UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
UNIDADE ACADÊMICA DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL

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Avaliação da resistência anti-helmíntica e diversidade de parasitos gastrintestinais em bovinos no Semiárido da Paraíba, Nordeste do Brasil

Patos/PB
2022

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Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal da Universidade Federal de Campina Grande, como pré-requisito para obtenção do grau de Doutor em Ciência e Saúde Animal.

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Patos/PB
2022

M528a Melo, Lídio Ricardo Bezerra de.

Avaliação da resistência anti-helmíntica e diversidade de parasitos gastrintestinais em bovinos no semiárido da Paraíba, Nordeste do Brasil / Lídio Ricardo Bezerra de Melo. – Patos, 2022.

94 f. : il. color.

Tese (Doutorado em Ciência e Saúde Animal) – Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, 2022.

"Orientação: Prof. Dr. Vinícius Longo Ribeiro Vilela".

Referências.

1. Coccídios. 2. Nematóides. 3. Resistência Parasitária. 4. Ruminantes. 5. Semiárido. I. Vilela, Vinícius Longo Ribeiro. II. Título.

CDU 595.752:631.467:636.2(043)

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECÁRIA MARIA ANTONIA DE SOUSA CRB 15/398

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
POS-GRADUACAO EM CIENCIA E SAUDE ANIMAL
Rua Aprigio Veloso, 882, - Bairro Universitario, Campina Grande/PB, CEP 58429-900

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LÍDIO RICARDO BEZERRA DE MELO

AVALIAÇÃO DA RESISTÊNCIA ANTI-HELMÍNTICA E DIVERSIDADE DE PARASITOS GASTRINTESTINAIS EM BOVINOS NO SEMIÁRIDO DA PARAÍBA, NORDESTE DO BRASIL

Tese apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutor em Ciência e Saúde Animal.

Aprovada em: 17/02/2022

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Referência: Processo nº 23096.080744/2021-16 SEI nº 2124974

Dedicatória

Aos meus pais, verdadeiros responsáveis pela minha formação pessoal e profissional.

AGRADECIMENTOS

Agradeço a Deus, que permitiu que tudo isso acontecesse ao longo de minha vida e não somente nestes anos como pós-graduando, mas em todos os momentos está comigo tornando-me sempre capaz de conquistar o que almejo.

A minha família, especialmente aos meus pais que me dão muita força e apoio, Manoel Bezerra e Maria de Lourdes, por sempre acreditarem no meu potencial. Vocês são verdadeiros responsáveis pelos primeiros passos da minha vida estudantil e por minha formação pessoal e profissional, coparticipantes desta conquista querida, amo vocês.

Ao IFPB campus Sousa-PB, pela receptividade de todos os funcionários e disponibilidade das instalações, principalmente o Laboratório de Parasitologia Veterinária (LPV), primordial para realização da pesquisa, no comando do meu orientador, prof. Vinícius Vilela. A todos os colegas que contribuíram para o desenvolvimento da minha pesquisa, especialmente os amigos do LPV/IFPB, campus Sousa-PB, sem a ajuda de vocês seria ainda mais difícil a conclusão em tempo hábil.

Ao meu orientador, prof. Vinícius Vilela, o qual eu tenho muito orgulho de ter a sua orientação e o apoio incansável que sempre me deu, desde quando nos conhecemos, assim como a professora Thaís Feitosa, sua esposa, pelo apoio e torcida pelas minhas conquistas, sinto-me muito orgulho de poder contar sempre. Não posso deixar de lembrar quando iniciei na pesquisa sob orientação de vocês (Vinícius e Thais) e da professora Ana Célia, pessoa essa que sempre me foi solicita a qual tenho grande apresso e admiração.

Agradeço demais a minha noiva, Mayla, a qual ao longo desses quatro anos de doutorado não mediu esforços para me ajudar e me apoiar nas horas mais difíceis, sempre acreditando e me fazendo ter certeza que eu seria capaz de vencer.

A todos que fazem o Programa de Pós-graduação em Ciência e Saúde Animal, docentes e funcionários, pela oportunidade de poder alcançar um título tão importante.

Agradeço aos pecuaristas e seus colaboradores que abriram às portas das suas propriedades cedendo o gado para que eu pudesse realizar a minha pesquisa, amigavelmente muito solícitos e prestativos, embora também beneficiados, estavam sempre com um sorriso no rosto e uma generosa boa vontade.

Às demais pessoas que deixaram de ser aqui mencionadas, mas que tiveram e têm uma importante participação na realização deste trabalho de Tese de Doutorado.

Os meus sinceros agradecimentos a todos àqueles que contribuíram para esta realização, por estarem presentes na minha vida, o meu muito obrigado.

SUMÁRIO

RESUMO

Dentre os fatores que afetam a bovinocultura do Brasil, destacam-se as parasitoses gastrintestinais. A presente tese foi dividida em três capítulos. No capítulo I, avaliou-se a eficácia de quatro classes de anti-helmínticos sobre nematóides gastrintestinais de bovinos, utilizaram-se 20 fazendas com 40 animais em cada, totalizando 800 animais. Foram testados quatro grupos compostos por dez animais: I, sulfóxido de albendazol 15%; II, ivermectina 1%; III, closantel 25%; IV, cloridrato de levamisole 7,5%. No Teste de Redução da Contagem de Ovos Fecais (TRCOF), fezes individuais foram coletadas nos dias 0 e 14. Observou-se multirressistência e resistência anti-helmíntica à ivermectina e ao albendazol em 95% das fazendas; ao closantel, em 75% e, ao levamisole, em 20%. O gênero mais prevalente foi *Haemonchus* spp., (76,7%). Constatou-se alta multirressistência anti-helmíntica nos rebanhos bovinos do Semiárido da Paraíba. No capítulo II, realizou-se levantamento epidemiológico de infecções gastrintestinais por nematóides e coccídios de rebanhos bovinos. Foram amostrados 832 bovinos procedentes de 21 municípios. Foram realizadas às contagens de Ovos Por Grama (OPG) e Oocistos Por Grama (OoPG) de fezes. A freqüência de infecção foi de 59,6% e 36,9% para nematóides e coccídios, respectivamente. Os fatores de risco relevantes para nematóides foi à aptidão para corte e o sistema de criação extensivo, para coccídios, os fatores de risco foram a idade $\leq$ 12 meses e o escore corporal entre 1 e 2. Portanto, é elevada a frequência de coccídios e nematóides gastrintestinais em rebanhos bovinos da Paraíba. No capítulo III, descreveu-se a diversidade de espécies de *Eimeria* spp., também nos rebanhos bovinos da Paraíba. Foram pesquisadas 20 fazendas e utilizados de forma aleatória 40 bovinos de cada. As amostras fecais coletadas dos animais foram submetidas a técnica de centrífugo-flutuação em solução de sacarose, à 3.000 rpm. Os coccídios foram visualizados por microscópio óptico MAX-300® com objetiva de 40X. De cada animal positivo, foram fotografados e mensurados 20 coccídios, sendo detectados em 17,12% das amostras analisadas. No total, foram fotografados e mensurados 2.740 coccídios, totalizando 14 espécies de *Eimeria* spp., cujas freqüências das principais foram: *Eimeria bovis* (35,10%), seguida de *Eimeria canadensis* (17,48%), *Eimeria auburnensis* (14,70%), *Eimeria ellipsoidallis* (9,70%), *Eimeria zuernii* (7,22%), *Eimeria brasiliensis* (4,56%), *Eimeria bukidnonensis* (3,97%), *Eimeria illinoisensis* (2,91%). Os bovinos do Semiárido da Paraíba encontram-se parasitados por 14 espécies de *Eimeria*, correções no manejo devem diminuir a infecção por esses parasitas. Concluiu-se com o estudo inédito que é elevada multirressistência anti-helmíntica em nematóides gastrintestinais de bovinos no Semiárido da Paraíba e o levamisole apresentou menor resistência. A frequência de coccídios e nematóides gastrintestinais nos rebanhos bovinos também é elevada, havendo a ocorrência de infecções por uma alta diversidade de *Eimeria* spp. com animais apresentando poliparasitismo por até sete espécies. Sugere-se a adoção de um manejo sanitário adequado para a realidade de cada rebanho e a assistência técnica periódica por um médico veterinário capacitado, contribuindo para a diminuição dos índices parasitários e melhor desempenho dos animais, gerando maior lucratividade aos pecuaristas.

