**LUCIA OLIVEIRA DE MACEDO**

# EPIDEMIOLOGIA DA INFECÇÃO POR *Eimeria* spp. EM PEQUENOS RUMINANTES NA MICRORREGIÃO DE GARANHUNS, PERNAMBUCO, BRASIL

**GARANHUNS**

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**UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO**
**PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO**
**PROGRAMA DE PÓS-GRADUAÇÃO EM SANIDADE E REPRODUÇÃO DE RUMINANTES**

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Dissertação apresentada ao programa de Pós-Graduação em Sanidade e Reprodução de Ruminantes da Universidade Federal Rural de Pernambuco, como requisito parcial para obtenção do grau de Mestre em Sanidade e Reprodução de Ruminantes.

Orientador: Profª Drª Gílcia Aparecida de Carvalho

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Dissertação elaborada por

**LUCIA OLIVEIRA DE MACEDO**

Aprovada em: 27/02/2019

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# RESUMO

*Eimeria* spp. é um protozoário, que acomete ruminantes, equinos, suínos, lagomorfos e aves. Dentre os hospedeiros, os caprinos e ovinos podem ser infectados por diversas espécies de *Eimeria*, causando importantes perdas econômicas na produção, devido ao atraso do desenvolvimento e mortalidade de animais. Assim, o objetivo deste estudo foi analisar os aspectos epidemiológicos relacionados ao parasitismo por *Eimeria* spp. em pequenos ruminantes na microrregião de Garanhuns, Pernambuco, Brasil. Para tanto, amostras de fezes (n=822) foram coletadas da ampola retal de caprinos (n=414) e ovinos (n=408), estas amostras foram analisadas pelo método de flutuação e contagem de oocistos por grama de fezes (OoPG) em câmara de McMaster. Posteriormente foi realizada a cultura para a esporulação em dicromato de potássio a 2,5% e realizada análise morfométrica dos oocistos para identificação das espécies. A avaliação dos fatores de risco foi realizada por meio de análise univariada. De todas as amostras analisadas, 62,90% (517/822) foram positivas para oocistos de *Eimeria* spp., com prevalência geral de 77,79% (322/414) em caprinos e 47,79% (195/408) em ovinos. Oito espécies foram identificadas em caprinos (*E. arloingi*, *E. ninakohlyakimovae*, *E. alijevi*, *E. jolchijevi*, *E. caprina*, *E. chirstenseni*, *E. caprovina* e *E. hirci*) e oito em ovinos (*E. ovinoidalis*, *E. parva*, *E. crandallis*, *E. granulosa*, *E. bakuensis*, *E. ashata*, *E. faurei*, *E. pallida*). Para os caprinos, o tamanho do rebanho (OR=5,52), sistema de criação (OR=1,57), local de alimentação (OR=2,60), ausência de sal mineral na dieta (OR=2,54), piso de instalação (OR=2,83) e periodicidade de limpeza (OR=5,39) foram considerados fatores de risco. Por outro lado, em ovinos, apenas o tamanho do rebanho (OR=3,16) e o sistema de criação (OR=2,45) foram importantes fatores associados à infecção por este protozoário. Os dados aqui relatados são essenciais para entender melhor a dinâmica da infecção por esse coccídeo nesses rebanhos, bem como um alerta quanto à presença de espécies patogênicas (*E. arloingi* e *E. ninakohlyakimovae*) em caprinos e (*E. ovinoidalis* e *E. crandallis*) em ovinos. Nas propriedades estudadas foram observadas precárias condições higiênico sanitárias, principalmente nas criações de caprinos favorecendo a disseminação de espécies de *Eimeria.* Portanto, este estudo, inédito na região, ressalta a extrema importância em adotar medidas de manejo apropriadas para prevenir a infecção por essas espécies e reduzir o impacto econômico para produção de pequenos ruminantes.



**Palavras-chave:** Caprinos; Ovinos; *E. ninakohlyakimovae*; *E. ovinoidalis*; Epidemiologia.

# ABSTRACT

*Eimeria* spp. is a protozoan that affects ruminants, horses, swine, lagomorphs and birds. Among the hosts, goats and sheep can be infected by several species of *Eimeria*, representing a problem for production of small ruminants in terms of treatment costs, as well as unsatisfactory performance of herds due to delayed development and mortality of animals. Thus, the aim of this study was to analyze epidemiological aspects related to parasitism by *Eimeria* spp. in small ruminants in the Garanhuns microregion, Pernambuco, Brazil. Faecal samples (n = 822) were collected from the rectum of goats (n = 414) and sheep (n = 408), samples were individually processed using the technique described by Gordon and Whitlock. Subsequently, the sporulation culture was carried out in 2.5% potassium dichromate and morphometric analysis of the oocysts was carried out to identify the species. The evaluation of the risk factors was performed through univariate analysis. Of all the analyzed samples, 62.90% (517/822) were positive for *Eimeria* oocysts, with a general prevalence of 77.79% (322/414) in goats and 47.79% (195/408) in sheep. Eight species were detected in goats (*E. arloingi*, *E. ninakohlyakimovae*, *E. alijevi*, *E. jolchijevi*, *E. caprine*, *E. christenseni*, *E. caprovina* and *E. hirci*) and eight in sheep (*E. ovinoidalis*, *E. parva*, *E. crandallis*, *E. granulosa*, *E. bakuensis*, *E. ashata*, *E. faurei* and *E. pallida*). For goats, herd size (OR = 5.52), rearing system (OR = 1.57), feeding place (OR = 2.60), absence of mineral salt in the diet (OR = 2.54), installation floor (OR = 2.83) and periodicity of cleaning (OR = 5.39) were considered risk factors. On the other hand, in sheep, only herd size (OR = 3.16) and rearing system (OR = 2.45) were important factors associated with infection by this protozoan. The data reported here are essential for a better understanding of the dynamics of the coccidian infection in these herds, as well as an alert to veterinarians regarding the presence of pathogenic species (*E. arloingi* and *E. ninakohlyakimovae*) in goats and (*E. ovinoidalis* e *E. crandallis*) in sheep. In the studied rural properties, precarious hygienic and sanitary conditions were observed, mainly in the goat farms favoring the dissemination of *Eimeria* species. Therefore, it is extremely important to adopt appropriate sanitary measures to prevent infection by these species and to reduce the economic impact on the production of small ruminants.



**Keywords**: Goats; Sheep; *E. ninakohlyakimovae; E. ovinoidalis*; Epidemiology.

# LISTA DE FIGURAS

Página

# LISTA DE TABELAS

Página

# SUMÁRIO

Página

# 1 INTRODUÇÃO

A criação de pequenos ruminantes é uma atividade que tem importância econômica mundial, especialmente nos países em desenvolvimento. Sabe-se que além desta atividade desenvolver um papel socioeconômico e cultural, as carnes caprinas e ovinas são utilizadas como fonte de proteína animal para boa parte da população, como também representa uma atividade de subsistência (Diniz et al., 2014; Monteiro et al., 2016).

O efetivo de ovinos e caprinos no Brasil é de 18.410.551 milhões e 9.614.722 milhões de cabeças respectivamente, sendo a Região Nordeste responsável por 92,7% do rebanho caprino, enquanto o rebanho ovino 60,5%, ambos encontram-se bastantes concentrados na região semiárida, demonstrando a grande importância da caprinovinocultura, seja para produção de carne ou de leite (IBGE, 2015). Também é expressiva a criação de caprinos e ovinos na microrregião de Garanhuns, Pernambuco, onde esta atividade apresenta importância econômica, entretanto, muitas vezes o desempenho dos rebanhos é comprometido devido a enfermidades (Diniz et al., 2014).

Dentre os problemas enfrentados pelos produtores de pequenos ruminantes está a ocorrência de parasitos gastrintestinais, os quais representam um dos maiores e mais graves problemas sanitários dos pequenos ruminantes, chegando a inviabilizar economicamente a criação (Keeton e Navarre, 2018). Neste contexto, as parasitoses gastrintestinais assumem relevância, considerando-se as elevadas perdas econômicas decorrentes da baixa produtividade dos animais e do atraso no desenvolvimento corporal dos jovens, reduzindo a lucratividade dos rebanhos (Chagas et al., 2005; Alencar et al., 2010).

As protozooses constituem um obstáculo para produção de caprinos e ovinos particularmente, as causadas por *Eimeria* spp. que levam à perdas econômicas decorrentes da mortalidade de animais jovens, queda na produtividade, como também ao baixo desempenho dos animais que se recuperam da enfermidade, necessitando de tempo adicional para atingir o peso daqueles não infectados da mesma idade e mantidos na mesma condição de manejo (Vieira, 2005; Maciel et al., 2006; Vieira e Chagas, 2009). Estima-se que as perdas econômicas cheguem aos 341 milhões de dólares por ano nos Estados Unidos e geralmente deve-se às infecções subclínicas, resultando em baixo desempenho produtivo (Grilo e Carvalho, 2014).

Os caprinos e ovinos podem ser infectados por várias espécies de *Eimeria*, entretanto poucas são consideradas patogênicas. Os caprinos são acometidos por espécies de maior patogenicidade, onde *Eimeria ninakohlyakimovae, Eimeria arloingi* e *Eimeria christenseni* que são responsáveis por óbitos em rebanhos caprinos, entretanto as de maior patogenicidade são *Eimeria ninakohlyakimovae* e *Eimeria arloingi* (Cavalcante et al., 2012; Chartier e Paraud, 2012, Macedo et al., 2019). Entre as diferentes espécies capazes de infectar ovinos, *Eimeria ovinoidalis* e *Eimeria crandallis* causam sintomatologia clínica (Gregory e Catchpole, 1990; Macedo et al., 2019).

Tendo em vista a importância da infecção por *Eimeria* spp. em pequenos ruminantes, bem como, o impacto econômico que representa e a escassez de informações epidemiológicas na região estudada, objetiva-se com este estudo, estudar os aspectos epidemiológicos relacionados ao parasitismo por *Eimeria* spp. em caprinos e ovinos na Microrregião de Garanhuns, Pernambuco, Brasil.

# 2 OBJETIVOS

## 2.1 Geral

Estudar os aspectos epidemiológicos relacionados ao parasitismo por *Eimeria* spp. em pequenos ruminantes na Microrregião de Garanhuns, Pernambuco, Brasil.

## 2.2 Específicos

Determinar a prevalência da infecção por *Eimeria* spp. em caprinos e ovinos na Microrregião de Garanhuns, Pernambuco;

Identificar as espécies do gênero *Eimeria* que acometem rebanhos caprinos e ovinos na Microrregião de Garanhuns, Pernambuco;

Identificar possíveis fatores de risco associados à infecção por *Eimeria* spp. em caprinos e ovinos.

# 3 REVISÃO DE LITERATURA

## 3.1 Caprinovinocultura no Nordeste

A Caprinovinocultura é uma atividade praticada por séculos, a capacidade de adaptação dos animais as condições da região semiárida no Brasil, tem contribuído para que esta atividade desempenhe grande importância para desenvolvimento econômico e social do Nordeste. Assim como, representa uma função de caráter familiar, sendo fundamental na geração de renda, voltada para pequenos produtores (Guilherme et al., 2017; Pimentel Neto et al., 2018; Ribeiro e Alencar, 2018).

A sanidade dos rebanhos representa um fator fundamental para produtividade. Entretanto, falhas no manejo sanitário comprometem o desempenho de caprinos e ovinos. Entre os principais entraves para esta atividade estão as enfermidades parasitárias, com destaque para infecções causadas por *Eimeria* spp., que acomete principalmente animais jovens e caracteriza-se por causar lesões intestinais, redução no desenvolvimento corporal e mortalidade de animais (Souza et al.,2015; Fthenakis e Papadopoulos, 2018).

## 3.2 Agente etiológico e taxonomia

*Eimeria* spp. é parasito intracelular do epitélio intestinal (Smith e Sherman, 2009) sendo responsável pela enfermidade parasitária denominada eimeriose. A posição taxonômica de *Eimeria* spp. de acordo com Taylor et al. (2010) é:

Reino Protista
Subreino Protozoa
Filo Apicomplexa
Classe Conoidasida
Ordem Eucoccidiorida
Família Eimeriidae
Gênero *Eimeria*

## 3.3 Morfologia de *Eimeria* spp.

Os oocistos desse gênero possuem características morfológicas peculiares, tais como a forma, cor, aspecto da parede do oocisto e presença de elementos estruturais, tais como capuz polar e micrópila. O oocisto não esporulado é composto por uma massa

embrionária e parede com duas camadas. Já o oocisto esporulado apresenta quatro esporocistos com dois esporozoítos cada, perfazendo um total de oito esporozoítos por oocisto esporulado (Figura 1). De acordo com o ciclo biológico desenvolve para formas distintas, tais como trofozoíto quando o esporozoíto entra na célula epitelial do hospedeiro e perde o complexo apical, seguido de formação do esquizonte, estrutura que produzirá os merozoítos (Eckert et al., 1995; Taylor et al., 2010; Chartier e Paraud, 2012).

