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Epidemiology of *Eimeria* infections in sheep raised extensively in a semiarid region of Brazil

Epidemiologia da infecção por *Eimeria* em ovinos criados extensivamente em região semiárida no Brasil

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Abstract

The aim of this study was to identify and determine the prevalence of *Eimeria* species affecting sheep raised extensively in a semiarid region of Brazil. Fecal samples of native sheep were collected during the rainy and dry seasons. The degree of infection was determined by counting oocysts per gram (OPG) of feces, and the morphometric method was used for species identification. Oocysts were found in all the properties assessed, in which 68.3% of the animals were infected. The prevalence of oocysts was influenced by the season and animal category (P<0.05). It was higher during the rainy season than the dry season (80.2% vs. 55.8%) and highest in young animals than the adults animals (68.2% vs. 39.6%). The OPG was lower during the dry season (1,269 ± 312 vs. 4,400 ± 1,122). Ten species were found; of these, *E. ovinoidalis, E. granulosa, E. faurei,* and *E. crandallis* were the most frequent. *E. ovinoidalis* and *E. crandallis* were found in all properties, with their prevalences being 19.4% and 13.6% respectively. The high prevalence of pathogenic species shows that eimeriosis is a risk for animals raised extensively in the semiarid region.

Keywords: Coccidiosis, morphometry, oocyts, OPG, parasitosis.

Resumo

Objetivou-se neste estudo identificar e determinar a prevalência de espécies de *Eimeria* que parasitam ovinos criados extensivamente em região semi-árida. Amostras de fezes de ovinos nativos foram coletados durante as estações chuvosa e seca. O grau de infecção foi determinado pela contagem de oocistos por grama de fezes (OoPG)e o método morfométrico foi utilizado para a identificação das espécies. Foram encontrados oocistos em todas os rebanhos avaliados e observou-se que 68,3% dos animais estavam infectados. A prevalência de oocistos foi influenciada pela estação climática e pela categoria dos animais (P<0,05), sendo mais alta durante a estação chuvosa em relação a estação seca (80,2% vs. 55,8%) e em animais jovens em relação aos animais adultos (68,2% vs. 39,6%). O OoPG foi menor durante a estação seca (1.269 ± 312 vs. 4.400 ± 1.122). Dez espécies foram encontradas sendo a *E. ovinoidalis*, *E. granulosa*, *E. faurei*, e *E. crandallis* as mais frequentes. *E. ovinoidalis* e *E. crandallis* foram encontrados em todas as propriedades, com prevalências de 19,4% e 13,6%, respectivamente. A alta prevalência de espécies patogênicas mostra que eimeriose é um risco para os animais criados extensivamente na região semiárida.

Palavras-chave: Coccidiose, morfometria, oocistos, OoPG, parasitose.

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Introduction

Eimeriosis is an endoparasitosis, which, in ruminants, is characterized by diarrhea that can lead to death. The subclinical form causes intestinal function impairment, which delays the animal's growth (DENIZ, 2009) and decreases its performance (TAYLOR et al., 2011).

In sheep, eimeriosis has great economic importance due to its high prevalence in many parts of the world (BAKUNZI et al., 2010). Even though eimeriosis is considered an emergent disease with a growing sanitary risk (TAYLOR, 2012), there are few studies about the epidemiology of eimeriosis in sheep raised extensively in semiarid regions, where the sheep industry plays a significant economic role (COSTA et al., 2011).

Knowledge of the prevalence of parasitic species and the predisposing factors for infections by these species is essential for evaluating the infection potential and minimizing the economic impact of eimeriosis (YAKHCHALI & GOLAMI, 2008). Conversely, the degree of infection depends on environmental conditions, the immunological response of the animal (CAVALCANTE et al., 2012), and parasitic species (REEG et al., 2005). Species vary with respect to pathogenicity, pre-patent period, and the rate of oocyst elimination (GAULY et al., 2001). Thus, eimeriosis diagnoses based on the quantification of oocysts in fecal samples does merit a relative strategic value (LIMA, 2004).