PALAVRAS-CHAVE: Coccídios; Nematóides; Resistência Parasitária, Ruminantes; Semiárido.

ABSTRACT

Among the factors that affect cattle farming in Brazil, gastrointestinal parasites stand out. This thesis has been divided in three chapters. In chapter I, the efficacy of four classes of anthelmintics on bovine gastrointestinal nematodes was evaluated, using 20 farms with 40 animals each, totaling 800 animals. Four groups composed of ten animals were tested: I, 15% albendazole sulfoxide; II, 1% ivermectin; III, 25% closantel; IV, 7,5% levamisole hydrochloride. In the Fecal Egg Count Reduction Test (FECRT), individual feces were collected on days 0 and 14. Multidrug resistance and anthelmintic resistance to ivermectin and albendazole were observed in 95% of the farms; to closantel, by 75% and, to levamisole, by 20%. The most prevalent genus was *Haemonchus* spp. (76,7%). High anthelmintic multidrug resistance was observed in cattle herds in the semiarid region of Paraíba. In chapter II, an epidemiological survey of gastrointestinal infections by nematodes and coccidia in bovine herds was carried out. A total of 832 cattle from 21 municipalities were sampled. Counts of Eggs Per Gram (EPG) and Oocysts Per Gram (OoPG) of feces were performed. The frequency of infection was 59,6% and 36,9% for nematodes and coccidia, respectively. The relevant risk factors for nematodes were cutting suitability and the extensive rearing system, for coccidia, the risk factors were age $\leq$ 12 months and body score between 1 and 2. Therefore, the frequency of coccidia and gastrointestinal nematodes in cattle herds in Paraíba. In chapter III, the diversity of *Eimeria* spp. species was described, also in the bovine herds of Paraíba. Twenty farms were surveyed and 40 cattle from each were used randomly. The fecal samples collected from the animals were submitted to the centrifuge-float technique in sucrose solution, at 3.000 rpm. Coccidia were visualized using a MAX-300® optical microscope with a 40X objective. From each positive animal, 20 coccidia were photographed and measured, being detected in 17,12% of the analyzed samples. In total, 2.740 coccidia were photographed and measured, totaling 14 species of *Eimeria* spp., whose main frequencies were: *Eimeria bovis* (35,10%), followed by *Eimeria canadensis* (17,48%), *Eimeria auburnensis* (14,70%), *Eimeria ellipsoidallis* (9,70%), *Eimeria zuernii* (7,22%), *Eimeria brasiliensis* (4,56%), *Eimeria bukidnonensis* (3,97%), *Eimeria illinoisensis* (2,91%). Cattle in the semiarid region of Paraíba are parasitized by 14 species of *Eimeria*, management corrections should reduce infection by these parasites. It was concluded with the unpublished study that anthelmintic multiresistance is high in bovine gastrointestinal nematodes in the semiarid region of Paraíba and levamisole showed lower resistance. The frequency of coccidia and gastrointestinal nematodes in cattle is also high, with infections caused by a high diversity of *Eimeria* spp. with animals showing polyparasitism by up to seven species. It is suggested the adoption of an adequate sanitary management for the reality of each herd and the periodic technical assistance by a qualified veterinarian, contributing to the reduction of the parasitic indices and better performance of the animals, generating greater profitability for the ranchers.

KEY-WORDS: Coccidia; Nematodes; Parasitic Resistance, Ruminants; Semiarid.

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INTRODUÇÃO GERAL

A bovinocultura ocupa lugar de destaque no cenário mundial, sendo o Brasil um dos maiores produtores de leite e exportadores de carne bovina do mundo (BRASIL, 2021). Na região Nordeste, os produtores utilizam seus produtos e subprodutos na alimentação e no comércio, gerando estabilidade e desenvolvimento. No estado da Paraíba, a produção de ruminantes é uma atividade viável, fornecendo uma das principais fontes de proteína animal para a alimentação humana. Entretanto, existem alguns limitantes na produtividade dos rebanhos, dentre eles, as endoparasitoses gastrintestinais, responsáveis pela diminuição no consumo de alimentos e absorção de nutrientes, crescimento retardado, queda na produção de carne e leite e mortalidade (CEZAR et al., 2008; ALMEIDA et al., 2020).

A principal forma de controle parasitário é feita pelo uso de compostos químicos de amplo espectro de atuação, na maioria das vezes administrados de forma empírica (CEZAR et al., 2010). Dentre os anti-helmínticos mais utilizados, destacam-se a Ivermectina, do grupo das lactonas macrocíclicas, o Cloridrato de Levamisol, do grupo dos imidazotiazóis (COLES et al., 2006). Seguido pelo Closantel, do grupo das salicilanilidas e o Sulfóxido de Albendazol, do grupo dos benzimidazois (TAYLOR; COOP; WALL, 2013).

A utilização irracional de anti-helmínticos vem contribuindo para a resistência das parasitoses gastrintestinais dos bovinos à maioria das drogas disponíveis no mercado (RAMOS et al., 2018; 2019). Vem sendo demonstrada em vários continentes e evidenciam a problemática no controle dos nematódeos gastrintestinais em bovinos (SUTHERLAND; LEATHWICK 2011; DEMELER et al., 2009; LOBAYAN et al., 2017; BAIAK et al., 2019). Em alguns países existem altas taxas de resistência, a exemplo da Nova Zelândia (LEATHWICK; LUO, 2017), em partes do Brasil (RAMOS et al., 2019), Argentina (CRISTEL et al., 2017) e Estados Unidos (GASBARRE et al., 2014).

Simultaneamente aos helmintos gastrintestinais, os protozoários entéricos são verdadeiras ameaças para a produção de bovinos, e tendem a aumentar a gravidade do parasitismo gastrintestinal (HILLESHEIM; FREITAS, 2016), afetando principalmente animais de até dois anos de idade (BOWMAN et al., 2010).

Os principais protozoários de interesse médico veterinário pertecem ao filo Apicomplexa, caracterizados pelo parasitismo intracelular obrigatório, que causam doenças destruindo a célula hospedeira (DUBEY, 2019). Os membros mais importantes são os coccídios, representados principalmente pelo gênero Eimeria, são transmitidas por contaminação feco-oral e se desenvolvem nas células epiteliais do trato digestivo causando uma enterite denominada eimeriose ou coccidiose (BOWMAN et al., 2010; FLORIÃO et al., 2016).