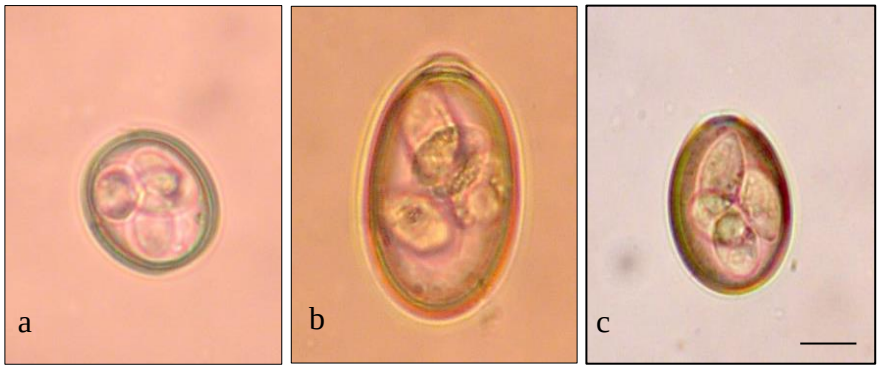


**Figura 1.** Oocistos esporulado de *E. parva* (a), *E. ashata* (b), *E. caprovina* (c). (Fonte: Macedo, 2019)

### 3.4 Hospedeiros

Acomete bovinos, bubalinos, pequenos ruminantes, equinos, suínos, lagomorfos e aves. Dentre os hospedeiros, caprinos e ovinos podem ser infectados por várias espécies de *Eimeria*, entretanto poucas são consideradas patogênicas (Taylor et al., 2010; Vieira e Berne, 2007).

#### 3.4.1 Ovinos

Os prejuízos causados pela eimeriose em ovinos são bastante significativos principalmente devido a diminuição do desempenho e desenvolvimento dos animais acometidos, podendo ainda levá-los à morte (Carrau et al., 2018). Foram identificadas onze espécies de *Eimeria* em ovinos, distinguidas pela morfologia dos oocistos, tais como ausência de capuz polar: *E. pallida*, *E. parva*; presença de capuz polar: *E. ahsata*, *E. bakuensis*, *E. crandallis*, *E. faurei*, *E. granulosa*, *E. intricata*, *E. marsica*, *E. ovinoidalis*, e *E. weybridgensis* (Eckert et al., 1995).

Cada estágio de desenvolvimento do protozoário tem suas preferências conforme as células e as partes do intestino que infectam. Aquelas que infectam a parte posterior do

intestino tendem a ser mais nocivas (Taylor et al., 2010). Entre as diferentes espécies capazes de infectar ovinos, *E. ovinoidalis* e *E. crandallis* causam sintomatologia clínica (Gregory e Catchipole, 1990).

### 3.4.2 Caprinos

Dentre as espécies de *Eimeria*, que parasitam caprinos, as mais citadas em estudos epidemiológicos são nove, onde cada uma apresenta características particulares, tais como ausência de capuz polar *E. ninakohlyakimovae* e *E. alijevi*, presença de capuz polar *E. arloingi, E. jolchijevi, E. chistenseni, E. hirci,* ausência de capuz polar e presença de micrópila *E. caprina, E. aspheronica,* e *E. caprovina* (Eckert et al., 1995).

Entretanto, em estudos pontuais, são descritas outras espécies, porém não apresentam informações quanto a sua epidemiologia: *E. capralis, E. masseyensis, E. charlestoni,* na Nova Zelândia (Soe e Pomroy, 1992); *E. minasensis*, no Brasil (Silva e Lima, 1998).

Em caprinos, assim como em ovinos, existem espécies de maior patogenicidade, entre as quais, *E. ninakohlyakimovae, E. arloingi* e *E. christenseni* são responsáveis por óbitos em rebanhos caprinos (Vieira, 1996). Destacando-se as de maior patogenicidade *E. ninakohlyakimovae* e *E. arloingi* (Cavalcante et al., 2012; Chartier and Paraud, 2012).

## 3.5 Ciclo biológico e transmissão

O ciclo biológico das espécies de *Eimeria* é autoxeno, completando seu ciclo em um único hospedeiro (Figura 2). Inclui uma fase exógena de maturação do oocisto (esporogonia), que ocorre fora do hospedeiro e uma fase endógena com infecção e merogonia (esquizogonia), seguido de uma multiplicação sexual (gametogonia) e formação do oocisto (Soulsby, 1982; Taylor et al., 2010). O potencial proliferativo no hospedeiro é muito alto uma vez que, de acordo com um cálculo teórico, cada oocisto ingerido poderia dar origem de 30 milhões de oocistos liberados em material fecal (Gregory e Catchpole, 1987).

Os hospedeiros infectados eliminam os oocistos não-esporulados, consistindo em uma massa nucleada de protoplasma circundado por uma parede resistente. Em condições adequadas de oxigenação, umidade elevada e temperaturas ideais ao redor de 27°C, o núcleo divide-se em duas massas protoplasmática forma quatro corpos cônicos irradiando-se da massa central (Chartier e Paraud, 2012). Cada um desses cones nucleados torna-se

arredondado para formar o esporoblasto, que secreta uma parede de material refratário e se torna conhecido como esporocisto. O tempo de esporulação altera de acordo com a espécie e condição ambiental. O oocisto esporulado, possui quatro esporocistos, cada um deles envolvendo dois esporozoítos, sendo o estágio infectante (Taylor et al., 2010). A transmissão ocorre de modo fecal-oral, quando oocistos esporulados são ingeridos juntamente com alimentos ou água contaminados (Lima, 2004).

Uma vez ingerido pelo hospedeiro, o oocisto esporulado passa por um processo excisão. Os esporozoítos são ativados pela tripsina e pela bile e deixam o esporocisto, penetram na célula epitelial e passa ser conhecido como trofozoíto. Passa então a dividir-se por divisão binária para formar o meronte (esquizonte), estrutura que contém os merozoítos. Após várias divisões, a célula do hospedeiro se rompe e os merozoítos invadem outras células (Foreyt, 1990; Taylor et al., 2010).

O número de gerações do meronte depende da espécie envolvida e termina quando os merozoítos originam os gametócitos masculinos e femininos. Entretanto os fatores que desencadeiam estas mudanças para gametogonia são desconhecidos. Os macrogametócitos (feminino) permanecem unicelulares, porém aumentam de tamanho para preencher a célula parasitada. Os microgametócitos (masculinos) sofrem divisão repetida para formar um grande número de microgametas flagelados. Os microgametas são liberados por ruptura da célula do hospedeiro penetra no macrogameta, e ocorre fusão dos núcleos do micro e do macrogameta, formando o oocisto que é eliminado juntamente com as fezes para o ambiente (Levine, 1970; Lima, 2004; Daugschies Najdrowski, 2005).

Para a espécie mais importante em caprinos, *E. ninakohlyakimovae* a primeira merogonia ocorre no íleo (10 dias pós infecção), a segunda nas células da cripta do ceco e do colón (12 dias pós infecção) e eventualmente a gametogonia ocorre no intestino grosso (13 dias pós infecção), com um período pré-patente de 15 dias (Vieira, 1997). Desenvolvimento semelhante é descrito em ovelhas com *E. ovinoidalis* (Gregory e Catchpole, 1987).

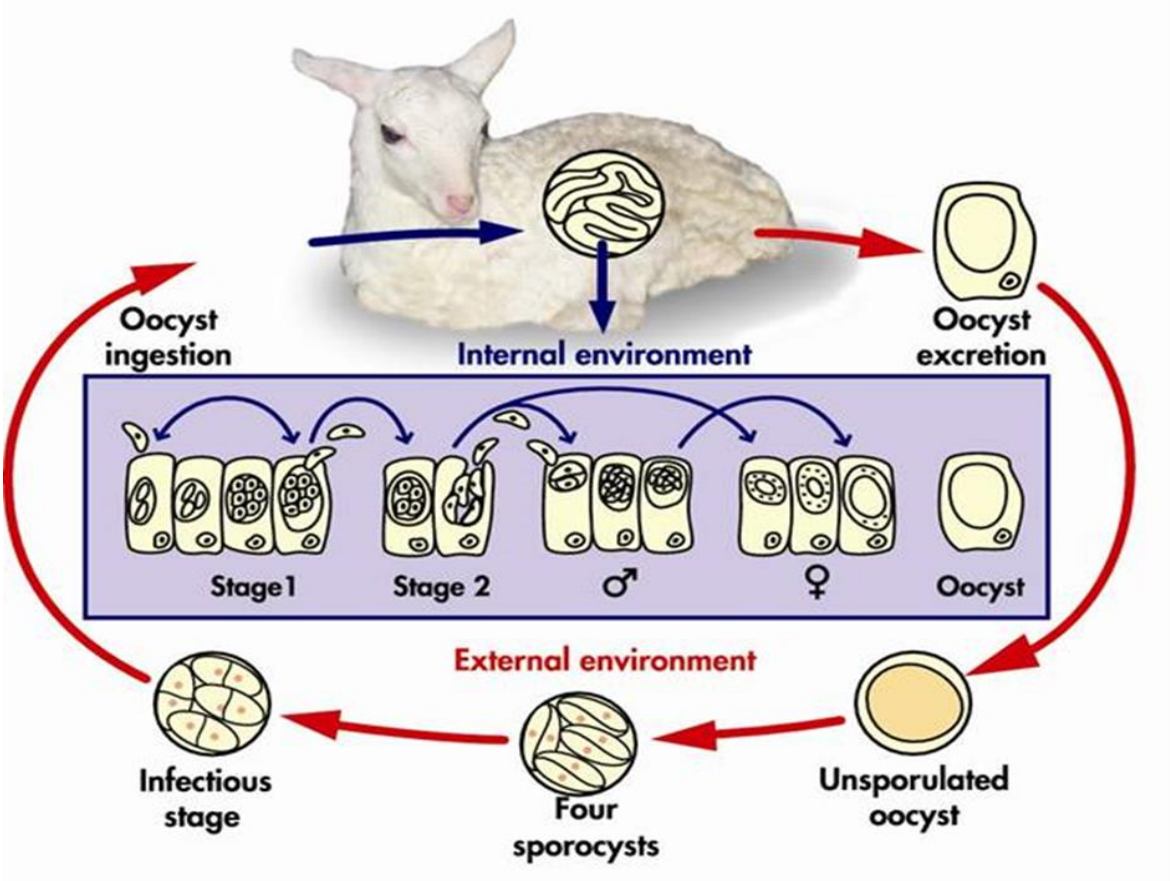


**Figura 2.** Ciclo biológico das espécies de *Eimeria*. (Fonte: Bayer, 2012)

### 3.6 Epidemiologia

A epidemiologia da eimeriose relaciona-se com diversos fatores ligados ao parasito, ao hospedeiro e ao ambiente (Rehman et al., 2011). As espécies de *Eimeria* acometem principalmente animais jovens, levando a queda na produtividade, como também baixo desempenho dos animais que se recuperam da enfermidade, necessitando de tempo adicional para atingir o peso daqueles não infectados da mesma idade e mantidos na mesma condição de manejo (Vieira e Chagas, 2009; Andrade Júnior et al., 2012).

A idade do hospedeiro é um fator importante para *Eimeria*, caprinos e ovinos infectam-se muito jovens, apresentando oocistos nas fezes a partir da segunda semana de vida (Saratsis et al., 2011; Khan et al., 2011). Os animais adultos são fontes de infecção, devido ao fato da frequente eliminação de baixo número de oocisto nas fezes por longos períodos (Foreyt, 1990). Ovelhas e cabras no pós-parto eliminam grandes quantidades de oocistos, onde fezes amolecidas e frequentemente diarreicas das pós-parturientes facilitam a disseminação dos oocistos no ambiente, bem como a contaminação fecal do úbere (Silva et al., 2007). Assim como locais de fornecimento de alimentação, condições dos apriscos,

tipos de piso da instalações e tamanho do rebanho são fatores que influenciam para ocorrência da infecção pelo gênero *Eimeria* (Khan et al., 2011).

A infecção causada por *Eimeria* spp. depende das condições ambientais, da resposta imunológica do animal e da virulência das espécies do parasito. Desta forma, é importante o conhecimento dos fatores predisponentes para infecções por este coccídeo, bem como a identificação das espécies de *Eimeria* para avaliar o potencial da infecção (Souza et al., 2015). Eventos estressantes como desmame, prenhez, parto, transporte, mudanças na alimentação, alterações climáticas (temperatura e umidade) e lotações com alta densidade, facilitam o desenvolvimento da enfermidade (Chartier e Paraud, 2012; Saratsis et al., 2011).

A eimeriose ocorre com maior frequência em animais mantidos em criação intensiva, quando comparados àqueles sob criação extensiva, devido a concentração dos animais no mesmo ambiente (Cai e Bai, 2009; Tomczuk et al., 2015).

Nas infecções em pequenos ruminantes, embora diversas espécies de *Eimeria* possam estar envolvidas, não ocorre infecção cruzada por este protozoário de caprinos para ovinos ou vice-versa, devido ao fato de serem espécies com alta especificidade pelos hospedeiros (Bakunzi et al., 2010; Taylor et al., 2010; Chartier e Paraud, 2012; Andrews, 2013). Contudo, outras espécies, como por exemplo a *Eimeria* (*Globidium*) *gilruthi*, é responsável por infecções incidentais dos abomasos de ovinos e caprinos (Maratea e Miller, 2007).