Identification of *Eimeria* species may be based on biological and morphological characteristics (MARTYNOVA-VANKLEY et al., 2008). Biological identification, which uses molecular methods for protein and amino acid identification, requires specific and costly equipment (BARKWAY et al., 2011). Thus, due to its practicality, morphological characterization utilizing morphometry of sporulated oocysts has been the most common identification method used to differentiate species in many epidemiological studies (AHID et al., 2009).

The infection by coccidia from genus *Eimeria* generally has a multi-specific character (CAVALCANTE et al., 2012), yet 15 species that parasitize sheep have been described (SARATSIS et al., 2011). Among the different species capable of infecting sheep, only *E. ovinoidalis* and *E. crandallis* cause the clinical symptomatology of eimeriosis; there is little evidence that other species are pathogenic (GREGORY & CATCHIPOLE, 1990). Consequently, the objective of this study was to identify and quantify the *Eimeria* species that parasitize sheep raised extensively in a semiarid region of Brazil, and the roles of animal category and the climatic season in the severity of infection.

Materials and Methods

The study was carried out in 20 rural properties located in the southwest of Bahia, Brazil (14° 36' S, 41° 08' O), at an altitude of 353 m. The climate in that region is classified as tropical-Aw according to the Köepen-Geiger classification (PEEL et al., 2007), with very well-defined dry seasons (from April to October) and wet seasons (from November to March) (Figure 1). The pluviometric annual mean index is 656 mm (INMET, 2012) and the region predominantly contains open arboreal caatinga vegetation (SEI, 2012).

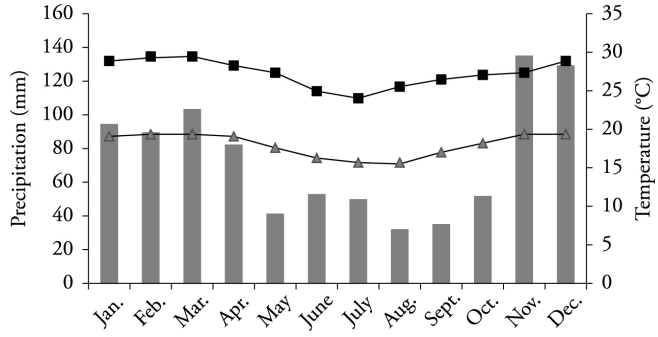

Figure 1. Minimum (-■-) and maximum (-▲-) temperatures and pluvial precipitation (▪) from January to December 2011 (INMET, 2012).

The sampled herds were composed of adaptated local breed sheep raised extensively in Caatinga pastures, supplemented with cactus forage (*Opuntia ficus*) during the most critical period of the dry season. The animals without a history of anti-coccidial treatment were grouped into four categories: ram, ewe, and male and female lambs (age, ≤6 months). The number of sampled animals corresponded to 20% of the total category, except the ram category, which was 100%. A total of 464 individual fecal samples were collected from 20 herds; among these, 238 were obtained during the rainy season (from December to January) and 226 during the dry season (from June to July).

Fecal samples collected directly from the rectum were placed in plastic bags and kept under refrigeration prior to laboratory analysis. The oocyst count expressed in oocysts per gram of feces (OPG) was performed using the McMaster technique of flotation in saturated saline solution developed by Gordon & Whitlock (1939) and modified by Ueno & Gonçalves (1998). After gaze filtration, the samples containing *Eimeria* were grouped by collection site, placed in Petri dishes containing potassium dichromate (2.5%), and kept for seven days at room temperature. *Eimeria* species were identified by the form aspects, the presence or absence of micropyle and micropylar caps, major and minor diameter measurements of the oocysts and sporocysts, and the morphometric index (MI). The MI was obtained by the equation MI = larger diameter/smaller diameter (LEVINE et al., 1980; HASSUM et al., 2002; AHID et al., 2009). A hundred randomly sampled oocysts from each farm were photographed using an Olympus Camera DP71 attached to an Olympus BX51 light microscope (Olympus, Center Valley, PA, USA) at 40× magnification. The oocysts were analyzed using Image Pro-Express 6.0 software (Media Cybernetics, Silver Springs, MD, USA). Fecal oocyst counts were transformed into the logarithm of OPG plus one [log (OPG + 1)] to obtain normal distribution. The average OPG, according to the category of animals and the season, as well as the diameters of oocysts and sporocysts and the morphometric index were compared by Scott-Knott test (SAEG, 9.1). The prevalence of *Eimeria* species was compared by the Chi-square test (EXCEL, 2007), while the frequencies on the oocystograms were compared by the Kruskal-Wallis test. All differences were considered significant at $P<0.05$.