Em sua forma clínica, as coccidioses são responsáveis por quadros de diarreias fétidas, desidratação, febre e óbito quando não há uma intervenção rápida pelo médico veterinário (LIMA, 2004; FLORIÃO et al., 2016). Os animais adultos são hospedeiros assintomáticos, servindo como fontes de infecção para os animais jovens que são mais susceptíveis ao parasita, apresentando distúrbios gastrintestinais e consequentemente retardo no crescimento (DAUGSCHIES; NAJDROWSKI, 2005; HILLESHEIM; FREITAS, 2016).

Todos os bovinos desenvolvem a infecção por uma ou mais espécies de Eimeria, especialmente durante os primeiros dois anos de vida, no entanto, surtos da doença podem ocorrer com frequência, atribuídos a *Eimeria zuernii* ou a *Eimeria bovis* (DUBEY, 2019), consideradas as espécies de maior importância clínica em bovinos em todo o mundo (DAUGSCHIES; NAJDROWSKI, 2005; DAS et al., 2015; HILLESHEIM; FREITAS, 2016). *Eimeria alabamensis* e *Eimeria auburnensis* foram relatadas em surtos de coccidiose clínica de patogenicidade moderada (DAUGSCHIES; NAJDROWSKI, 2005; BOWMAN et al., 2010; HILLESHEIM; FREITAS, 2016).

Outras espécies como *Eimeria ellipsoidalis*, ocasionalmente causadora de diarréia (MIELKE et al., 1993), *Eimeria brasiliensis*, *Eimeria bukidnonensis*, *Eimeria canadenses*, *Eimeria cylindrica*, *Eimeria ildefonsoi*, entre outras, já foram descritas, porém são caracterizadas como pouco patogênicas, manifestando-se de forma subclínica (LIMA 2004; DAUGSCHIES; NAJDROWSKI, 2005; ALMEIDA et al., 2011; DAS et al., 2015; FLORIÃO et al., 2016; HILLESHEIM; FREITAS, 2016).

Diante disso, a identificação das várias espécies de *Eimeria* spp., e da intensidade das infecções em bovinos, tornam-se muito importantes, pois direcionam as medidas de controle e prevenção da doença, favorecendo a adequada administração de medicamentos e desinfecção das instalações dos animais nas produções convencionais (DAUGSCHIES;

NAJDROWSKI, 2005), principalmente sob condições de manejo intensivo e confinamentos, devido a maior taxa de lotação (LIMA, 2004).

Devido à importância da bovinocultura e aos prejuízos econômicos que as endoparasitoses causam nos rebanhos, faz-se necessário um estudo sobre a resistência anti-helmíntica e os parasitos gastrintestinais de bovinos no semiárido da Paraíba. Servirá como base para definir o controle da verminose e protozooses, pois será determinada a real eficácia anti-helmíntica dos principais fármacos do mercado utilizados no rebanho bovino paraibano, os níveis de infecção e a frequência dos nematóides e enteroprotozoários que parasitam os bovinos e promovem a diminuição dos índices produtivos do rebanho.

Além disso, é importante o conhecimento das práticas de manejo de rebanho atuais adotadas no controle da verminose, bem como a avaliação da susceptibilidade dos nematóides aos anti-helmínticos em propriedades rurais das principais mesorregiões paraibanas de bovinocultura. Esse conhecimento irá contribuir efetivamente com a diminuição dos problemas de controle da verminose e o retardo no desenvolvimento de resistência aos fármacos anti-helmínticos mais utilizados, minimizando assim os prejuízos econômicos na bovinocultura da Paraíba, Brasil.

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CAPÍTULO I

Resistance of bovine gastrointestinal nematodes to four classes of anthelmintics in the semiarid region of Paraíba state, Brazil

Manuscito publicado no periódico “Brazilian Journal of Veterinary Parasitology”
ISSN: 0103-846X; Fator de impacto: 1.458; Qualis A2
Vol. 30, número 3, jun.-ago. 2021 Doi: https://doi.org/10.1590/S1984-29612021077

Resistance of bovine gastrointestinal nematodes to four classes of anthelmintics in the semiarid region of Paraíba state, Brazil

Resistência de nematódeos gastrintestinais de bovinos à quatro classes de anti-helmínticos no semiárido do Estado da Paraíba, Brasil

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Abstract

The effectiveness of four anthelmintic classes on cattle gastrointestinal nematodes in the semi-arid region of Paraiba State, Brazil, was evaluated. Twenty farms were used, testing 40 animals in each one, totaling 800 animals. Cattle were divided into four groups composed with ten animals: I, treated with albendazole sulfoxide 15%; II, treated with ivermectin 1%; III, treated with closantel 25%; IV, treated with levamisole hydrochloride 7.5%. All treatments were administered subcutaneously. For the Fecal Egg Count Reduction Test (FECRT), individual fecal samples were collected on days 0 and 14, and sent for analysis of egg count per gram of feces (EPG) and larval cultures. It was observed that multiresistance was present in 95% (19/20) of the farms. Resistance to ivermectin and albendazole was observed in 95% (19/20), to closantel in 75% (15/20) and to levamisole in 20% (4/20). The most used management system was semi-intensive (75%; 15/20) and the ivermectin was the most reported drug for controlling helminths (65%; 13/20). *Haemonchus* spp. was the most prevalent helminth genus. It was concluded that the anthelmintic resistance of bovine gastrointestinal nematodes is high in the semi-arid of

Paraíba State, Brazil, with multiresistance observed mainly to ivermectin, albendazole and closantel.

Keywords: Antiparasitics, helminthiasis, multiresistance, ruminants.

Resumo

Avaliou-se a eficácia de quatro classes de anti-helmínticos sobre nematódeos gastrintestinais de bovinos na região semiárida da Paraíba, Brasil. Foram utilizadas 20 fazendas, sendo testados 40 animais em cada uma, totalizando 800 animais. Os bovinos foram distribuídos em quatro grupos compostos por dez animais: I, tratado com sulfóxido de albendazol 15%; II, tratado com ivermectina 1%; III, tratado com closantel 25%; IV, tratado com cloridrato de levamisole 7,5%. Para o Teste de Redução da Contagem de Ovos Fecais (TRCOF), amostras fecais individuais foram coletadas nos dias 0 e 14 e enviadas para análises de contagem de ovos por grama de fezes (OPG) e coproculturas. Observou-se que a multirressistência estava presente em 95% (19/20) das fazendas. Foi observada resistência à ivermectina e ao albendazol, em 95% das fazendas (19/20); ao closantel, em 75% (15/20) e, ao levamisole, em 20% (4/20). O sistema de manejo mais utilizado foi o semi-intensivo (75%; 15/20) e a ivermectina foi o fármaco mais relatado para controle de verminose (65%; 13/20). O gênero de helminto mais prevalente foi *Haemonchus* spp. (76,7%). Conclui-se que é alta a resistência anti-helmíntica por nematódeos gastrintestinais de bovinos no Semiárido da Paraíba, Brasil, com multirressistência observada principalmente à ivermectina, ao albendazol e ao closantel.

Palavras-chave: Anti-parasitários, helmintoses, multirressistência, ruminantes.

