokale *et al.,* 2020). It is essential to emphasize the importance of studies of species prevalence for the development of regional vaccines, as in this study. The use of antimicrobials has been analyzed with a view to replacing them in order to avoid the resistance problem, and several alternatives have been proposed (Kim *et al.,* 2017; Bortoluzzi *et al.,* 2019; Lu *et al.,* 2019; Park *et al.,* 2020). The low amount of oocysts present in the samples in this study (score 1) and the few observations of the zero score (no oocysts/sample) may indicate the effectiveness of chemical anticoccidials' strategic use via feed.

In addition to the loss of performance, coccidiosis is a predisposing factor for an even more harmful pathology in birds, such as necrotic enteritis (Williams, 2005; Adhikari *et al.*, 2020). *E. maxima*'s presence was considered by Paiva and McElroy (2014) to be an essential risk factor for promoting *Clostridium perfringens*, and this must be considered in the search for effective control methods for farms in Brazil.

For herd monitoring, the mucosa visualization and oocyst counting, and classification technique remains the most viable for the Brazilian agribusiness, to the detriment of the qPCR test, also available for diagnosis and quantification (Velkers *et al.,* 2010). This would considerably increase the monitoring and require differential care of the samples.

CONCLUSIONS

In conclusion, subclinical coccidiosis has a worrying prevalence, with every ten birds 3.5 having coccidiosis. This is one of the factors that likely causes reductions in the broiler batches' productive performance as well as being a predisposing factor for clostridiosis. Because attempts to eradicate the parasite by quarantine, disinfection, and sanitation have not been successful (McDougald, 2008), it is of fundamental importance that professionals in the poultry sector to carry out subclinical coccidiosis assessments routinely, thereby being able to intervene if necessary, to maintain the productive performance of broilers.

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