### 3.7 Distribuição geográfica

*Eimeria* spp. apresenta grande importância econômica em pequenos ruminantes devido à sua alta ocorrência e às perdas ocasionadas em diversas partes do mundo (Bakunzi et al., 2010). Várias espécies já foram reportadas na maioria dos continentes: Africano (Vercruysse, 1982; Chhabra et al., 1991; Matjila e Penzhorn, 2002; Mohamaden et al., 2018), Americano (Penzhon et al., 1994, Tembue et al., 2009; Macedo et al., 2019), Asiático (Abo-Shehada e Abo-Farieha, 2003; Jalila et al., 1998; Mat Yusof e Md Isa, 2016), Europeu (Koudela e Boková, 1998; Von Samson-Himmelstjerna et al., 2006).

Estudos no Brasil com a identificação de espécies foram realizados em diversos estados brasileiros, conforme a Tabela 1.

Tabela 1. Identificação de espécies de *Eimeria* em caprinos e ovinos no Brasil.

| Autor | Estado | N Amostral | Hospedeiros | N Espécies identificadas |
|---|---|---|---|---|
| Cardoso e Oliveira (1993) | RS | 53 | Caprinos | 11 |
| Barbosa et al., (2003) | RN | 478 | Caprinos | 9 |
| Silva et al., (2007) | MG | 30 | Ovinos | 11 |
| Ahid et al., (2009) | RN | 357 | Caprinos | 9 |
| | | 209 | Ovinos | 8 |
| Ramirez et al., (2009) | RJ | 370 | Caprinos | 8 |
| Tembue et al., (2009) | PE | 319 | Caprinos | 8 |
| | | 81 | Ovinos | 7 |
| Silva et al., (2011) | RN | 191 | Ovinos | 8 |
| Cavalcante et al., (2012) | CE | 215 | Caprinos | 8 |
| Coelho et al., (2012) | MG | 202 | Caprinos | 8 |
| Lopes et al., (2013) | PR | 210 | Ovinos | 9 |
| Brinker et al., (2014) | RS | 120 | Ovinos | 3 |
| Souza et al., (2015) | BA | 464 | Ovinos | 10 |
| Macedo et al., (2019) | PE | 414 | Caprinos | 8 |
| | | 408 | Ovinos | 8 |

### 3.8 Patogenia

O epitélio do intestino apresenta microvilosidades, que são estruturas responsáveis pela absorção e excreção com efeito direto na digestão e aproveitamento de nutrientes (Samuelson, 2007). A patogenia da eimeriose envolve a destruição das microvilosidades intestinais, devido à multiplicação dos estágios do coccídeo (Foreyt, 1990). Os esporozoítos invadem as células devido a sua capacidade de ligação com receptores da membrana plasmática do epitélio intestinal (Augustine, 2001). No interior das células inicia-se a multiplicação, que origina os esquizontes, provocando uma resposta imunológica com infiltração leucocitária, desencadeando hiperplasia da cripta e perdas epiteliais, com atrofia das vilosidades (Gregory e Cacthpole, 1987).

Como consequência da atrofia das vilosidades, ocorre a diminuição de absorção de lipídeos, proteínas, vitaminas e outros nutrientes. As células alteradas são eliminadas e aparecem áreas de micro ulcerações. Além disso, as células restantes possuem menor

capacidade de absorção, por não se encontrarem completamente diferenciadas, resultando na síndrome da má absorção. As alterações funcionais e intensidade das lesões teciduais dependem da espécie envolvida, bem como da quantidade ingerida de oocistos esporulados. Quando a infeção é intensa, ocorre destruição de áreas do intestino, levando ao desprendimento da mucosa intestinal, ocasionando enterite hemorrágica (Lima, 2004).

Macroscopicamente as lesões mais notáveis são mudanças na coloração da mucosa intestinal de branca para cinza, identificação de nódulos e espessamento multifocal principalmente na região afetada no jejuno, íleo (Hashemnia et al., 2015). Microscopicamente a principal lesão histopatológica em animais afetados é enterite proliferativa associada à presença do desenvolvimento dos estágios do coccídeo, incluindo esquizontes maduros, microgametócitos, macrogametócitos e oocistos imaturos nas células epiteliais e criptas do jejuno, íleo e ceco (Khodakaram-Tafti e Mansourian 2008; Hashemnia et al., 2012).

### 3.9 Achados clínicos

A infecção subclínica é a forma mais comum da enfermidade, o que acarreta impacto significativo na saúde e produção do rebanho (Silva et al., 2011; Grilo e Carvalho, 2014). Um dos primeiros sinais de que a doença se disseminou no rebanho é o atraso no desenvolvimento em relação ao esperado, principalmente nos animais mais jovens (Andrade Júnior et al., 2012).

Entre os sinais clínicos de animais infectados por *Eimeria* spp., estão: febre, depressão, perda de apetite, diarreia aquosa, algumas vezes presença de muco ou sangue, desidratação progressiva, emagrecimento, palidez de mucosas, podendo evoluir a óbito (Daí et al., 2006).

Em caprinos a prevalência e intensidade de eliminação de oocistos nas fezes são mais elevadas em animais jovens entre 4-6 meses de idade, o principal sinal é a diarreia, onde as fezes são aquosas com aglomerados de muco, podendo ser hemorrágica em ovinos. Há perda de peso e desidratação, a condição geral do animal é agravada devido a diminuição do apetite, em algumas vezes a eimeriose pode ser caracterizada por mortalidade súbita sem precedentes de sinais digestivos, em particular em animais jovens entre 2 e 4 meses de idade (Chartier e Paraud, 2012).

### 3.10 Diagnóstico

O diagnóstico de *Eimeria* spp. em pequenos ruminantes é baseado na anamnese, levando em consideração questões sobre o manejo e sistema de criação, aspectos clínicos, exame coproparasitológico, lesões macroscópicas na necropsia e presença de formas endógenas do parasito nos tecidos afetados (Andrade Júnior et al., 2012). Devido a possibilidade de haver animais infectados com coronavírus, rotavírus, *Cryptosporidium* spp., *Clostridium perfringens* tipo C e *Salmonela* que podem apresentar sinais semelhantes aos da infecção por *Eimeria* spp., é essencial realizar o diagnóstico para a diferenciação das espécies patogênicas e não patogênicas (Bangoura et al., 2012).

O exame coproparasitológico deve ser realizado por meio de técnicas de flutuação em soluções saturadas de sacarose ou de sais, seguida da identificação das espécies. Frequentemente o método mais utilizado é a técnica de flutuação e contagem de oocistos por grama de fezes (OoPG) em câmara de McMaster (Gordon; Whitlock 1939) e posteriormente cultura em dicromato de potássio a 2,5%.

Atualmente a técnica de Mini-FLOTAC, mostra-se como uma alternativa promissora na detecção e contagem de oocistos de *Eimeria* spp., assim como a técnica de FLOTAC, particularmente usada em laboratórios especializados apresentando um papel importante para fins de pesquisas (Cringoli et al., 2010; Silva et al., 2013).

Em estudos epidemiológicos a morfometria tem sido utilizada para identificação das espécies de *Eimeria,* avaliando os oocistos esporulados quanto as características morfológicas, dimensões e hospedeiro infectado (Levine e Ivens, 1970; Hassum et al., 2002; Taylor, 2002; Ahid et al., 2009). Os critérios confiáveis para a identificação e diferenciação das espécies são a forma e a presença de elementos estruturais, tais como capuz polar, micrópila, cor, aspecto da parede do oocisto, resíduos de oocistos e de esporocistos (Hassum et al., 2007; Chartier e Paraud, 2012). Cada espécie tem suas peculiaridades, como por exemplo, a forma esférica em *E. ninakohlyakimovae* e *E. pallida*, ovoide em *E. faurei*, quanto a coloração amarelada em *E. ashata*, castanha em *E. intricata* ou incolor em *E. crandallis* e *E. parva* (Taylor et al., 2010). Testes sorológicos como ELISA e Western Blot, bem como, testes moleculares por meio da reação em cadeia da polimerase (PCR) também podem ser utilizados (Daugschies, Najdrowski, 2005). Apesar da disponibilidade de identificação através de métodos moleculares, requer informações

específicas e proteínas e aminoácidos, além de custos elevados e equipamentos especializados (Barkway et al., 2011).

### 3.11 Profilaxia

*Eimeria* spp. em pequenos ruminantes pode ser controlada através do tratamento dos animais com sinais clínicos, uso preventivo de drogas anticoccídicas incluindo decoquionato e ionóforos (como monensina) e práticas de manejo (Lima, 2004; Saratsis et al., 2011). O conhecimento detalhado dos aspectos inerentes ao curso da infecção causada por *Eimeria* spp. é importante para definição das medidas apropriadas, uma vez que apenas a administração de medicamentos, na maioria das vezes, não representa êxito no controle do protozoário (Young et al., 2011; Chartier e Paraud, 2012).

Na profilaxia devem ser consideradas práticas sanitárias que promovam diminuição da contaminação ambiental como higienização criteriosa das instalações por remoção do material fecal, manter os animais em ambientes limpos, secos, alimentação adequada. Assim como, adequação das instalações para redução do estresse, evitar superlotação, animais separados de acordo com a faixa etária, elevação dos cochos e bebedouros, por exemplo, ajudam na redução da contaminação alimentar e hídrica com oocitos (Saratsis et al., 2011; Macedo et al., 2019).

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# 5 ARTIGOS CIENTÍFICOS

## 5.1 Artigo 1

## MORPHOLOGICAL AND EPIDEMIOLOGICAL DATA ON *Eimeria* SPECIES INFECTING SMALL RUMINANTS IN BRAZIL

**(Artigo publicado na revista Small Ruminant Research)**

**(Anexo 1)**

# Morphological and epidemiological data on *Eimeria* species infecting small ruminants in Brazil


## Abstract

Parasites of the genus *Eimeria* induce relevant economic losses in small ruminants worldwide. The aim of this study was to investigate the epidemiology of goats and sheep coccidiosis in a semi-arid region of North-eastern Brazil and to assess whether the rearing system represents a risk factor for the distribution of the infection. A total of 822 fresh faecal samples were collected from the rectum of goats (n = 414) and sheep (n = 408) All samples were individually processed using the technique described by Gordon and Whitlock, and after sporulation in 2.5% Potassium dichromate ($K_2Cr_2O_7$), an accurate morphometric analysis was performed, with oocysts morphologically identified at species level. The analysis of the risk factors associated with *Eimeria* infection and system of rearing was performed through univariate analysis and logistic regression. Out of all animals sampled, 70.07% (576/822) scored positive for coccidia oocysts, with an overall prevalence of the infection of 73.91% (306/414) in goats and 66.18% (270/408) in sheep ($\chi^2 = 5.502$; p = 0.0190). Eight *Eimeria* species were identified either in goats (i.e., *E. arloingi*, *E. ninakohlyakimovae*, *E. alijevi*, *E. jolchijevi*, *E. caprina*, *E. chirstenseni*, *E. caprovina* and *E. hirci*) and in sheep (i.e., *E. ovinoidalis*, *E. parva*, *E. crandallis*, *E. granulosa*, *E. bakuensis*, *E. ashata*, *E. faurei*, *E. pallida*). The analysis of risk factors revealed that the semi-intensive system (OR = 1.57) was a risk factor for goats, and, incontrast, intensive system for sheep (OR = 2.45) was safer, relative to *Eimeria* species transmission. Data reported in this study indicate that a wide species diversity and frequency of coccidia affect small ruminants in the study area. Finally, these findings are pivotal to better understand the dynamics of infection by this coccidian in these herds, as well as sound as an alert for practice veterinarians of the region, regarding the most common pathogenic species.

**Keywords:** protozoa; *Eimeria*; coccidiosis; morphology; goats; sheep.

## 1. Introduction

Parasites of the genus *Eimeria* (Apicomplexa: Eimeriidae) are accounted as an important cause of intestinal infection in animals worldwide (Ahmad et al., 2016). These protozoa are responsible for the so-called "coccidiosis", which represent a very important disease in vertebrate animals, including small ruminants (Mohamaden et al., 2018). Amongst these animals, goats and sheep are commonly infected by *Eimeria* species causing relevant economic losses, either in terms of direct costs and productivity, directly affecting the animal welfare (Silva et al., 2014).

*Eimeria* parasites usually affect young animals of up to 4 months of age, but are also diagnosed in adult animals, acting as reservoirs of the infection (Lopes et al., 2013). Coccidiosis can induce a wide plethora of clinical signs, such as weight loss, fever, diarrhea, dehydration, pale mucous, and even death (Andrade Júnior et al., 2012; Keeton and Navarre, 2017). The prepatent period of coccidiosis in small ruminants depends upon the species involved, with infected animals shedding unsporulated oocysts in about 7 days. Oocysts sporulate in the environment, becoming infective in a very short time span depending on the species. Once the sporogony is completed in the environment, susceptible hosts ingest oocysts while grazing or drinking contaminated water (Chartier and Paraud, 2012).