Introduction

Brazil stands out among the milk and beef-producing countries, with the fastest-growing agribusiness in the world. This country finished 2020 with a record final balance of US$ 87.7 billion, which contributed positively and decisively to the total trade balance (Kreter et al., 2021). In this country, the profitability of livestock activities can be significantly reduced by the effects of parasites, which affect the welfare and productivity

of cattle. Infections with gastrointestinal nematodes have an annual economic impact of 7.11 billion dollars (Grisi et al., 2014).

The main genera of nematodes that parasitize cattle are *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., belonging to the *Trichostrongylidae* family; *Oesophagostomum* sp., belonging to the *Strongylidae* family; *Strongyloides* sp., to the *Strongyloididae* family; and *Trichuris* spp., to the *Trichuridae* family. In cattle, the parasitic infection occurs under ideal temperature and humidity conditions, and transmission occurs during the ingestion of pasture contaminated with infective larvae, which, in the animal's gastrointestinal tract, become adults, reaching reproductive maturity, reproducing and eliminating their eggs in the environment through feces (Bowman, 2010; Neves, 2014). This cycle is completed around 28 to 35 days. However, the infecting larvae can remain in the environment for a few months (Taylor et al., 2013). Usually, cattle nematode infections are mixed, in which more than one species parasitizes the same animal (Fávero et al., 2020). The absence of adequate anthelmintic control can lead to significant losses, which gives rise to decreased food intake, gastrointestinal disorders and impaired animal development, leading to death in extreme cases (Cezar et al., 2008; Almeida et al., 2020).

To avoid losses relating to helminth infections, the main means of control is through use of chemical compounds with a broad spectrum of activity, mainly comprising macrocyclic lactones, benzimidazoles, imidazothiazoles and salicylanilides (Taylor et al., 2013; Silva et al., 2017; Ramos et al., 2020). Most of the time, these are administered without applying any technical criteria for drug selection, in an empirical and indiscriminate manner. This has additional implications for the effectiveness of anthelmintic treatments, since it causes the emergence and dissemination of parasitic resistance (Neves et al., 2014; Geurden et al., 2015. Ramos et al., 2018; 2020).

The resistance of bovine gastrointestinal nematodes to most drugs available on the market has become an emerging problem worldwide (O'Shaughnessy et al., 2014; Rose et al., 2015; Berk et al., 2016; Lobayan et al., 2017; Baiak et al., 2019). In several countries, there are high rates of resistance, such as in New Zealand (Leathwick & Luo, 2017), Australia (Bullen et al., 2016), Germany, Belgium and Sweden (Demeler et al., 2009), Sudan (Mohammedsalih et al., 2021), United States (Gasbarre, 2014) and Argentina (Cristel et al., 2017). In Brazil, there have been reports of resistance to benzimidazoles

(Ramos et al., 2020; Fávero et al., 2020), macrocyclic lactones (Neves et al., 2014; Borges et al., 2015; Ramos et al., 2020), imidazothiazoles (Neves et al., 2014; Ramos et al., 2020) and salicylanilides (Silva et al., 2017; Ramos et al., 2020).

In the semiarid region of Brazil, information about the effectiveness of anthelmintics is scarce. Therefore, the objective of the present study was to obtain more information on anthelmintic resistance in cattle in the semiarid region of the state of Paraíba, northeastern Brazil. The efficacy of injectable formulations of albendazole sulfoxide, ivermectin, closantel and levamisole hydrochloride was evaluated in naturally infected cattle in 20 farms from 20 different municipalities across the state of Paraíba.

Material and Methods

Ethical approval and location of the study

This study was approved by our institution's research ethics committee, under registration number 23000.000663.2019-81.

It was carried out in the semi-arid region of the state of Paraíba, northeastern Brazil. This area forms part of the Caatinga biome. Its rains are irregular and are concentrated in the months from January to May, with average annual precipitation between 250 and 800 mm. The average maximum temperature is 32 °C and the average minimum temperature is 20 °C. There are high rates of evaporation and the relative humidity of the air is around 70% (IBGE, 2019).

Fecal samples were collected between January and December 2020, on 20 cattle farms that had herds of more than 40 animals, with a history of problems with nematode control or indiscriminate anthelmintic use, and on which the cattle had not been dewormed for at least 90 days. Each farm was located in a different municipality, as shown in Figure 1.

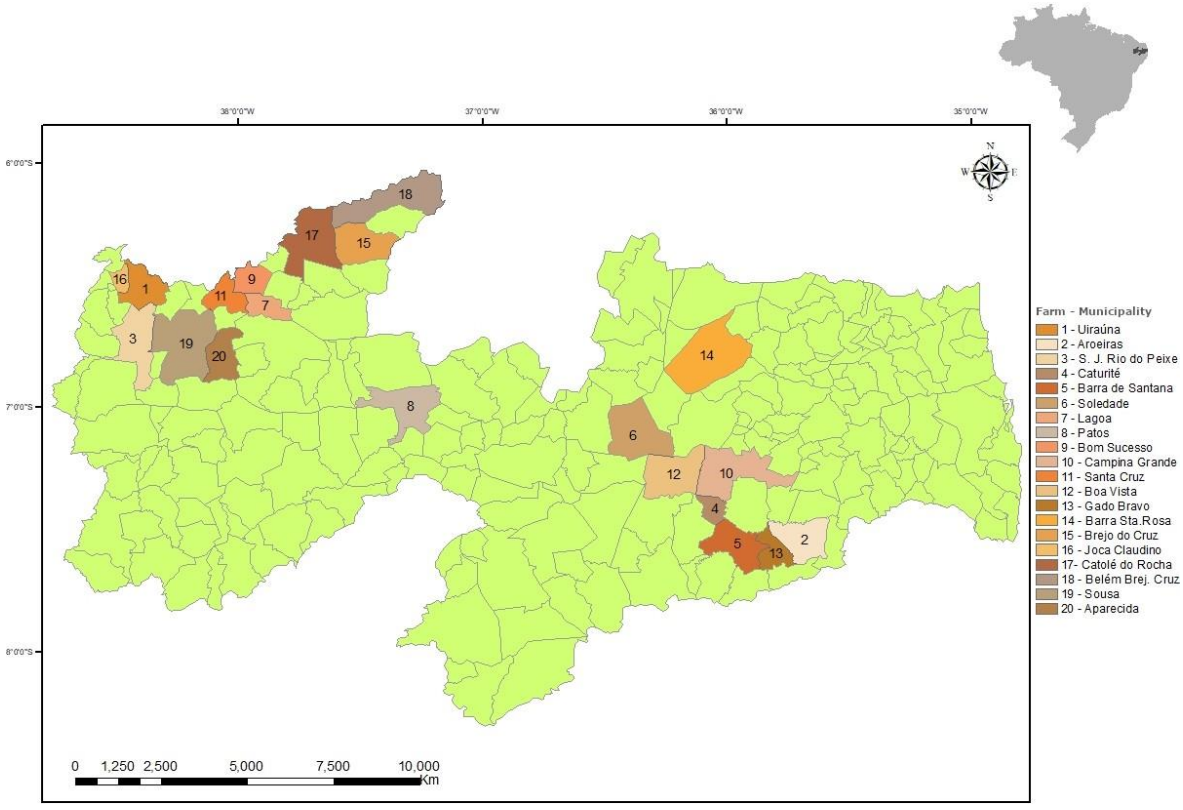

Figure 1. Geographical distribution of the municipalities in which farms were visited for undertaking anthelmintic resistance tests on bovine gastrointestinal nematodes, in the semiarid region of Paraíba state, Brazil.