Results

Eimeria species oocysts were found in 68.3% of the fecal samples, and parasites were verified in at least one of the animal categories on all of the properties. Furthermore, in 90% of the properties, all animal categories (young, adults, male, female) showed infection by *Eimeria*.

The animal category significantly influenced oocyst prevalence (P<0.05). During the dry season, young animals (males and females) showed values higher than adults (68.2% vs. 39.6%, respectively). During the rainy season, young females showed higher prevalence than adults (P<0.05). In the rainy season, sex also contributed to oocyst prevalence, with males showing higher values than females (P<0.05). Similarly, the season influenced the prevalence of oocysts, since it was higher during the rainy season than the dry season (P<0.05), with average values of 80.2% and 55.8%, respectively (Table 1).

In young animals in both seasons, the intensity of *Eimeria* infection (4,620.4 ± 1,029.6 OPG) was relatively high. The maximum OPG values for lambs (male and female) and adults (rams and ewes) were observed during the rainy season (17×10^4; 11×10^4; 9×10^3; and 4.4×10^3 OPG, respectively). The average infection observed in dry and rainy seasons (1,269 ± 312 vs. 4,400 ± 1,123 OPG, respectively) showed that the season had influence on the infection intensity (P<0.05).

The rate of animals that showed high infection intensity varied according to the season. During the dry season, 23.7% of the young and 1.0% of the adult animals had OPG≥1.10^3, and during the rainy season, the corresponding numbers rose to 48.2% and 12.4%, respectively. The animal category influenced the infection intensity. All the samples that showed OPG>1.10^4 (5.1%) were from young animals. Individuality also influenced the infection intensity since an expressive variation in the OPG was noted among individuals of the same category, especially among young animals, regardless of the weather season (Table 2).

Ten *Eimeria* species were identified in the samples of feces analyzed: *E. ovinoidalis*, *E. granulosa*, *E. faurei*, *E. crandallis*, *E. ahsata*, *E. parva*, *E. bakuensis*, *E. intricata*, *E. pallida*, and *E. punctata*.

The most prevalent five species (*E. ovinoidalis*, *E. granulosa*, *E. faurei*, *E. crandallis,* and *E. ahsata*) corresponded to 80.1% of the oocysts identified. *E. crandallis* and *E. ovinoidalis*, with higher pathogenic potential, represented 33.0% of the total oocysts identified, from which *E. ovinoidalis* was the most prevalent (19.5%) in both seasons (P<0.05) and was present in 100.0% of the studied properties. The prevalence of *E. ovinoidalis, E. ntricata*, and *E. bakuensis* was influenced by the season: the first two species were more prevalent during the dry season and *E. bakuensis* during the rainy season (P<0.05). Nevertheless, the prevalence of most of the species was not influenced by the season. *E. intricata*, *E. pallida,* and *E. punctata* were the least prevalent species, together totaling 3.4% of the oocysts identified during the two seasons (Table 3).

Discussion

The prevalence of *Eimeria* speciesoocysts is influenced by the husbandry system, showing a direct relationship between the technification level and infection intensity (CAI & BAI, 2009). Previous studies reported 92.7% prevalence of *Eimeria* sp. oocysts in an intensive system (SARATSIS et al., 2011), 78.3% in a semi-intensive system (HASSUM & MENEZES, 2005), and 25.3% and 58.9% in extensive system (AHID et al., 2009; BRITO et al., 2009). Limiting factors related to the environment (climate and management) or to the animal (genetic and immunologic status) may promote dissemination and increase

Table 1. Prevalence and number of *Eimeria* sp. oocysts in fecal samples of sheep raised in extensively extensively in the semiarid region.