The diagnosis of coccidiosis in small ruminants is usually achieved *via* copromicroscopic methods, with oocysts detected during routine analyses. However, the differentiation of these parasites at species level still represents a challenge since requires an accurate morphometrical analysis performed by skilled and trained laboratory technicians (Al-Habsi et al., 2017). To date, 17 *Eimeria* species have been

reported in goats (Silva and Lima, 1998), with nine of major epidemiological and veterinary importance. These species are differentiated according to their morphology and size, with the presence/absence of the polar cap being of diagnostic relevance for the identification of *E. arloingi, E. jolchijevi, E. chistenseni* and *E. hirci*. Similarly, the presence of the micropile allows detecting *E. caprina, E. apsheronica* and *E. caprovina* (Eckert et al., 1995). In regard to sheep, 15 *Eimeria* species have been described (Saratsis et al., 2011).

A proper identification of *Eimeria* spp. is fundamental to estimate the importance of any anticoccidicidal treatments as only selected species show a marked pathogenic role in goats (e.g., *E. arloingi* and *E. ninakohlyakimovae*) (Cavalcante et al., 2012; Chartier and Paraud, 2012), or in sheep (e.g., *E. ovinoidalis* and *E. crandallis*). Nonetheless, this assessment has not fully clarified, since does not take into account the interaction between the parasite development, concomitant diseases and/or the animal nutrition. Accordingly, epidemiological studies on small ruminant coccidiosis are lacking, with no information reported from regions where the farming system of goats and sheep is progressively expanding. This is the example of Brazil, where only a few data are published (Barbosa et al., 2003; Silva et al., 2011). Therefore, the aim of this study was to investigate the diversity of coccidia infecting goats and sheep in a semi-arid region of North-eastern Brazil and to assess whether the system of rearing was a risk factor.

## 2. Material and methods

### 2.1. Study area and ethical aspects

A transversal study was performed in 36 small ruminants farms located in Garanhuns (8°53′25″S and 36°29′34″W), state of Pernambuco, North-eastern Brazil.

Animals enrolled in this study originated from three different production systems represented by intensive (n = 11), semi-intensive (n = 21) and extensive (n = 4) rearing systems. Animals in the intensive system (n = 263) were confined in wood pens during the whole day. Conversely, those in the semi-intensive system (n = 468) were partially confined at night and maintained at grazing during the day. Finally, livestock in the extensive system (n = 91) were maintained exclusively grazing. Independently from the rearing system, the pasture area was cultivated with *Cynodon dactylon* (Tifton grass). In addition, in the intensive and semi-intensive system, the diet of animals was supplemented with mineral salt. The consortium of animals was not observed in this study.

The area of Garanhuns is featured by a semi-arid climate with an annual average of temperature of 22°C (from 17°C to de 30°C), rainfall mean of 147 mm (from 25 mm to 295mm), and air relative humidity of 90%.

The Ethics Committee for Animal Experimentation (ECAE) of *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (license number: 06/2017).

**2.2. Animals, sampling and laboratorial procedures**

The minimum sample size (n = 384) was estimated based on the goat population (n = 35770) and the sheep population (n = 99606) of the study area (IBGE, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004).

From March 2017 to May 2018, fresh faecal samples (n = 822) were collected from the rectum of goats (n = 414) and sheep (n = 408) using plastic gloves. The history of each animal was recorded in a clinical chart (Diffay et al., 2005). Of all goats enrolled, 144 were young (≤ 12 months old) and 270 adult animals (> 12 months old),

whereas among sheep 172 were young and 236 adult animals. After collection, samples were stored in isothermal boxes (4° C) until laboratory processing.

Each sample was individually processed by the technique of counting eggs/oocysts of Gordon and Whitlock (1939). Afterwards, in order to allow the sporulation of oocysts all positive samples were stored in Petri dishes containing 2.5% Potassium dichromate ($K_2Cr_2O_7$) and maintained at 26ºC for seven days. Finally, the material was transferred into plastic tubes, centrifuged at 200 g for 15 minutes and the supernatant analyzed at different magnifications (10x and 40x) (Menezes and Lopes, 1995). A total of 10 oocysts per sample were measured using the AxioVision software (release 4.8). The identification of oocysts was based on morphometrical features previously described (Eckert et al., 1995).

**2.3. Statistical analysis**

Descriptive statistical analysis was performed to obtain relative and absolute frequency. In addition, the Lilliefors test was used to verify the normality of the data. The Chi-square test with Yates correction ($\chi^2$) was used to compare the occurrence of *Eimeria* species infecting goats and sheep, as well as the occurrence of the protozoan in different ages of the small ruminants and rearing system. A 5% significance level was considered. The BioEstat software version 5.3 was used for statistical evaluation (Ayres et al., 2007). In addition, the analysis of the risk factor associated with *Eimeria* infection and system of rearing was performed through univariate analysis and logistic regression considering as dependent variable the results of test (Gordon and Whitlock technique). Odds ratio (OR) values were obtained and the significance level was set up at 5%. All analyzes were carried out using the EPIINFO$^{TM}$7.2.2.6 software.

## 3. Results

Out of all samples analyzed, 70.07% (576/822) scored positive for *Eimeria* oocysts. The overall results of positivity for any *Eimeria* species are shown on Table 1. A morbidity of 13.40% (41/306) for goats and 9.63% (26/270) for sheep was observed, since some animals presented at least one suggestive clinical sign (e.g., diarrhea, apathy and/or rough hairs) of coccidiosis.

The analysis of risk factors revealed that the semi-intensive system (OR = 1.57) was a risk factor for goats, and in contrast, intensive system for sheep (OR = 2.45) was considered safer for the transmission of *Eimeria* species.

Sixteen different *Eimeria* species were diagnosed in this study **(Figure 1)**. Data describing the frequency and all morphometric features of *Eimeria* species herein detected are summarized in Table 2.

## 4. Discussion

This study assessed the diversity of the *Eimeria* species infecting small ruminants in North-eastern Brazil. Data indicate that animals living in this region are parasitized by a wide range of *Eimeria* species (up to 16). The overall prevalence herein obtained (i.e., 70.07%) is lower than other studies conducted in North-eastern Brazil where 99.1% (Tembue et al., 2009) and 91.2% were detected in small ruminants (Cavalcante et al., 2012). This high positivity observed in the present study is reflecting the poor hygienic sanitary conditions, which may be considered an aggravating factor for the spreading of coccidiosis. It is known that this condition allows that a high burden of infecting *Eimeria* to be present on the environment contaminating food and water, and exposing animals to the infection (Khodakaram-Tafti and Hashemnia, 2017).

Statistical difference ($\chi^2 = 5.50$; $p = 0.01$) was observed between the positivity of goats and sheep, demonstrating that both species are stickered by these coccidia. For both animal species, adults were most frequently affected than youngsters, thus suggesting that the former individuals might have a huge epidemiological burden, especially in conditions of stress such as childbirth, transportation, food and climate changes, besides of high density stocking (Chartier and Paraud, 2012).

In the present study, the highest infection in animals over 12 months of age may be due to high number parturient females (31.98%), as well as the different immune responses, the breeding and overcrowding system observed in the different properties. Further studies are needed to elucidate the hypotheses above. From a clinical point of view, animals presented some clinical signs suggestive of the infection by *Eimeria* species, however data obtained in this study are not enough to associate these signs with the infection status of animals. Coinfections were observed in almost all positive goats (86.60%) and sheep (95.19%). This finding suggests that infection by this genus usually occur by more than one species (Kheirandish et al., 2014; Mohamaden et al., 2018). It is known that the rearing system may play a pivotal role on the occurrence of coccidiosis, showing a close relationship between the level of technification and the intensity of infection. It has already been demonstrated that the intensive and semi-intensive rearing systems, where high population density occur, facilitate the dissemination of coccidia species (Tomczuk et al., 2015). Although, no statistical difference had been observed among the overall results of different systems of rearing ($\chi^2 = 2.74$; $p = 0.25$), the risk factor observed in semi-intensive and intensive system for goats and sheep revealed the importance of confinement of animals and occurrence of coccidiosis. It is important to highlight that the majority of animals herein analyzed were subjected to a total (intensive system) or partial confinement (semi-intensive system). A wide diversity of

*Eimeria* species was detected in goats (see Table 2). It is important to note the predominance of pathogenic species such as *E. arloingi* and *E. ninakohlyakimovae.* Both species have been considered important causes of gut lesion and diarrhea on goats, and are widespread in different regions of Brazil (Ramirez et al., 2009; Ahid et al., 2009; Cavalcante et al., 2012; Coelho et al., 2012). In particular, *E. arloingi*, the most pathogenic species, it has been responsible for lesions featured by formation of schizonts in endothelial cells of vessels of small intestine of hosts, resulting in severe destruction of the intestinal mucosa (Silva et al., 2017). In general the morphometric analysis revealed that species herein identified were similar to other species from previous studies, with divergence only for *E. caprovina* and *E. arloingi* (Ramirez et al., 2009, Ahid et al., 2009). Discrete individual intraspecific variations may be found due to intense infections, environmental factors, as well as different ages of hosts (Fonseca et al., 2012; Ramirez et al., 2009).

On the other hand, the most common species detected in sheep were *E. ovinoidalis*, *E. parva* and *E. crandallis* (see Table 2). All species herein diagnosed have already been reported in North-eastern Brazil (Silva et al., 2011; Souza et al., 2015). Previous reports suggest that infection by different species and their respective frequencies vary according to region (Khan et al., 2011). Among the eight species identified, four exhibited polar cap (*E. crandallis, E. granulosa, E. bakuensis* and *E. ashata*). The presence or absence of this cap, associated with the presence of micropyle, diameters (oocysts and sporocysts) and oocyst shape are reliable criteria for species differentiation (Hassum et al., 2007). It is important to highlight that *E. ovinoidalis*, the most common in this study, presents a high fertility when compared to other species (Kyriánová et al., 2017).

## 5. Conclusions

This research assessed for the first time the diversity of *Eimeria* species infecting small ruminants in the study area. Data reported here are pivotal to better understand the dynamics of the infection by this coccidian in these herds, as well as an alert for veterinarians regarding the presence of pathogenic species (*E. arloingi* and *E. ninakohlyakimovae*) in goats and (*E. ovinoidalis* and *E. crandallis*) in sheep. Although, no association had been observed among the production systems and the positivity of animals, it is extremely important to adopt sanitary measures appropriated in order to prevent the infection by theses species and to reduce the economic impact that they cause.

## Conflict of interest

The authors declare no conflict of interest.

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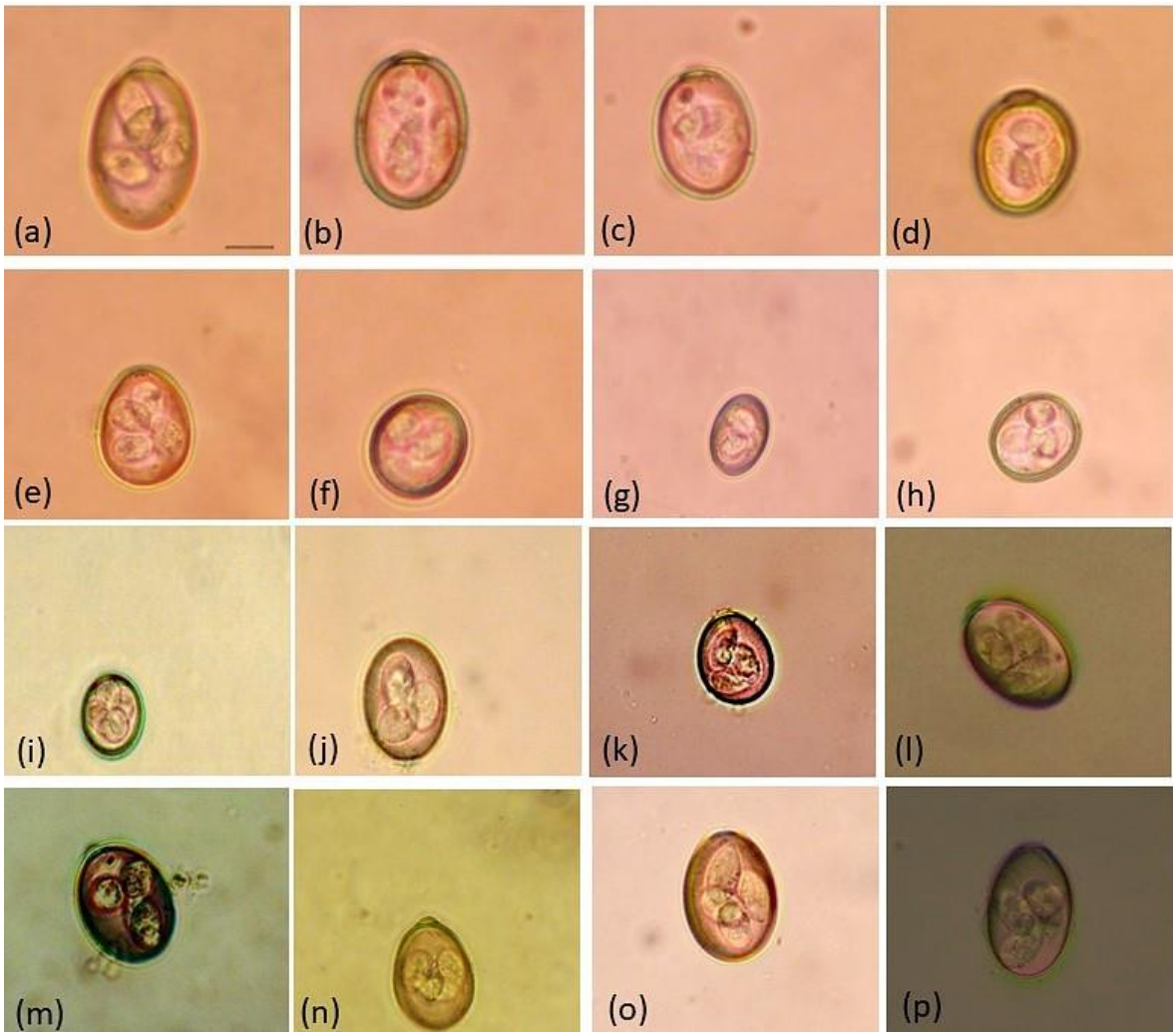
Table 1. Positivity for *Eimeria* species infecting goats and sheep in Microregion of Garanhuns, semi-arid region of North-eastern Brazil.