Diagnosis of anthelmintic resistance

On each farm, 40 naturally infected animals were used, of both sexes, aged between four months and eight years, regardless of breed. These 40 animals had an egg count per gram of feces (EPG) ≥ 150. Subsequently, the animals were identified individually through ear tags and were randomly distributed into four groups composed of ten animals each: group I, treated with albendazole sulfoxide 15% (3.4 mg / kg) (Agebendazol®, Agener União); group II, treated with ivermectin 1% (0.2 mg / kg) (Ivomec®, Boehringer Ingelheim); group III, treated with closantel 25% (5 mg / kg) (Taitec®, Calbos); group IV, treated with levamisole hydrochloride 7.5% (3.75 mg / kg) (Ripercol®, Zoetis). Prior to the treatments, all animals underwent weight estimation by means of a weighing tape (Fita Torácia para Pesar Gado®, MultitecAgro). All treatments were performed as a single dose, administered subcutaneously after previous antisepsis of the application site, in accordance with the manufacturers' recommendations.

Fecal samples were collected individually and directly from the rectal ampoule, on the same day, before the anthelmintic treatment (day 0) and 14 days after (day 14) (FAO, 2004). Subsequently, these were placed in plastic bags, labeled, kept refrigerated in an isothermal box and promptly sent to the Veterinary Parasitology Laboratory (VPL) of the Instituto Federal da Paraíba (IFPB), Sousa, Paraíba, for analysis.

EPG counts were performed by a McMaster modified technique (Gordon & Whitlock, 1939), with a sensitivity of 50 EPG. Larval cultures (Roberts & O'Sullivan, 1950) were carried out per group and per collection, from a pool of samples. However, when the mean EPG was zero in a given group on day 14, larval cultures were not performed.

Were used eggCounts package version 2.3, which is a "R" package developed to analyze faecal egg count reduction using Bayesian hierarhical models (Wang & Paul, 2018). The percentage efficacy was calculated, along with the lower (L95) and upper (U95) 95% confidence limits. The models are tailored for a variety of practical situations, including individual treatment efficacy, zero inflation, small sample size and potential outliers (Wang et al., 2018).

The anthelmintic resistance status was interpreted using the method described by Lyndal-Murphy et al. (2014), and based on the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines on anthelmintic resistance (Coles et al.,

1992), considering the EPG reduction percentage and the upper and lower 95% confidence limits.

- Efficacious (E): percentage reduction and upper 95% confidence limit above 95% and lower 95% confidence limit above 90%.
- Confirmed anthelmintic resistance (R): percentage reduction and upper 95% confidence limit below 95% and lower 95% confidence limit below 90%.
- Suspicion of resistance (S): neither of the above criteria fulfilled.

Epidemiological questionnaire

A structured epidemiological questionnaire was used at the farms visited, to collect information about the rearing system (extensive, intensive or semi-intensive), number of animals, type of herd exploitation, average daily milk production, deworming strategy, drugs used, frequency of drug use, routes of administration, use of endectocides and movements of animals (purchase and/or sale).

Results

The arithmetic mean (AM), minimum and maximum faecal egg counts before and after treatment, the percentage efficacy calculated based on AM, along with the lower (L95) and upper (U95) 95% confidence limits per anthelmintic and per farm are shown in Tables 1-4. Multidrug resistance was observed in 95% (19/20) of the farms evaluated in this study. Among these farms, 5% (1/20) showed resistance to one drug, 20% (4/20) to two drugs, 60% (12/20) to three drugs and 15% (3/20) to all four of the drugs evaluated (Tables 1-4). Anthelmintic resistance to ivermectin and albendazole was detected in 95% (19/20) of the herds, closantel in 75% (15/20) and levamisole in 20% (4/20) (Table 5).

In larval cultures, presence of five genera of gastrointestinal helminths was observed. These were predominantly *Haemonchus* spp. (76.7%), which was most prevalent in all larval cultures, followed by *Trichostrongylus* spp. (13.2%), *Oesophagostomum* sp. (8.5%), *Cooperia* spp. (1.1%) and *Strongyloides* sp. (0.5%) (Table 6).

It was observed that the management system most used was semi-intensive (75%; 15/20), followed by extensive (20%; 4/20) and intensive (5%; 1/20). Dairy farms were the most frequent type among the farms studied (40%; 8/20), followed by mixed farms (35%;

7/20) and beef farms (25%; 5/20). The number of cattle per farm ranged from 45 to 600 animals and the average daily milk production ranged from zero (beef herds) to 1700 liters.

Among the active ingredients most reported by the farmers for controlling worms, ivermectin was cited as the only drug used in 65% (13/20) of the farms, followed by doramectin (20%; 4/20), which are both in the group of macrocyclic lactones. In addition, ivermectin, doramectin and moxidectin were administered alone or in association also for controlling *Rhipicephalus microplus* and/or *Haematobia irritans* on 95% (19/20) of the farms. Levamisole was mentioned in 15% (3/20) of the farms, used separately on one farm, in association with ivermectin on another and ins association with ivermectin and doramectin on the third. There was no report of previous use of albendazole or closantel.

Anthelmintics were administered by means of injection on 100% of the farms. This took place in the entire herd at least once a year, mostly during the rainy season (January to May), on 75% (15/20) of the farms. On the remaining 25% (5/20) of the farms, this was only done when verminosis was suspected and, in these cases, administration was individual.

Discussion

In the semiarid region of northeastern Brazil, the present study was the first to test the anthelmintic efficacy of four distinct pharmacological groups against bovine gastrointestinal nematodes. Multiresistance was found in 19/20 herds tested. The phenomenon of multidrug resistance was also observed by Ramos et al. (2018, 2020) in the state of Rio Grande do Sul in 100% (10/10) of the farms studied and by Soutello et al. (2007) in 20% (5/25) of the farms assessed in the northwest of the state of São Paulo.

Table 1. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Ivermectin 1% (IVM) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	IVM									
	Pre			Post			Efficacy			
	AM	Min	Max	AM	Min	Max	%	L95	U95	Status
1	240	150	850	120	0	400	50.9	39.6	88.7	R
2	160	150	200	120	0	150	24.2	0	44.4	R
3	650	150	2500	200	0	500	68.1	32.1	82.0	R
4	155	150	200	155	0	450	0	0	0.19	R
5	155	150	200	155	0	250	0	0	0.9	R
6	233.3	150	500	133.3	0	550	42.7	12.6	75.9	R
7	165	150	200	15	0	50	91.2	83.3	98.6	S
8	175	150	400	120	0	200	31.8	0	87.5	R
9	160	150	250	160	0	250	0	0	0.7	R
10	170	150	350	170	0	600	0	0	0.23	R
11	155	150	200	155	0	200	0	0	0.15	R
12	230	150	900	85	0	300	62.8	48.8	87.1	R
13	200	150	600	160	0	550	21.9	0	37.2	R
14	395	150	1650	130	0	900	66.2	23.2	78.2	R
15	410	150	2450	90	0	400	76.1	44.1	91.6	R
16	160	150	200	160	0	250	0	0	0.7	R
17	255	150	1000	100	0	250	63.2	31.2	78.8	R
18	170	150	350	150	0	650	13.7	0	32.2	R
19	205	150	550	140	0	450	30.9	0	89.2	R
20	465	150	3450	50	0	250	86.2	52.2	92.1	R

The results regarding ivermectin demonstrated that anthelmintic resistance was present on 95% (19/20) of the farms, with 36.5% of efficacious mean for FECR. In São Paulo, Soutello et al. (2007) and Neves et al. (2014) detected anthelmintic resistance in 92% (23/25) and 100% (10/10), respectively, of cattle herds evaluated, after treatment with this drug. Ramos et al. (2020), in Rio Grande do Sul, also detected resistance on all the farms examined (7/7). In Argentina, resistance to ivermectin was seen on 93.5% (58/62) (Lobayan et al., 2017) and 100% (4/4) (Cristel et al., 2017) of the cattle farms evaluated.