Animal Category	Prevalence (%)*		Oopg (average ± EP)**	
	Dry Season	Rainy Season	Dry Season	Rainy Season
Rams	38.1^{Bb}	100^{Aa}	212.5 ± 44.0^{Bb}	$1{,}361.1 \pm 635.9^{Aa}$
Ewes	41.0^{Bb}	60.8^{Ca}	400.0 ± 102.9^{Bb}	695.8 ± 123.7^{Ba}
Male lambs	74.1^{Ab}	94.3^{Aa}	$1{,}981.3 \pm 694.1^{Ab}$	$7{,}040.9 \pm 2{,}673.1^{Aa}$
Female lambs	62.3^{Ab}	83.1^{Ba}	$2{,}493.0 \pm 725.6^{Ab}$	$5{,}386.4 \pm 1{,}992.4^{Aa}$

*Values followed by different letters, lowercase in the row and uppercase in the column, differ by Chi-square test (P<0.05). **Values followed by different letters, lowercase in the row and uppercase in the column, differ by the Scott-Knott test (P<0.05).

Table 2. Frequency of oocistograms with low (<1.10^3), medium (1.10^3-1.10^4) and high (>10^4) infection intensity in sheep raised extensively in the semiarid region.

Season	Category**	N	Oocistograms Frequency (%)		
			<1.10^3*	1.10^3-1.10^4*	>10^4*
Dry	Young	127	76.3^{Ac}	19.0^{Bb}	4.7^{Cb}
	Adult	99	99.0^{Aa}	1.0^{Bc}	0.0^{Cc}
Rainy	Young	141	51.8^{Ad}	36.2^{Ba}	12.0^{Ca}
	Adult	97	87.6^{Ab}	12.4^{Bb}	0.0^{Cc}
Total		464	76.1	16.4	7.5

*Number of eggs per gram of feces – Oopg. **Young = lambs (male and female) and adult = rams and ewes. Values followed by different uppercases in the row differ by Kruskal-Wallis test (P<0.05). Values followed by different lowercases in the column differ by Qui-square test (P<0.05).

Table 3. Oocysts and sporocysts diameters of *Eimeria* found in fecal samples of sheep raised extensively in the semiarid region.

Species	Oocysts diameter (µm)			Sporocysts diameter (µm)		
	Polar	Equatorial	MI[1]	Polar	Equatorial	IM[1]
With micropylar hood						
E. intricata	58.0±3.7[a]	41.1±3.3[a]	1.4±0.1[b]	21.5±2.4[a]	12.8±1.1[a]	1.7±0.2[a]
E. ahsata	40.4±2.9[b]	27.1±2.4[b]	1.5±0.1[a]	16.4±2.4[b]	9.9±1.1[b]	1.7±0.2[b]
E. bakuensis	33.9±2.7[c]	22.5±2.1[c]	1.5±0.1[a]	14.3±2.0[d]	8.5±1.1[e]	1.7±0.3[a]
E. granulosa	33.7±2.4[d]	24.7±2.7[c]	1.4±0.1[c]	15.0±3.1[c]	9.1±1.0[d]	1.6±0.3[b]
E. crandallis	27.3±1.5[f]	20.8±1.5[d]	1.3±0.1[d]	12.6±1,9[e]	8.0±0.9[g]	1.6±0.2[d]
E. punctata	21.9±1.2[j]	19.1±2.0[e]	1.1±0.1[f]	10.8±1.6[h]	6.8±0.9[i]	1.6±0.3[c]
Without micropylar hood						
E. faurei	32.5±2.9[e]	24.8±2.4[f]	1.3±0.1[d]	14.5±2.0[d]	9.3±1.1[c]	1.5±0.2[d]
E. ovinoidalis	25.6±1.6[g]	21.4±1.9[g]	1.2±0.2[e]	12.2±1.8[f]	8.1±1.3[f]	1.5±0.2[e]
E. pallida	18.2±1.1[h]	16.1±1.1[h]	1.1±0.1[h]	8.8±1.3[i]	6.1±0.8[j]	1.5±0.2[f]
E. parva	20.6±2.1[i]	18.2±2.1[i]	1.1±0.1[g]	10.1±.,7[g]	6.9±1.2[h]	1.5±0.2[f]

[1]MI: Morphometric Index = Ø polar/Ø equatorial. Values followed by different letters in the column differs by the Scott-Knott test (P<0.05).

the prevalence of coccidia even in extensive systems (CHARTIER & PARAUD, 2012).