| **Age** | **Goat % (n/N)** | **Sheep % (n/N)** |
|---|---|---|
| Young | 35.62 (109/306) | 43.70 (118/270) |
| Adult | 64.38 (197/306) | 56.30 (152/270) |
| **System of rearing** | | |
| Intensive | 55.56 (60/108) | 70.97 (110/155) |
| Semi-intensive | 82.28 (195/237) | 62.77 (145/231) |
| Extensive | 73.91 (51/69) | 68.18 (15/22) |
| **Co-infections** | | |
| Double | 23.53 (72/306) | 18.52 (50/270) |
| Triple | 37.58 (115/306) | 20.00 (24/270) |
| > Four | 25.49 (78/306) | 56.67 (153/270) |

Table 2. *Eimeria* species infecting goats and sheep in Microregion of Garanhuns, semi-arid region of Northeastern Brazil.

| Species (Goat) | Frequency % (n) | Form | Polar cap | Micropyle | Oocysts diameter (µm) | | Sporocyst diameter (µm) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Larger | Smaller | Larger | Smaller |
| *E. ninakohlyakimovae* | 84.31 (258) | Sub-spherical | Absent | Absent | 24.37±2.15 | 19.25±1.64 | 11.12±1.65 | 7.47±0.74 |
| *E. arloingi* | 80.07 (245) | Ellipsoid | Present | Present | 28.06±1.29 | 19.83±1.65 | 12.12±1.81 | 7.72±0.84 |
| *E. alijevi* | 52.61 (161) | Spherical | Absent | Absent | 18.82±5.20 | 16.87±2.36 | 8.79±2.23 | 6.49±1.09 |
| *E. jolchijevi* | 15.36 (47) | Ellipsoid | Present | Present | 31.55±1.32 | 21.17±2.08 | 13.53±1.85 | 8.10±0.78 |
| *E. caprina* | 19.93 (61) | Ellipsoid | Absent | Present | 30.37±2.65 | 21.22±1.75 | 12.78±1.76 | 8.19±1.04 |
| *E. chirstenseni* | 16.01 (49) | Oval | Present | Present | 38.4±2.69 | 24.72±2.21 | 13.9±1.47 | 9.13±0.78 |
| *E. caprovina* | 13.73 (42) | Oval | Absent | Present | 31.76±3.11 | 23.27±2.25 | 14.31±1.56 | 8.86±1.00 |
| *E. hirci* | 3.92 (12) | Sub-spherical | Present | Present | 23.00±1.75 | 18.26±1.31 | 9.88±1.23 | 7.10±0.74 |
| **Species (Sheep)** | | | | | | | | |
| *E. ovinoidalis* | 72.59 (196) | Ellipsoid | Absent | Indistinct | 24.15±1.79 | 19.10±1.43 | 10.51±1.67 | 7.15±0.86 |
| *E. parva* | 64.81 (175) | Spherical | Absent | Absent | 20.90±1.70 | 18.09±1.47 | 9.56±1.32 | 6.76±0.76 |
| *E. crandallis* | 58.89 (159) | Sub-spherical | Present | Present | 24.79±1.72 | 19.1±1.46 | 10.56±1.46 | 7.08±0.79 |
| *E. granulosa* | 56.30 (152) | Ellipsoid | Present | Present | 28.23±1.13 | 19.76±1.57 | 11.79±1.61 | 7.37±0.81 |
| *E. bakuensis* | 54.81 (148) | Ellipsoid | Present | Present | 30.84±1.18 | 20.81±1.27 | 12.69±1.22 | 7.87±0.92 |
| *E. ashata* | 51.85 (140) | Ellipsoid | Present | Present | 35.1±2.84 | 22.48±2.11 | 13.23±1.62 | 8.11±0.94 |
| *E. faurei* | 22.96 (62) | Oval | Absent | Present | 32.46±1.44 | 24.78±1.29 | 12.70±1.48 | 5.52±0.86 |
| *E. pallida* | 14.44 (39) | Spherical | Absent | Absent | 14.96±1.07 | 12.89±1.35 | 7.18±0.96 | 5.31±0.80 |

**Figure 1.** Different *Eimeria* species were diagnosed in this study. (a) *E. ashata*; (b) *E. bakuensis*; (c) *E. crandallis*; (d) *E. granulosa*; (e) *E. faurei*; (f) *E. ovinoidalis*; (g) *E. pallida*; (h) *E. parva* (i) *E. alijevi*; (j) *E. ninakohlyakimovae*; (k) *E. hirci*; (l) *E. arloingi*; (m) *E. jolchijevi*; (n) *E. christenseni*; (o) *E. caprovina*; (p) *E. caprina* (scale bar = 10 μm).



## 5.2 Artigo 2

# RISK FACTORS ASSOCIATED WITH INFECTION BY *Eimeria* SPP. IN GOATS AND SHEEP IN NORTH-EASTERN BRAZIL

**(Artigo submetido a revista Tropical Animal Health and Production)**

# Risk factors associated with infection by *Eimeria* spp. in goats and sheep in North-eastern Brazil

**Abstract**

*Eimeria* spp. infection leads to acute or chronic intestinal disorders responsible for important economic losses in small ruminant worldwide. The aim of this study was to assess the risk factors associated to *Eimeria* spp. infection in small ruminants of the microregion of Garanhuns, state of Pernambuco, North-eastern Brazil. Fecal samples (n = 822) were obtained of goats (n = 414) and sheep (n = 408), and evaluated by the modified Gordon and Whitlock technique. The risk factors assessment was performed through univariate analysis and logistic regression. Oocysts of *Eimeria* species were detected in 62.90% (517/822) of the animals, being 77.79% (322/414) in goats and 47.79% (195/408) in sheep. For goats, the herd size (OR = 5.52), rearing system (OR = 1.57), feeding place (OR = 2.60), absence of mineral salt in the diet (OR = 2.54), installation floor (OR = 2.83) and periodicity of cleaning (OR = 5.39) were considered risk factors. Conversely, for sheep only the herd size (OR = 3.16) and rearing system (OR = 2.45) were important factors associated with the infection by *Eimeria* spp. Data herein obtained brings meaningful information on the epidemiology of coccidiosis in small ruminants in North-eastern Brazil. The knowledge of these risk factors are useful to aid the development of measures of prevention reducing the economic impact caused by these protozoan in the small ruminant production.

**Keywords:** Protozoan; Coccidiosis; Small ruminants; Epidemiology.

## INTRODUCTION

Protozoa belonging to the genus *Eimeria* (Apicomplexa) are obligate intracellular parasites of the intestinal epithelium, responsible for an economically important parasitic disease commonly known as coccidiosis. Goats and sheep may become infected after ingestion of sporulated oocysts (Bakunzi et al. 2010; Keeton and Navarre, 2018). Once ingested, the protozoan invades the intestinal cells and undergoes multiple generations of asexual reproduction followed by sexual reproduction, resulting in damage to the intestinal lining and consequently inadequate absorption causing a reduction in weight gain (Ozmen et al. 2016).

For a long time, attention has been paid to coccidiosis due to its economical impact on the ruminant production. In fact, in the United States is estimated that the economical losses may achieve 341 millions dollars per year (Grilo and Carvalho, 2014). At the same time, a previous study conducted in Europe

demonstrated that the prophylactic treatment of coccidiosis represent a cost of 4.88 dollars per calf (Lassen and Østergaard, 2012). The cost for the small ruminant production has not been assessed, but it is believed that the losses are present worldwide, especially in young animals.

Several factors related to the host, environment and parasite are associated to the occurrence of *Eimeria* infection (Rehman et al. 2011). The age of the host has been considered an important risk factor, since it is known that small ruminants become infected within the first few days after birth, eliminating oocysts in faeces from the second week of life (Saratsis et al. 2011). On the other hand, older animals are sources of infection due to the frequent elimination of low numbers of oocysts in faeces for long periods (Foreyt, 1990). Similarly, post-partum females are also responsible for maintain a high burden of oocysts in the environment, facilitating the infection of young animals (Silva et al. 2007).

Subclinical infection is the most common manifestation of the disease, which has a significant impact on health and herd production (Silva et al. 2011). One of the first signs is the delay on development, especially in young animals (Andrade Júnior et al. 2012). However, when the clinical infection occurs, signs such as diarrhea, anorexia, weight loss and dehydration have been observed. The knowledge of the inherent aspects of the course of the infection is important to define the appropriate measures of prevention, since only the administration of drugs, most of the times does not represent success in the control of coccidiosis (Young et al. 2011; Chartier and Paraud, 2012).

The assessment of risk factors is pivotal, as it helps to adopt prophylactic measures, consequently reducing the occurrence of *Eimeria* infection in small ruminant (Souza et al. 2015; Macedo et al. 2018). However, risk factors have been few investigated in many regions where the rearing of goat and sheep is an important economic activity (Carrau et al. 2018). Therefore, the aim of this study was to assess the prevalence and risk factors associated with the infection by *Eimeria* spp. in small ruminants from North-eastern Brazil.

## MATERIAL AND METHODS

### Study area

A transversal study was performed in 36 small ruminant farms located in the microregion of Garanhuns (8°53′25″South and 36°29′34″West), state of Pernambuco, North-eastern Brazil. This area is

featured by a semi-arid climate with an annual average of temperature of 22°C (from 17°C to 30°C), rainfall mean of 147 mm (from 25 mm to 295mm), and air relative humidity of 90%.

**Animals, sampling and laboratorial procedures**

The minimum sample size (n = 384) was estimated based on the goat population (n = 35,770) and the sheep population (n = 99,606) of the study area (IBGE, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004). The farms were selected from non-probabilistic sampling by convenience (Reis, 2003). There were no exclusion criteria in relation to the breed, gender, age and rearing systems.

From March 2017 to May 2018, fresh faecal samples (n = 822) were collected from the rectum of goats (n = 414) and sheep (n = 408) using plastic gloves. Of all goats enrolled, 144 were young (≤ 12 months old) and 270 adult animals (> 12 months old), whereas among sheep 172 were young and 236 adult animals. After collection, samples were stored in isothermal boxes (4°C) until laboratory processing. For the analysis of the risk factors, an investigative questionnaire was applied, based on questions about individual animals' information, herd and sanitary conditions.

Each sample was individually processed by the modified Gordon and Whitlock technique (Gordon and Whitlock, 1939).

**Data analysis**

Descriptive statistical analysis was performed to obtain relative and absolute frequency. In addition, the Lilliefors test was used to verify the normality of the data. The Chi-square test with Yates correction ($\chi^2$) was used to compare the occurrence of *Eimeria* species infecting goats and sheep, as well as the occurrence of the protozoan in different ages of the small ruminants. Subsequently, for the analysis of the risk factors associated with *Eimeria* infection was performed a univariate analysis of the variables of interest and logistic regression analysis considering as dependent variable the results of test (Gordon and Whitlock technique). Odds ratio (OR) values were obtained for each parameter assessed. The significance level was set up at 5%. All analyzes were carried out using the EPIINFO$^{TM}$7.2.2.6 software.

**RESULTS**

Out of all samples analyzed, 62.90% (517/822) were positive for *Eimeria* spp. oocysts, being 77.79% (322/414) of goats (mean 5,601,55 ± 14,851,74) and 47.79% (195/408) of sheep (mean 1,667,69 ± 14,780,03) ($\chi^2$ = 77,881, p = 0.0000). No statistical difference was observed between the positivity and age of both species ($\chi^2$ = 1.027; p = 0.3109).

The univariate analysis of risk factors associated infection by *Eimeria* spp. in sheep and goats of North-eastern region of Brazil are shown on Tables 1 and 2.

**DISCUSSION**

This study describes risk factors associated with the infection by *Eimeria* spp. in small ruminants reared in North-eastern region of Brazil. The overall prevalence herein obtained (i.e., 77.79% for goats and 47.79% for sheep) revealed a high parasitism by *Eimeria* spp. in both animal species. Currently, coccidiosis is one of the most important threat for the small ruminant production, since it may cause relevant economic losses.

Several risk factors have been associated to the infection by *Eimeria* species in ruminants, especially in bovines. Data about small ruminants (i.e., goats and sheep) have been fewer exploited throughout the world. However, this study provides some risk factor associated to the infection by these coccids in these animals. It is known that the age is considered an important risk factor for infection by *Eimeria* species, however, in this study animals of different ages were equally exposed to *Eimeria* spp., differing from other studies in which young animals are more affected (Tomczuk et al. 2015, Carrau et al. 2018).