In line with the lower efficacy rates of ivermectin, it was observed that macrocyclic lactones, especially avermectins, were the drugs most used for anthelmintic treatments on the farms studied, similarly to what had been observed in other studies (Pereira, 2011; Suarez & Cristel, 2014). Soutello et al. (2007) and Ramos et al. (2018, 2020) stated that the higher rate of resistance to ivermectin, compared with other drugs, was probably related to its frequent use, and to its easy availability and ease of acquisition by farmers. Use of this drug has also been reported for controlling ticks (*R. microplus*) and horn flies (*H. irritans*), which indiscriminately boosts its use in cattle herds, thus generating greater pressure for selection and dissemination of resistant alleles in parasite populations (Graef et al., 2013; Neves et al., 2014; Vilela et al., 2020). These actions can promote faster development of drug resistance (Leathwick & Luo, 2017).

For albendazole, anthelmintic resistance was also observed on 95% (19/20) of the farms, with 51.5% of efficacious mean for FECR. Ramos et al. (2020) obtained similar values for the FECR (49.8%) and detected resistance to this drug on all the farms evaluated (7/7). On the other hand, Soutello et al. (2007) detected anthelmintic resistance on only 20% (5/25) of the farms examined, with an average FECR of 75.9%. In Sudan, Mohammedsalih et al. (2021) reported that a reduction (< 90%) in the effectiveness of albendazole on bovine gastrointestinal nematodes had occurred.

Resistance to closantel was observed on 75% (15/20) of the farms, with 55.8% of efficacious mean for FECR, which was similar to the results obtained by Ramos et al. (2020), of 55.2%, and Silva et al. (2017), of 45.45%. However, Bushra et al. (2019) and Maqbool et al. (2018) found reductions of 94.44% and 100%, respectively, in India. In the present study, despite the observed resistance, no use of this drug was reported in the herds. According to Neves (2014), the low use of closantel among cattle may be due to the

fact that most products sold are for oral use, thus limiting the possibility of administering them to cattle.

Table 2. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Albendazole Sulfoxide 15% (ALB) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as confirmed anthelminti resistance (R) or suspicion of resistance (S).

Farm	ALB									
	Pre			Post			Efficacy			
	AM	Min	Max	AM	Min	Max	%	L95	U95	Status
1	340	150	1950	145	0	650	57.8	42.3	78.7	R
2	155	150	250	100	0	150	36.1	0	73.2	R
3	355	150	1150	225	50	500	35.7	0	78.9	R
4	160	150	300	160	0	450	0	0	0.17	R
5	160	150	450	40	0	350	77.2	66.1	94.4	R
6	300	150	500	160	0	600	42.1	0	83.1	R
7	150	150	250	75	0	200	50.3	37.2	78.9	R
8	155	150	250	90	0	150	42.1	0	77.2	R
9	250	150	500	30	0	100	88.3	71.2	92.0	R
10	185	150	500	185	0	1050	0	0	0.3	R
11	200	150	350	65	0	100	67.9	61.2	91.1	R
12	315	150	1100	65	0	200	78.7	70.4	89.0	R
13	285	150	650	210	0	600	29.1	0	69.3	R
14	245	150	900	160	0	550	22.6	0	70.1	R
15	265	150	1300	10	0	100	97.7	87.2	100.0	S
16	195	150	300	75	0	100	62.3	50.7	88.2	R
17	420	150	2900	45	0	150	88.3	79.2	93.2	R
18	165	150	250	165	0	400	0	0	0.21	R
19	205	150	400	65	0	150	71.2	36.4	94.1	R
20	855	150	4900	135	0	450	81.9	54.3	89.8	R

Table 3. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Closantel 25% (CLO) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S).

	CLO									
Farm	Pre			Post			Efficacy			
	AM	Min	Max	AM	Min	Max	%	L95	U95	Status
1	210	150	750	70	0	250	67.2	28.3	83.2	R
2	150	150	150	100	0	200	31.7	0	44.2	R
3	170	150	250	25	0	100	86.9	64.7	97.3	S
4	180	150	250	40	0	50	77.6	41.3	94.3	R
5	345	150	1900	25	0	200	91.1	81.4	97.6	S
6	266,7	150	600	11,1	0	50	94.3	71.4	100.0	S
7	165	150	350	35	0	200	79.6	44.4	91.4	R
8	190	150	550	65	0	300	66.6	42.1	93.7	R
9	240	150	1050	295	0	1000	0	0	0.15	R
10	150	150	150	150	0	200	0	0	0.1	R
11	155	150	200	155	0	250	0	0	0.14	R
12	535	150	1550	295	0	600	47.2	0	83.3	R
13	220	150	600	195	0	350	12.3	0	44.1	R
14	490	150	1200	85	0	250	88.2	75.3	94.1	R
15	210	150	350	15	0	50	96.1	91.4	100.0	E
16	185	150	500	90	0	600	47.3	12.1	71.3	R
17	300	150	800	165	0	1150	49.1	84.6	91.3	R
18	245	150	600	170	0	300	32.2	0	66.2	R
19	375	150	1700	15	0	50	98.8	94.6	100.0	E
20	230	150	750	110	0	500	50.7	79.3	87.2	R

Resistance to levamisole was observed on 20% (4/20) of the farms. Similar results regarding resistance to this drug were reported by Ramos et al. (2020), of 28% (2/7), and Bullen et al. (2016), of 25% (5/20), in Australia. In the present study, levamisole demonstrated the highest efficacious mean (93.1%). Efficacious status was obtained on

55% (11/20) of the farms. The good efficacy of this drug in the herds tested may have resulted of its low selection pressure, as it was not used frequently by the farmers, such that it was only mentioned on 15% (3/20) of the farms.

The *Haemonchus* was the most prevalent parasite genus in all larval cultures, both before and after treatments. This has also been reported in several other studies evaluating bovine gastrointestinal nematodes (Borges et al., 2015; Lobayan et al., 2017; Silva et al., 2017; Ramos et al., 2020). This nematode probably acquires resistance faster due to its high biotic potential and great genetic variability. In addition, it harbors the allele that causes decreased susceptibility to a drug (Blackhall et al., 1998; Chaudhry et al., 2015). *Haemonchus placei* is the species that is considered most relevant in cattle (Borges et al., 2015; Santos et al., 2015). It causes gastric hemorrhagic lesions, due to its high capacity for hematophagy (Taylor et al., 2013).