The animal category was a significant factor influencing oocysts excretion, because young animals were more affected than adults, regardless of the season. The high susceptibility of young animals is related to immunological aspects once the species-specific immunity against *Eimeria* sp. occurs after the initial infection (SILVA et al., 2011).

Sex was also an factor influencing eimeriosis prevalence, especially in the rainy season. The ram's susceptibility to infection by *Eimeria* sp. can be attributed to immunosuppression caused by elevated plasma levels of androgens, mainly testosterone, throughout the breeding season (BHAT et al., 2012). During the rainy season, physical exhaustion from the intense reproductive activity, certainly contributed to the increased susceptibility of the male to eimeriosis.

Climate-related aspects, especially the moisture caused by rain in places where drainage is difficult, may influence the prevalence of *Eimeria* sp. A warm and moist environment provides ideal conditions for oocyst sporulation and thereby increases the potential for infection (TAYLOR, 2012). The effect of weather on oocyst sporulation can be potentiated by extreme variations in pluviometry and temperature that occur throughout the year (KHAN et al., 2011). This fact was observed in the area where the present study was carried out, where maximum and minimum monthly values of temperature and pluviometry during the dry and rainy season were 15-30 °C and 15-140 mm, respectively. Recognizing that an environment with high moisture and mild temperature leads to oocyst sporulation, promoting its higher elimination (TAYLOR, 2012), the rainy season in the semiarid region presents favorable environmental conditions for oocyst sporulation of *Eimeria,* suggesting the necessity of management targeted toward its control during this critical period of the infection.

The quantity of excreted oocysts can vary depending on the infecting dose of oocysts (GREGORY & CATCHIPOLE, 1987, 1990) and the animal's immune status (REEG et al., 2005). Therefore, individual influences play a role in the degree of infection. Oocystograms with values >10^4 indicate high infection intensity and are generally linked to typical diarrheal syndromes of this endoparasitosis (COZMA & TITILINCU, 2007). However, the diagnosis of eimeriosis cannot be excluded when the OPG is low or non-existent. Adult animals that excrete small amounts of oocysts are important in the epidemiology of eimeriosis; the oocysts released by these animals are usually the cause of infection in young animals (PLATZER et al., 2005).

Regarding the prevalence of *Eimeria* sp., there have been 15 species identified that parasitize sheep (SARATSIS et al., 2011). Previous studies suggest that species and their respective prevalences vary according to the region, probably due to the influence of climate (KHAN et al., 2011) and husbandry systems (CAI & BAI, 2009). In three studies carried out in the Rio Grande do Norte State of Brazil, eight species of *Eimeria* were identified, from which *E. bakuensis, E. ovinoidalis*, *E. parva,* and *E. faurei* were the most prevalent (AHID et al., 2009). In the Pernambuco State, *E. ahsata*, *E. crandallis*, *E. faurei,* and *E. intricata* (TEMBUE et al., 2009) and in the Rio Grande do Sul State, *E. parva, E. ashata, E. punctata,* and *E. granulosa* (SILVA et al., 2008) were the most prevalent species. The analysis of all this information shows that the survey of the species present in a determined region, especially the pathogenic ones, has great importance in facilitating our understanding of the epidemiology (LIMA, 2004) of eimeriosis and contributes to defining strategies for its control in a herd.