Interestingly the herd size and rearing system were risk factors for the infection of both species. It has already been indicated that intensive and semi-intensive breeding systems, where high population density occurs, contribute to the propagation of coccidia species (Sharma et al. 2017). A common practice adopted in the farms herein studied was the partial confinement (usually at night) of animals. This data demonstrates that in the present study the semi-intensive rearing system and the high density of animals might have directly influenced the infection by *Eimeria* spp. in goats and sheep.

Other risk factors such as feeding place, type of floor, absence of mineral salt in the diet and periodicity of cleaning were observed only for goats. It is important to note, that all factors mentioned above (except the use of mineral salt) are related to the hygienic conditions. The faecal contamination of water and

food (**Fig. 1**) is important for the transmission of many protozoan, especially in places where animals are fed directly over the soil, favoring the ingestion of sporulated oocysts (Sharma et al. 2017). Precarious hygiene conditions (e.g., uncemented floor) associated with overcrowding in intensive rearing systems result in high level of infection (Khan et al. 2011). Therefore, it is important to highlight that the place and conditions of food supply are fundamental, since the proximity with the soil favors the faecal contamination and consequently animal infection. The absence of mineral salt in the diet of goats has also been considered a risk factor. Currently, it is known that many formulations of mineral salt contain coccidiostatic compounds (e.g., decoquinate and monensin) that are used as preventive for coccidiosis (Andrade Júnior et al. 2012).

It is noteworthy that this study was the first to identify risk factors for infection by *Eimeria* spp. in goats and sheep in the study area. The data herein presented are important for understanding the factors that influence the occurrence of the infection by this protozoan. Accordingly, the knowledge of these parameters is of great importance for the development of prophylaxis strategies suitable for the different conditions and to minimize the economical losses for the small ruminant production.

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**Compliance with ethical standards**

**Statement of animal right**

The Ethics Committee for Animal Experimentation (ECAE) of the *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (license number: 06/2017).

**Conflict of interest**

The authors declare no conflict of interest.

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**Figure caption**

**Fig 1** Fecal contamination of water (a) and food (b)

Table 1 Univariate analysis of risk factors associated infection by *Eimeria* spp. in goats of North-eastern region of Brazil.

| Variable | N | OoPG Positive | Univariate analysis OR (C.I. 95%) | *p*-value |
|---|---|---|---|---|
| **Age (months)** | | | | |
| ≤ 12 | 144 | 112 (77.78%) | | |
| > 12 | 270 | 210 (77.78%) | 1.00 (0.60-1.68) | 0.546 |
| **Gender** | | | | |
| Male | 61 | 50 (81.97%) | | |
| Female | 353 | 272 (77.05%) | 1.35 (0.65-3.01) | 0.250 |
| **Herd size (animals)** | | | | |
| >50 | 244 | 206 (84.43%) | | |
| ≤50 | 170 | 116 (68.24%) | 2.52 (1.53-4.17) | 0.00008* |
| **Rearing System** | | | | |
| Intensive | 108 | 80 (74.07%) | - | |
| Semi-intensive | 237 | 194 (81.86%) | 1.57 (0.87-2.80) | 0.022* |
| Extensive | 69 | 48 (69.57%) | 0.50 (0.26-0.98) | |
| **Place of supply (food)** | | | | |
| Others | 224 | 191 (85.32%) | | |
| Trough | 190 | 131 (68.95%) | 2.60 (1.57-4.35) | 0.00005* |
| **Mineral salt** | | | | |
| Not | 253 | 213 (84.19%) | | |
| Yes | 161 | 109 (67.70%) | 2.54 (1.54-4.19) | 0.00007* |
| **Installation floor** | | | | |
| Cemented | 121 | 81 (66.94%) | - | |
| Ground | 175 | 149 (85.14%) | 2.83 (1.55-5.18) | 0.0002* |
| Ripped | 118 | 92 (77.97%) | 0.617 (0.32-1.18) | |
| **Periodicity of cleaning** | | | | |
| Daily | 158 | 102 (64.56%) | - | |
| Weekly | 130 | 118 (90.77%) | 5.39 (2.66-11.63) | 0.00000007* |
| Monthly | 126 | 102 (80.95%) | 0.43 (0.18-0.95) | |

N – Total samples; OR – Odds Ratio; C. I. – Confidence interval.

*p < 0.05, significant association.

Table 2 Univariate analysis of risk factors associated infection by *Eimeria* spp. in sheep of North-eastern region of Brazil.

| Variable | N | OoPG Positive | Univariate analysis OR (C.I. 95%) | *p*-value |
|---|---|---|---|---|
| **Old (months)** | | | | |
| ≤ 12 meses | 172 | 45 (51.16%) | | |
| >12 meses | 236 | 107 (45.34%) | 1.26 (0.83-1.90) | 0.143 |
| **Gender** | | | | |
| Male | 116 | 56 (48.28%) | | |
| Female | 292 | 139 (47.60%) | 1.02 (0.65-1.61) | 0.494 |
| **Herd size (animals)** | | | | |
| <50 | 365 | 164 (44.93%) | | |
| ≥50 | 43 | 31 (72.09%) | 3.16 (1.51-6.97) | 0.0005* |
| **Rearing System** | | | | |
| Semi-intensive | 231 | 89 (38.53%) | | |
| Intensive | 155 | 94 (60.65%) | 2.45 (1.58-3.81) | 0.00001* |
| Extensive | 22 | 12 (54.55%) | 0.77 (0.28-2.15) | |
| **Place of supply (food)** | | | | |
| Others | 395 | 188 (47.59%) | | |
| Trough | 13 | 7 (53.85%) | 0.77 (0.21-2.76) | 0.434 |
| **Mineral salt** | | | | |
| Not | 266 | 124 (46.62%) | | |
| Yes | 142 | 71 (50.00%) | 0.87 (0.56-1.34) | 0.291 |
| **Installation floor** | | | | |
| Ground | 281 | 137 (48.75%) | | |
| Ripped | 77 | 38 (49.35%) | 1.02 (0.59-1.75) | 0.514 |
| Cemented | 50 | 20 (40.00%) | 0.68 (0.31-1.49) | |
| **Periodicity of cleaning** | | | | |
| Daily | 232 | 117 (50.43%) | | |
| Weekly | 75 | 29 (38.67%) | 0.62 (0.33-1.14) | |
| Monthly | 101 | 49 (47.46%) | 1.49 (0.78-2.87) | 0.125 |

N – Total samples; OR – Odds Ratio; C. I. – Confidence interval.

*p < 0.05, significant association.

# 6 CONSIDERAÇÕES FINAIS

A diversidade de espécies de *Eimeria* que infectam pequenos ruminantes na área de estudo foi detectada pela primeira vez, assim como foram identificados fatores de risco que influenciam a ocorrência da infecção por este protozoário.

Há a necessidade de melhoria nas instalações dos pequenos ruminantes, principalmente nas de caprinos para minimizar a transmissão de *Eimeria* spp.

Os dados obtidos são essenciais para compreensão da dinâmica da infecção por *Eimeria* spp. na microrregião de Garanhuns, bem como um alerta para os veterinários em relação a infecções subclínicas e presença de espécies patogênicas (*E. arloingi* e *E. ninakohlyakimovae*) em caprinos e (*E. ovinoidalis* e *E. crandallis*) em ovinos.

Portanto, este estudo inédito na região ressalta a extrema importância em adotar medidas sanitárias apropriadas para prevenir a infecção por essas espécies e reduzir o impacto econômico para produção de pequenos ruminantes.

# APÊNDICES

## Apêndice A – Questionário investigativo

**UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO-UFRPE**
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Avenida Bom Pastor, s/n. – Boa Vista, Garanhuns/PE
55.293-901 – Telefone: (87) 3764-5505 / (87)3764-5551

**QUESTIONÁRIO INVESTIGATIVO PARA *Eimeria* spp. EM PEQUENOS RUMINANTES**

FICHA Nº: PROPRIEDADE Nº

DATA: / /

INVESTIGADOR:

IDENTIFICAÇÃO

Fazenda: Proprietário:

Endereço:

Município:

Contato:

DADOS DO REBANHO

**1) Tamanho do rebanho:**

a) Abaixo de 25 animais

b) Entre 26 e 50 animais

c) Entre 51 e 100 animais

d) Acima de 100 animais

**2) Espécie:**

a) Caprina

b) Ovina

**3) Tipo de criação:**

a) Extensivo

b) Semi-intensivo

c) Intensivo

**4) Tipo de exploração:**

a) Carne

b) Leite

**5) Alimentação:**

a) Com suplementação. Qual?_____

b) Sem suplementação

**6) Fornece silo:**

a) Sim

b) Não

**7) Fornece feno:**

a) Sim

b) Não

**8) Local de fornecimento:**

a) Cocho

b) Solo

c) Outro_____

**9) Fornece sal mineral:**

a) Sim_____

b) Não

**10) Fonte de água:**

a) Água tratada

b) Córregos e riachos

c) Açudes

d) Poço

INSTALAÇÕES

**11) Tipo de instalação:**

a) Piso ripado

b) Chão batido

c) Cimentado

**12) Instalação coletiva para todos os animais:**

a) Sim

b) Não

**13) Instalação separada para fêmeas e machos:**

a) Sim

b) Não

**14) Instalação separada para cabritos e borregos:**

a) Sim

b) Não

**14) Realiza limpeza das instalações:**

a) Sim

b) Não

**15) Periodicidade da limpeza:**

a) Diariamente

b) Semanalmente

c) Quinzenalmente

d) Mensalmente

MANEJO SANITÁRIO

**16) Procedimento dos animais:**

a) Rebanho autóctone

b) Exposição/leilão

c) Feira livre

**17) Realiza isolamento dos animais doentes:**

a) Sim

b) Não

**18) Possui assistência veterinária:**

a) Sim

b) Não

**19) Utiliza antiparasitário:**

a) Sim

b) Não (Pergunta 29)

**20) Grupo animal para desverminação:**

a) Toda população (caprinos e ovinos)

b) Somente cabritos e / ou borregos

c) Animais após desmame

d) Animais com sinais clínicos

c) Cabras e / ou ovelhas em lactação

**21) Período para desverminação:**

a) Logo após o parto

b) Uma vez ao ano

c) Duas vezes ao ano

d) Mais de três vezes ao ano

**22) Rotação de princípio ativo:**

a) Após um ano utilizando

b) Troca a cada desverminação

c) De acordo com o preço

d) Esporadicamente

e) Não rotaciona.

**Apêndice B - Termo de Consentimento Livre e Esclarecido**

**UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO-UFRPE**
**UNIDADE ACADÊMICA DE GARANHUNS-UAG**
Avenida Bom Pastor, s/n. – Boa Vista, Garanhuns/PE
55.293-901 – Telefone: (87) 3764-5505 / (87)3764-5551

**TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

Eu, _____, portador de RG nº_____ residente em _____, abaixo assinado, atesto que entendi o conteúdo desse consentimento informado e concordo de livre e espontânea vontade em participar do estudo sobre parasitos gastrintestinais em fezes de caprinos e ovinos da microrregião do agreste meridional de Pernambuco. Atesto que fornecerei fezes de meus animais (caprinos e ovinos) e informações verídicas ao questionário aplicado pelo grupo de pesquisa da UAG/UFRPE. Declaro ainda, que esclareci todas as minhas dúvidas com os responsáveis pela pesquisa e autorizo a publicação dos dados e/ou fotos.

_____de_____ 20____.

_____

Proprietário responsável dos animais

_____

Assinatura de um dos responsáveis pela pesquisa

**Apêndice C – Folder Eimeriose**

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO-UFRPE
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM SANIDADE E REPRODUÇÃO DE RUMINANTES

# Eimeriose

Garanhuns / PE

# EIMERIOSE EM CAPRINOS E OVINOS

**Sabe os prejuízos que a eimeriose pode causar no seu rebanho?**


*Eimeria caprovina*

Eimeriose é uma enfermidade parasitária causada por protozoários do gênero *Eimeria*, também conhecida por coccidiose, acomete animais de todas as idades, ocasionando sinais clínicos principalmente em animais jovens. Causa alterações intestinais, resultando em importantes prejuízos econômicos na criação de caprinos e ovinos.

## SINAIS E PREJUÍZOS

- ✓ Diarreia
- ✓ Perda de peso (emagrecimento)
- ✓ Pelos arrepiados
- ✓ Animais apáticos (tristeza)
- ✓ Atraso no desenvolvimento
- ✓ Diminuição na produção
- ✓ Cansaço
- ✓ Óbito


Figura 1. Animais com atraso no desenvolvimento (A e B).

## TRANSMISSÃO

- ✓ Fecal-oral
- ✓ Ingestão do parasito com água ou alimentos contaminados


Figura 2. Contaminação fecal de água (A) e alimentação (B).

## CONTROLE

- ✓ Tratamento dos animais doentes
- ✓ Animais separados por idade
- ✓ Ambiente limpo e seco
- ✓ Remoção das fezes
- ✓ Alimentação adequada
- ✓ Redução do estresse
- ✓ Evitar superlotação


Figura 3. Animais em locais adequados (A); Animais em condições indesejáveis (B e C).