Visual estimation of the animals' weight was the practice generally used by the farmers. According to Leathwick & Luo (2017), this can be reflected in inefficiency of the treatment, given that doses below or above those recommended by the manufacturers might be estimated. In addition, most of the farmers (75%; 15/20) used anthelmintics at the beginning of the rainy season. However, if the animals are treated and transferred to clean pastures, or if they are treated during the dry season, resistance can develop quickly, even if the animals receive few annual treatments with anthelmintics (Martin et al., 1981; Papadopoulos et al., 2001). Therefore, management methodologies and strategies should be designed to keep parasites in *refugia* in herds, thereby prolonging the effectiveness of current anthelmintics and preserving susceptible nematode genotypes (Berk et al., 2016).

Movement of animals without previous knowledge of the clinical history was a common practice reported by all the farmers. Moreover, no investigations or parasitological examinations were conducted on newly acquired animals. Both of these practices contribute to dispersion of resistant nematode populations. Bullen et al., (2016) suggested that, without prior knowledge of the farm's anthelmintic resistance status, movement of dairy cattle constitutes a considerable risk with regard to introduction of anthelmintic resistance on unaffected farms.

In addition, through PCR, Ramos et al. (2020) demonstrated the presence of co-infections of species of *Haemonchus* spp. that affect different cattle and sheep that share pastures. This matter deserves further study and may explain the high rates of anthelmintic

resistance found on the farms studied, especially in relation to the drugs albendazole and closantel. Although no previous use of these drugs was reported in the herds evaluated, they are widely used among small ruminants and anthelmintic resistance to them in the semi-arid region of Paraíba has already been reported (Lima et al., 2010; Silva et al., 2018).

Table 4. The arithmetic mean (AM), minimum (Min) and maximum (Max) faecal egg counts before (pre) and after (post) treatment of 10 animals per farm with Levamisole Hydrochloride 7.5% (LEV) on the 20 farms (1-20) in the semiarid region of Paraíba state, Brazil. The percentage (%) efficacy calculated based on the arithmetic group mean is provided, along with the lower (L95) and upper (U95) 95% confidence limits. The anthelmintic resistance status (Status) is provided as efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S).

Farm	LEV									
	Pre			Post			Efficacy			
	AM	Min	Max	AM	Min	Max	%	L95	U95	Status
1	255	150	2150	0	0	0	100	100.0	100.0	E
2	175	150	350	10	0	50	93.1	87.2	100.0	S
3	485	150	1500	40	0	250	95.7	90.1	99.2	E
4	230	150	800	0	0	0	100	100.0	100.0	E
5	170	150	600	25	0	200	84.2	63.2	94.1	R
6	261	150	950	6	0	50	98.9	91.3	100.0	E
7	155	150	200	0	0	0	100	100.0	100.0	E
8	165	150	300	55	0	200	72.3	37.3	91.7	R
9	175	150	300	20	0	150	90.5	82.3	100.0	S
10	300	150	700	20	0	150	91.9	83.9	96.0	S
11	235	150	400	15	0	100	92.7	66.2	98.3	S
12	240	150	750	0	0	0	100	100.0	100.0	E
13	165	150	300	45	0	150	73.9	36.4	89.1	R
14	225	150	850	0	0	0	100	100.0	100.0	E
15	290	150	1500	0	0	0	100	100.0	100.0	E
16	180	150	350	0	0	0	100	100.0	100.0	E
17	160	150	250	35	0	350	77.6	36.3	93.2	R
18	320	150	1550	20	0	150	94.2	90.6	98.0	S
19	370	150	1400	0	0	0	100	100.0	100.0	E
20	978	150	3000	5	0	50	98.1	98.6	100.0	E

Table 5. The number of farms with efficacious (E), confirmed anthelmintic resistance (R) or suspicion of resistance (S) for Ivermectin 1% (IVM), Albendazole Sulfoxide 15% (ALB), Closantel 25% (CLO) and Levamisole Hydrochloride 7.5% (LEV), and the percentage of efficacious mean (EM%) of the anthelmintics on cattle gastrointestinal nematodes in the semiarid region of Paraíba state, Brazil.

Treatment	N animals	N farms	EM (%)	R	S	E
IVM	200	20	36.5	19	1	0
ALB	200	20	51.5	19	1	0
CLO	200	20	55.8	15	3	2
LEV	200	20	93.1	4	5	11
Total				57	10	13

Table 6. Percentage (%) of cattle gastrointestinal nematodes by genus and farm recovered from larval cultures before (pre) and after (post) anthelmintic treatments with Ivermectin 1% (IVM), Albendazole Sulfoxide 15% (ALB), Closantel 25% (CLO) and Levamisole Hydrochloride 7.5% (LEV) in the semi-arid region of Paraíba state, Brazil.

		Farms																			
		IVM																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Pre	H	84	91	85	75	71	94	50	72	80	75	100	89	51	82	80	62	74	90	100	80
	T	10	6	9	16	14	4	40	13	18	25	0	3	49	0	4	23	4	5	0	19
	O	6	3	0	6	11	0	10	11	2	0	0	1	0	18	16	15	22	5	0	1
	C	0	0	6	3	3	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	0	0	0	7	0	0	0	0	0	0	0	0
Post	H	94	74	78	95	97	61	93	75	98	70	60	100	81	85	76	95	74	74	94	58
	T	6	7	12	3	2	34	7	8	2	30	30	0	1	5	18	2	6	21	4	42
	O	0	9	5	2	1	5	0	17	0	0	0	0	18	10	6	3	20	5	2	0
	C	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	10	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
		ALB																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Pre	H	92	95	93	83	70	100	82	71	80	51	94	93	100	76	64	86	87	85	80	57
	T	2	5	2	9	13	0	8	14	19	49	4	2	0	0	30	7	3	15	20	33
	O	6	0	5	6	13	0	6	11	0	0	2	0	0	24	5	7	10	0	0	10
	C	0	0	0	2	3	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	1	0	0	5	0	0	1	0	0	0	0	0
Post	H	98	89	90	69	100	79	97	75	80	64	79	100	76	94	100	82	89	86	98	85
	T	2	7	5	25	0	7	2	25	20	30	13	0	21	4	0	0	11	5	1	15
	O	0	4	5	0	0	14	1	0	0	6	8	0	2	2	0	18	0	9	1	0
	C	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		CLO																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Pre	H	75	70	71	100	85	65	94	70	69	71	70	51	66	75	81	84	79	82	95	86
	T	17	13	12	0	10	0	6	12	20	13	13	37	3	0	6	0	8	6	2	13
	O	6	13	14	0	5	11	0	14	11	12	14	12	31	25	13	16	13	12	3	1
	C	0	3	2	0	0	24	0	3	0	3	2	0	0	0	0	0	0	0	0	0
	S	2	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
Post	H	90	95	100	76	98	71	91	87	77	91	64	90	90	97	97	68	90	95	72	91
	T	8	3	0	9	2	0	9	0	21	4	13	3	0	3	1	27	5	2	22	9
	O	2	2	0	12	0	29	0	13	2	0	23	5	10	0	2	5	5	3	6	0
	C	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	5	0	2	0	0	0	0	0	0	0	0
		LEV																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Pre	H	95	87	86	67	70	75	72	71	51	94	72	90	51	96	74	91	100	80	75	52
	T	4	13	9	18	14	0	6	14	16	3	13	0	32	0	5	7	0	19	25	39
	O	1	0	5	6	12	25	17	11	33	3	11	1	17	4	21	2	0	1	0	9
	C	0	0	0	9	3	0	5	3	0	0	3	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	1	0	0	1	9	0	0	0	0	0	0	0	0
Post	H	-	95	100	-	98	100	-	95	80	100	95	-	100	-	-	-	100	83	-	97
	T	-	5	0	-	2	0	-	3	8	0	5	-	0	-	-	-	0	0	-	3
	O	-	0	0	-	0	0	-	1	12	0	0	-	0	-	-	-	0	17	-	0
	C	-	0	0	-	0	0	-	0	0	0	0	-	0	-	-	-	0	0	-	0
	S	-	0	0	-	0	0	-	0	0	0	0	-	0	-	-	-	0	0	-	0