Among the ten species identified in the present study, six exhibited a micropylar cap (*E. intricata, E. ahsata, E. bakuensis, E. granulosa, E. crandallis*, and *E. punctata*). The presence or absence of a micropylar cap, the oocyst and sporocyst diameters, and the shape of the oocysts are reliable criteria to differentiate *Eimeria* species (HASSUM et al., 2007). Regarding *E. intricata*, the polar (>50 µm) and equatorial (>35 µm) diameter of the oocysts were decisive for the identification. The measurements of the major and minor diameter of the oocysts and sporocysts are sufficient to identify *E. intricata*, *E. ahsata,* and *E. pallida* (LEVINE et al., 1980). *E. ahsata* was differentiated from the other species with a micropylar cap by its ovoid format (HIDALGO-ARGÜELLO & CORDERO-DEL-CAMPILLO, 1988).

Differentiation of *E. ovinoidalis* from the other species without a micropylar cap was determined by the diameter and the form (spherical or ovoid) of the oocysts; identification of *E. crandallis*, *E. bakuensis,* and *E. granulosa* was performed by the form (elliptical or spherical) of the oocysts (DENIZ, 2009). The spherical form of the *E. parva* oocysts differs from the elliptical shape of the *E. pallida* (LEVINE et al., 1980). The MI was strategic to differentiate some species (HASSUM et al., 2002), in conjunction with the oocyst form; for example, in the differentiation between *E. bakuensis* and *E. granulosa* (Table 4), *E. granulosa* a presents pyriform shape and *E. bakuensis* shows an urn format, that is, elliptical with straight sides (LEVINE et al., 1980). Although the morphometric method has limitations in differentiating the species due to overlap of some parameters (BERRIATUA et al., 1995), the combined use of these elements increases the efficiency of this method and provides satisfactory reliability to identify the species of *Eimeria* that parasitize sheep.

Although species such as *E. intricata*, *E. pallida,* and *E. punctata* are not considered pathogenic to sheep (LE SUEUR et al., 2009), the high prevalence of *E. ovinoidalis* and *E. crandallis* identified in the present study suggest that eimeriosis is an emerging disease condition of sheep raised under extensive systems in the semiarid region. *E. ovinoidalis* is the species that presented higher pathogenic potential for sheep, since even in low quantities (1.10^3) it is able to cause damage to the small intestine, with epithelium loss and atrophy of microvilli membranes (GREGORY & CATCHIPOLE, 1987). Intense infection by this species causes disequilibrium of the intestinal microflora, allowing the proliferation of Gram-positive bacteria and worsening the scope of diarrhea (YAKHCHALI & GOLAMI, 2008).

The high prevalence of *E. ovinoidalis* in different regions and husbandry systems (GUL & DEGER, 2002; TOULAH, 2007; HASAN & ABED, 2012) may be attributed to its high reproduction potential when compared to the other species (REEG et al., 2005). *E. crandallis*, found in 95.0% of the evaluated properties, also presents considerable pathogenic potential; in high infection ($\geq 1.10^5$), it is capable of causing intestinal microvilli destruction (GREGORY & CATCHIPOLE, 1990) and compromises regeneration of the mucous membrane, which causes irreversible changes to the animal's development (TAYLOR et al., 2003). Thus, the high infection intensity in young animals during the rainy season, coupled with the high prevalence of pathogenic species, show that eimeriosis is a potential risk for sheep raised under extensive systems in semiarid region.

Table 4. Prevalence of *Eimeria* species oocysts in fecal samples of sheep raised extensively in the semiarid region.

Specie	Prevalence (%)	
	Dry season	Rainy Season
Eimeria ovinoidalis	21.3^{Aa}	17.6^{Ba}
Eimeria granulosa	16.9^{Ab}	16.7^{Aab}
Eimeria faurei	16.5^{Abc}	14.8^{Ab}
Eimeria crandallis	14.4^{Acd}	12.7^{Ac}
Eimeria ahsata	14.1^{Ad}	15.2^{Ab}
Eimeria parva	8.6^{Ae}	10.1^{Ad}
Eimeria bakuensis	4.1^{Bf}	10.2^{Ad}
Eimeria intricata	2.8^{Ag}	0.8^{Bef}
Eimeria pallida	1.1^{Ah}	1.4^{Ae}
Eimeria punctata	0.3^{Ai}	0.4^{Af}

Values followed by different, uppercase in the row and lowercase in column, differ by Chi-square test ($P < 0.05$).

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