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# Morphological and epidemiological data on *Eimeria* species infecting small ruminants in Brazil

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ABSTRACT

Parasites of the genus *Eimeria* induce relevant economic losses in small ruminants worldwide. The aim of this study was to investigate the epidemiology of goats and sheep coccidiosis in a semi-arid region of North-eastern Brazil and to assess whether the rearing system represents a risk factor for the distribution of the infection. A total of 822 fresh faecal samples were collected from the rectum of goats (n = 414) and sheep (n = 408) All samples were individually processed using the technique described by Gordon and Whitlock, and after sporulation in 2.5% Potassium dichromate ($K_2Cr_2O_7$), an accurate morphometric analysis was performed, with oocysts morphologically identified at species level. The analysis of the risk factors associated with *Eimeria* infection and system of rearing was performed through univariate analysis and logistic regression. Out of all animals sampled, 70.07% (576/822) scored positive for coccidia oocysts, with an overall prevalence of the infection of 73.91% (306/414) in goats and 66.18% (270/408) in sheep ($\chi^2$ = 5.50; p = 0.01). Eight *Eimeria* species were identified either in goats (i.e., *E. arloingi*, *E. ninakohlyakimovae*, *E. alijevi, E. jolchijevi*, *E. caprina*, *E. chirstenseni*, *E. caprovina* and *E. hirci*) and in sheep (i.e., *E. ovinoidalis*, *E. parva*, *E. crandallis*, *E. granulosa*, *E. bakuensis*, *E. ashata*, *E. faurei*, *E. pallida*). The analysis of risk factors revealed that the semi-intensive system (OR = 1.57) was a risk factor for goats, and, incontrast, intensive system for sheep (OR = 2.45) was safer, relative to *Eimeria* species transmission. Data reported in this study indicate that a wide species diversity and frequency of coccidia affect small ruminants in the study area. Finally, these findings are pivotal to better understand the dynamics of infection by this coccidian in these herds, as well as sound as an alert for practice veterinarians of the region, regarding the most common pathogenic species.

## 1. Introduction

Parasites of the genus *Eimeria* (Apicomplexa: Eimeriidae) are accounted as an important cause of intestinal infection in animals worldwide (Ahmad et al., 2016). These protozoa are responsible for the so-called “coccidiosis”, which represent a very important disease in vertebrate animals, including small ruminants (Mohamaden et al., 2018). Amongst these animals, goats and sheep are commonly infected by *Eimeria* species causing relevant economic losses, either in terms of direct costs and productivity, directly affecting the animal welfare (Silva et al., 2014).

*Eimeria* parasites usually affect young animals of up to 4 months of age, but are also diagnosed in adult animals, acting as reservoirs of the infection (Lopes et al., 2013). Coccidiosis can induce a wide plethora of clinical signs, such as weight loss, fever, diarrhea, dehydration, pale mucous, and even death (Andrade et al., 2012; Keeton and Navarre, 2018). The prepatent period of coccidiosis in small ruminants depends upon the species involved, with infected animals shedding unsporulated oocysts in about 7 days. Oocysts sporulate in the environment, becoming infective in a very short time span depending on the species. Once the sporogony is completed in the environment, susceptible hosts ingest oocysts while grazing or drinking contaminated water (Chartier and Paraud, 2012).

The diagnosis of coccidiosis in small ruminants is usually achieved via copromicroscopic methods, with oocysts detected during routine analyses. However, the differentiation of these parasites at species level

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still represents a challenge since requires an accurate morphometrical analysis performed by skilled and trained laboratory technicians (Al-Habsi et al., 2017). To date, 17 *Eimeria* species have been reported in goats (Silva and Lima, 1998), with nine of major epidemiological and veterinary importance. These species are differentiated according to their morphology and size, with the presence/absence of the polar cap being of diagnostic relevance for the identification of *E. arloingi, E. jolchijevi, E. chistenseni* and *E. hirci*. Similarly, the presence of the micropile allows detecting *E. caprina, E. apsheronica* and *E. caprovina* (Eckert et al., 1995). In regard to sheep, 15 *Eimeria* species have been described (Saratsis et al., 2011).

A proper identification of *Eimeria* spp. is fundamental to estimate the importance of any anticoccidicidal treatments as only selected species show a marked pathogenic role in goats (e.g., *E. arloingi* and *E. ninakohlyakimovae*) (Cavalcante et al., 2012; Chartier and Paraud, 2012), or in sheep (e.g., *E. ovinoidalis* and *E. crandallis*). Nonetheless, this assessment has not fully clarified, since does not take into account the interaction between the parasite development, concomitant diseases and/or the animal nutrition. Accordingly, epidemiological studies on small ruminant coccidiosis are lacking, with no information reported from regions where the farming system of goats and sheep is progressively expanding. This is the example of Brazil, where only a few data are published (Barbosa et al., 2003; Silva et al., 2011). Therefore, the aim of this study was to investigate the diversity of coccidia infecting goats and sheep in a semi-arid region of North-eastern Brazil and to assess whether the system of rearing was a risk factor.

## 2. Material and methods

### 2.1. Study area and ethical aspects

A transversal study was performed in 36 small ruminants farms located in Garanhuns (8°53′25″S and 36°29′34″W), state of Pernambuco, North-eastern Brazil. Animals enrolled in this study originated from three different production systems represented by intensive (n = 11), semi-intensive (n = 21) and extensive (n = 4) rearing systems. Animals in the intensive system (n = 263) were confined in wood pens during the whole day. Conversely, those in the semi-intensive system (n = 468) were partially confined at night and maintained at grazing during the day. Finally, livestock in the extensive system (n = 91) were maintained exclusively grazing. Independently from the rearing system, the pasture area was cultivated with *Cynodon dactylon* (Tifton grass). In addition, in the intensive and semi-intensive system, the diet of animals was supplemented with mineral salt. The consortium of animals was not observed in this study.

The area of Garanhuns is featured by a semi-arid climate with an annual average of temperature of 22 °C (from 17 °C to de 30 °C), rainfall mean of 147 mm (from 25 mm to 295 mm), and air relative humidity of 90%.

The Ethics Committee for Animal Experimentation (ECAE) of *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (license number: 06/2017).

### 2.2. Animals, sampling and laboratorial procedures

The minimum sample size (n = 384) was estimated based on the goat population (n = 35,770) and the sheep population (n = 99,606) of the study area (IBGE-Brazilian Institute of Geography and Statistics, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004).

From March 2017 to May 2018, fresh faecal samples (n = 822) were collected from the rectum of goats (n = 414) and sheep (n = 408) using plastic gloves. The history of each animal was recorded in a clinical chart (Diffay et al., 2005). Of all goats enrolled, 144 were young (≤ 12 months old) and 270 adult animals (> 12 months old), whereas among sheep 172 were young and 236 adult animals. After collection, samples were stored in isothermal boxes (4 °C) until laboratory processing.

Each sample was individually processed by the technique of counting eggs/oocysts of Gordon and Whitlock (1939). Afterwards, in order to allow the sporulation of oocysts all positive samples were stored in Petri dishes containing 2.5% Potassium dichromate ($K_2Cr_2O_7$) and maintained at 26 °C for seven days. Finally, the material was transferred into plastic tubes, centrifuged at 200 *g* for 15 min and the supernatant analyzed at different magnifications (10x and 40x) (Menezes and Lopes, 1995). A total of 10 oocysts per sample were measured using the AxioVision software (release 4.8). The identification of oocysts was based on morphometrical features previously described (Eckert et al., 1995).

### 2.3. Statistical analysis

Descriptive statistical analysis was performed to obtain relative and absolute frequency. In addition, the Lilliefors test was used to verify the normality of the data. The Chi-square test with Yates correction ($\chi^2$) was used to compare the occurrence of *Eimeria* species infecting goats and sheep, as well as the occurrence of the protozoan in different ages of the small ruminants and rearing system. A 5% significance level was considered. The BioEstat software version 5.3 was used for statistical evaluation (Ayres et al., 2007). In addition, the analysis of the risk factor associated with *Eimeria* infection and system of rearing was performed through univariate analysis and logistic regression considering as dependent variable the results of test (Gordon and Whitlock technique). Odds ratio (OR) values were obtained and the significance level was set up at 5%. All analyzes were carried out using the EPIINFO™7.2.2.6 software.

## 3. Results

Out of all samples analyzed, 70.07% (576/822) scored positive for *Eimeria* oocysts. The overall results of positivity for any *Eimeria* species are shown on Table 1. A morbidity of 13.40% (41/306) for goats and 9.63% (26/270) for sheep was observed, since some animals presented at least one suggestive clinical sign (e.g., diarrhea, apathy and/or rough hairs) of coccidiosis.

The analysis of risk factors revealed that the semi-intensive system (OR = 1.57) was a risk factor for goats, and in contrast, intensive system for sheep (OR = 2.45) was considered safer for the transmission of *Eimeria* species.

Sixteen different *Eimeria* species were diagnosed in this study (Fig. 1). Data describing the frequency and all morphometric features of *Eimeria* species herein detected are summarized in Table 2.

## 4. Discussion

This study assessed the diversity of the *Eimeria* species infecting small ruminants in North-eastern Brazil. Data indicate that animals living in this region are parasitized by a wide range of *Eimeria* species

**Table 1**
Positivity for *Eimeria* species infecting goats and sheep in Microregion of Garanhuns, semi-arid region of North-eastern Brazil.

| Age | Goat % (n/N) | Sheep % (n/N) |
|---|---|---|
| Young | 35.62 (109/306) | 43.70 (118/270) |
| Adult | 64.38 (197/306) | 56.30 (152/270) |
| **System of rearing** | | |
| Intensive | 55.56 (60/108) | 70.97 (110/155) |
| Semi-intensive | 82.28 (195/237) | 62.77 (145/231) |
| Extensive | 73.91 (51/69) | 68.18 (15/22) |
| **Co-infections** | | |
| Double | 23.53 (72/306) | 18.52 (50/270) |
| Triple | 37.58 (115/306) | 20.00 (24/270) |
| > Four | 25.49 (78/306) | 56.67 (153/270) |

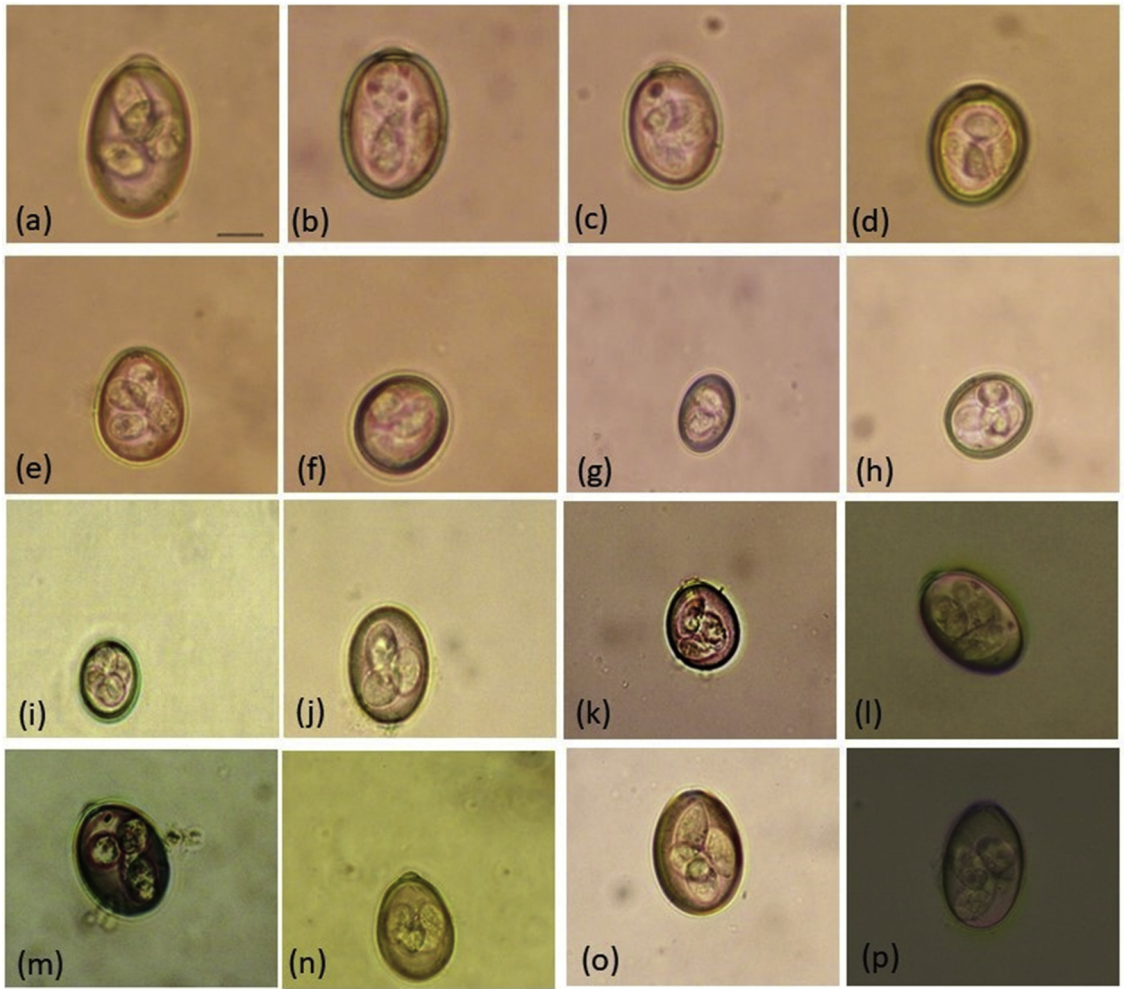


**Fig. 1.** Different *Eimeria* species were diagnosed in this study. (a) *E. ashata*; (b) *E. bakuensis*; (c) *E. crandallis*; (d) *E. granulosa*; (e) *E. faurei*; (f) *E. ovinoidalis*; (g) *E. pallida*; (h) *E. parva* (i) *E. alijevi*; (*j*) *E. ninakohlyakimovae*; (k) *E. hirci*; (l) *E. arloingi*; (m) *E. jolchijevi*; (n) *E. christenseni*; (o) *E. caprovina*; (p) *E. caprina* (scale bar = 10 μm).

(up to 16). The overall prevalence herein obtained (i.e., 70.07%) is lower than other studies conducted in North-eastern Brazil where 99.1% (Tembue et al., 2009) and 91.2% were detected in small ruminants (Cavalcante et al., 2012). This high positivity observed in the present study is reflecting the poor hygienic sanitary conditions, which may be considered an aggravating factor for the spreading of coccidiosis. It is known that this condition allows that a high burden of infecting *Eimeria* to be present on the environment contaminating food

**Table 2**
*Eimeria* species infecting goats and sheep in Microregion of Garanhuns, semi-arid region of Northeastern Brazil.

| | Frequency % (n) | Form | Polar cap | Micropyle | Oocysts diameter (μm) Larger | Oocysts diameter (μm) Smaller | Sporocyst diameter (μm) Larger | Sporocyst diameter (μm) Smaller |
|---|---|---|---|---|---|---|---|---|
| **Species (Goat)** | | | | | | | | |
| *E. ninakohlyakimovae* | 84.31 (258) | Sub-spherical | Absent | Absent | 24.37 ± 2.15 | 19.25 ± 1.64 | 11.12 ± 1.65 | 7.47 ± 0.74 |
| *E. arloingi* | 80.07 (245) | Ellipsoid | Present | Present | 28.06 ± 1.29 | 19.83 ± 1.65 | 12.12 ± 1.81 | 7.72 ± 0.84 |
| *E. alijevi* | 52.61 (161) | Spherical | Absent | Absent | 18.82 ± 5.20 | 16.87 ± 2.36 | 8.79 ± 2.23 | 6.49 ± 1.09 |
| *E. jolchijevi* | 15.36 (47) | Ellipsoid | Present | Present | 31.55 ± 1.32 | 21.17 ± 2.08 | 13.53 ± 1.85 | 8.10 ± 0.78 |
| *E. caprina* | 19.93 (61) | Ellipsoid | Absent | Present | 30.37 ± 2.65 | 21.22 ± 1.75 | 12.78 ± 1.76 | 8.19 ± 1.04 |
| *E. chirstenseni* | 16.01 (49) | Oval | Present | Present | 38.4 ± 2.69 | 24.72 ± 2.21 | 13.9 ± 1.47 | 9.13 ± 0.78 |
| *E. caprovina* | 13.73 (42) | Oval | Absent | Present | 31.76 ± 3.11 | 23.27 ± 2.25 | 14.31 ± 1.56 | 8.86 ± 1.00 |
| *E. hirci* | 3.92 (12) | Sub-spherical | Present | Present | 23.00 ± 1.75 | 18.26 ± 1.31 | 9.88 ± 1.23 | 7.10 ± 0.74 |
| **Species (Sheep)** | | | | | | | | |
| *E. ovinoidalis* | 72.59 (196) | Ellipsoid | Absent | Indistinct | 24.15 ± 1.79 | 19.10 ± 1.43 | 10.51 ± 1.67 | 7.15 ± 0.86 |
| *E. parva* | 64.81 (175) | Spherical | Absent | Absent | 20.90 ± 1.70 | 18.09 ± 1.47 | 9.56 ± 1.32 | 6.76 ± 0.76 |
| *E. crandallis* | 58.89 (159) | Sub-spherical | Present | Present | 24.79 ± 1.72 | 19.1 ± 1.46 | 10.56 ± 1.46 | 7.08 ± 0.79 |
| *E. granulosa* | 56.30 (152) | Ellipsoid | Present | Present | 28.23 ± 1.13 | 19.76 ± 1.57 | 11.79 ± 1.61 | 7.37 ± 0.81 |
| *E. bakuensis* | 54.81 (148) | Ellipsoid | Present | Present | 30.84 ± 1.18 | 20.81 ± 1.27 | 12.69 ± 1.22 | 7.87 ± 0.92 |
| *E. ashata* | 51.85 (140) | Ellipsoid | Present | Present | 35.1 ± 2.84 | 22.48 ± 2.11 | 13.23 ± 1.62 | 8.11 ± 0.94 |
| *E. faurei* | 22.96 (62) | Oval | Absent | Present | 32.46 ± 1.44 | 24.78 ± 1.29 | 12.70 ± 1.48 | 5.52 ± 0.86 |
| *E. pallida* | 14.44 (39) | Spherical | Absent | Absent | 14.96 ± 1.07 | 12.89 ± 1.35 | 7.18 ± 0.96 | 5.31 ± 0.80 |

and water, and exposing animals to the infection (Khodakaram-Tafti and Hashemnia, 2017).

Statistical difference ($\chi^2 = 5.50$; $p = 0.01$) was observed between the positivity of goats and sheep, demonstrating that both species are stickered by these coccidia. For both animal species, adults were most frequently affected than youngsters, thus suggesting that the former individuals might have a huge epidemiological burden, especially in conditions of stress such as childbirth, transportation, food and climate changes, besides of high density stocking (Chartier and Paraud, 2012).

In the present study, the highest infection in animals over 12 months of age may be due to high number parturient females (31.98%), as well as the different immune responses, the breeding and overcrowding system observed in the different properties. Further studies are needed to elucidate the hypotheses above. From a clinical point of view, animals presented some clinical signs suggestive of the infection by *Eimeria* species, however data obtained in this study are not enough to associate these signs with the infection status of animals. Coinfections were observed in almost all positive goats (86.60%) and sheep (95.19%). This finding suggests that infection by this genus usually occur by more than one species (Kheirandish et al., 2014; Mohamaden et al., 2018). It is known that the rearing system may play a pivotal role on the occurrence of coccidiosis, showing a close relationship between the level of technification and the intensity of infection. It has already been demonstrated that the intensive and semi-intensive rearing systems, where high population density occur, facilitate the dissemination of coccidia species (Tomczuk et al., 2015). Although, no statistical difference had been observed among the overall results of different systems of rearing ($\chi^2 = 2.74$; $p = 0.25$), the risk factor observed in semi-intensive and intensive system for goats and sheep revealed the importance of confinement of animals and occurrence of coccidiosis. It is important to highlight that the majority of animals herein analyzed were subjected to a total (intensive system) or partial confinement (semi-intensive system). A wide diversity of *Eimeria* species was detected in goats (see Table 2). It is important to note the predominance of pathogenic species such as *E. arloingi* and *E. ninakohlyakimovae*. Both species have been considered important causes of gut lesion and diarrhea on goats, and are widespread in different regions of Brazil (Ramirez et al., 2009; Ahid et al., 2009; Cavalcante et al., 2012; Coelho et al., 2012). In particular, *E. arloingi,* the most pathogenic species, it has been responsible for lesions featured by formation of schizonts in endothelial cells of vessels of small intestine of hosts, resulting in severe destruction of the intestinal mucosa (Silva et al., 2017). In general the morphometric analysis revealed that species herein identified were similar to other species from previous studies, with divergence only for *E. caprovina* and *E. arloingi* (Ramirez et al., 2009; Ahid et al., 2009). Discrete individual intraspecific variations may be found due to intense infections, environmental factors, as well as different ages of hosts (Fonseca et al., 2012; Ramirez et al., 2009).

On the other hand, the most common species detected in sheep were *E. ovinoidalis*, *E. parva* and *E. crandallis* (see Table 2). All species herein diagnosed have already been reported in North-eastern Brazil (Silva et al., 2011; Souza et al., 2015). Previous reports suggest that infection by different species and their respective frequencies vary according to region (Khan et al., 2011). Among the eight species identified, four exhibited polar cap (*E. crandallis, E. granulosa, E. bakuensis* and *E. ashata*). The presence or absence of this cap, associated with the presence of micropyle, diameters (oocysts and sporocysts) and oocyst shape are reliable criteria for species differentiation (Hassum et al., 2007). It is important to highlight that *E. ovinoidalis*, the most common in this study, presents a high fertility when compared to other species (Kyriánová et al., 2017).

## 5. Conclusions

This research assessed for the first time the diversity of *Eimeria* species infecting small ruminants in the study area. Data reported here are pivotal to better understand the dynamics of the infection by this coccidian in these herds, as well as an alert for veterinarians regarding the presence of pathogenic species (*E. arloingi* and *E. ninakohlyakimovae*) in goats and (*E. ovinoidalis* and *E. crandallis*) in sheep. Although, no association had been observed among the production systems and the positivity of animals, it is extremely important to adopt sanitary measures appropriated in order to prevent the infection by theses species and to reduce the economic impact that they cause.

## Conflict of interest

The authors declare no conflict of interest.

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**Anexo B - Instruções aos autores (Tropical Animal Health and Production)**

TYPES OF ARTICLES

Manuscripts should be presented preferably in Times New Roman font, double spaced, using A4 paper size. Please use the automatic page and line numbering function to number the pages and lines in your document and number the lines in a single continuous sequence.

Regular Articles: Articles should be as concise as possible and should not normally exceed approximately 4000 words or about 8 pages of the journal including illustrations and tables.

Articles should be structured into the following sections;

(a) Abstract of 150-250 words giving a synopsis of the findings presented and the conclusions reached. The Abstract should be presented as a single continuous paragraph without subdivisions.

(b) Introduction stating purpose of the work

(c) Materials and Methods

(d) Results

(e) Discussion (conclusions should be incorporated in the discussion!)

(f) Acknowledgements

(g) Statement of Animal Rights

(h) Conflict of Interest Statement

(i) References

ETHICAL STANDARDS

Manuscripts submitted for publication must contain a statement to the effect that all human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted.

These statements should be added in a separate section before the reference list. If these

statements are not applicable, authors should state: The manuscript does not contain clinical studies or patient data.
The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The author will be held responsible for false statements or failure to fulfill the above-mentioned requirements.

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Online Submission

Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

Title Page

The title page should include: The name(s) of the author(s); A concise and informative title; The affiliation(s) and address(es) of the author(s); The e-mail address, and telephone number(s) of the corresponding author; If available, the 16-digit ORCID of the author(s)

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT

Text Formatting

Manuscripts should be submitted in Word.
Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Please note:
Use the automatic page and line numbering function to number the pages and lines in your document.

REFERENCES

1. All publications cited in the text should be presented in the list of references. The typescript should be carefully checked to ensure that the spelling of the authors 'names and dates are exactly the same as in the reference list.
2. In the text, refer to the author's name (without initial) and year of publication, followed, if necessary, by a short reference to appropriate pages. Examples: 'Peters (1985) has shown that … ''This is in agreement with results obtained later (Kramer, 1984, pp. 12--16)
3. If reference is made in the text to a publication by three or more authors, the abbreviation et al. should be used. All names should be given in the list of references.
4. References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically by authors' surname(s) and chronologically by author. If an author in the list is also mentioned with co-authors the following order

should be used: publications by the single author, arranged according to publication dates; publications of the same author with co-authors. Publications by the same author(s) in the same year should be listed as 1986a, 1986b, etc.

5. Use the following system for arranging each reference in the list:

• For journal articles:

Ahl, A.S., 1986. The role of vibrissae in behaviour: a status review, Veterinary Research Communications, 10, 245--268

• For books:

Fox, J.G., Cohen, B.J. and Lowe, F.M., 1984. Laboratory Animal Medicine, (Academic Press, London)

• For a paper in published symposia proceedings or a chapter in multi-author books: Lowe, K.F. and Hamilton, B.A., 1986. Dairy pastures in the Australian tropics and subtropics. In: G.T. Murtagh and R.M. Jones (eds), Proceedings of the 3rd Australian conference on tropical pastures, Rockhampton, 1985, (Tropical Grassland Society of Australia, St. Lucia; Occasional Publication 3), 68--79

• For unpublished theses, memoranda etc:

Crowther, J., 1980. Karst water studies and environment in West Malaysia, (unpublished PhD thesis, University of Hull)

• For Online documents:

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999

6. Do not abbreviate the titles of journals mentioned in the list of references.

7. Titles of references should be given in the original language, except for the titles of publications in non-Latin alphabets, which should be transliterated, and a notation such as ‘(in Russian)‘ or ‘(in Greek, with English abstract)‘ added.

8. Citations of personal communications should be avoided unless absolutely necessary. When used, they should appear only in the text, using the format: ‘E. Redpath, personal communication, 1986‘ and should not appear in the Reference List. Citations to the unpublished data of any of the authors should not be included unless the work has already been accepted for publication, in which case a reference should be given in the usual way with "in press" in place of the volume and page numbers.

TABLES

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

FIGURE

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,

"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted separately from the text, if possible.

When preparing your figures, size figures to fit in the column width.

For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.