H: *Haemonchus* spp.; T: *Trichostrongylus* spp.; O: *Oesophagostomum* sp.; C: *Cooperia* spp.; S: *Strongyloides* sp. For those groups with high efficacy no coproculture was performed (-)

To optimize the effectiveness of anthelmintics in populations of multidrug-resistant nematodes, Ramos et al. (2016) suggested that combinations of two drugs belonging to different chemical groups should be used. However, they stressed the importance of conducting anthelmintic efficacy tests in order to choose the chemical groups to be used, as well as introduction of control measures for gastrointestinal worms within the management of cattle. Acquisition of cattle without adequate sanitary management before or after transporting them needs to be avoided. This is especially important in relation to acquisition from farms on which grazing combined with goats and/or sheep is practiced: this can be considered to be a critical point regarding worm control. Chaudhry et al. (2015) reported the first genetic confirmation of hybridization between *H. contortus* and *H. placei* in the field, thus increasing the possibility of inter-species transmission of anthelmintic resistance mutations.

Conclusion

It was concluded that the anthelmintic resistance of bovine gastrointestinal nematodes in the semiarid region of Paraíba, northeastern Brazil, is high. Multidrug resistance was observed on almost all the farms evaluated, especially in relation to the drugs ivermectin, albendazole and closantel. Levamisole was considered to be the drug with the best anthelmintic efficacy. It can be suggested that the sanitary management of cattle herds in the semiarid region should be adapted so as to avoid mass deworming without applying technical criteria. Moreover, animals should only be transported after a parasitological diagnosis has been made, especially in the case of acquisition from farms with a history of problems regarding worm control and from farms use pastures intercropped between cattle and small ruminants.

Acknowledgements

The authors acknowledge finding from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and IFPB (Instituto Federal da Paraíba), for scholarship grants.

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CAPÍTULO II

Epidemiological survey of gastrointestinal infections by nematodes and coccidia in cattle in the semiarid region of northeastern Brazil

Manuscrito submetido ao periódico "Acta Parasitologica"
ISSN: 1230-2821; Fator de impacto: 1.440; Qualis B3

Epidemiological survey of gastrointestinal infections by nematodes and coccidia in cattle in the semiarid region of northeastern Brazil

Levantamento epidemiológico de infecções gastrintestinais por nematóides e coccídios em bovinos no Semiárido do Nordeste do Brasil

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Abstract

Purpose The aim of the present study was to carry out an epidemiological survey on gastrointestinal infections by nematodes and coccidia in cattle herds in the semiarid region of Paraíba, northeastern Brazil.

Methods A total of 832 cattle were sampled, of both sexes and different ages, on 21 farms in 21 municipalities in this state. Stool samples were collected individually to make eggs per gram (EPG) and oocysts per gram (OoPG) counts in feces, and to perform stool cultures. Epidemiological questionnaires were applied to the farmers.

Results The total frequency of nematodes was 59.6% (496/832) and it was 36.9% (307/832) for coccidia. The levels of infection by nematodes and coccidia were most frequently low (EPG > 0 ≤ 300; OoPG > 0 ≤ 1000), in 72.4% (359/496) and 75.2% (231/307), respectively. The most frequent nematode genus was *Haemonchus* spp. (78.8%). The risk factors for nematode infections in cattle were beef suitability (odds ratio = 2.99) and extensive rearing system (odds ratio = 3.8); the factors for coccidia were age ≤ 12 months (odds ratio = 2.9) and body score between 1 and 2 (odds ratio = 5.2).

Conclusion These results prove that there are high frequencies of presence of nematodes and coccidia and gastrointestinal infections in cattle herds in the semiarid region of Paraíba, with

predominantly low levels of infection. However, given the importance of subclinical infections, adjustments to sanitary management measures can contribute to reduction of parasite indices and better performance among the animals, thus generating greater profitability for farmers.

Keywords: cattle, frequency, parasitic infections, semiarid.

Resumo

Objetivo O objetivo do presente estudo foi realizar um levantamento epidemiológico das infecções gastrointestinais por nematóides e coccídios em rebanhos bovinos no semiárido da Paraíba, nordeste do Brasil.

Métodos Foram amostrados 832 bovinos, de ambos os sexos e diferentes idades, em 21 fazendas de 21 municípios deste estado. Amostras de fezes foram coletadas individualmente para realização de contagens de ovos por grama (OPG) e oocistos por grama (OoPG) de fezes e para realização de coproculturas. Questionários epidemiológicos foram aplicados aos produtores**.**

Resultados A frequência total de nematoides foi de 59,6% (496/832) e de 36,9% (307/832) para coccídios. O nível de infecção por nematóides e coccídios mais freqüente foi o baixo (EPG > 0 ≤ 300; OoPG > 0 ≤ 1000), em 72,4% (359/496) e 75,2% (231/307), respectivamente. O gênero de nematoide mais frequente foi *Haemonchus* spp. (78,8%). Os fatores de risco para infecções por nematoides em bovinos foram a aptidão para corte (odds ratio = 2,99) e o sistema de criação extensivo (odds ratio = 3,8); os fatores para coccídios foram a idade ≤ 12 meses (odds ratio = 2,9) e o escore corporal entre 1 e 2 (odds ratio = 5,2).

Conclusão Esses resultados comprovam que é alevada a frequência de nematóides e coccídios e infecções gastrintestinais em rebanhos bovinos do semiárido paraibano, com índices predominantemente baixos de infecção. No entanto, dada a importância das infecções subclínicas, ajustes nas medidas de manejo sanitário podem contribuir para redução dos índices parasitários e melhor desempenho entre os animais, gerando assim maior lucratividade para os pecuaristas.

Palavras-chave: bovinos, frequência, infeccções parasitárias, semiárido.

Introduction

Brazil is considered to be the second largest producer of beef in the world and has a herd of over 214 million head. Its agribusiness sector is currently the fastest growing worldwide and ended the year 2020 with a record final balance of US$ 87.7 billion [25]. This country also stands out with regard to milk production, which is one of the six most important products of Brazilian agribusiness and is an important generator of food and income for the population [7].

Among the factors that affect the cattle industry in Brazil, gastrointestinal parasites stand out. Several genera of parasites inhabit the digestive tract of cattle and are responsible for causing major economic losses in herds. These parasites compromise the animals' health through causing clinical pictures of inappetence, anemia, diarrhea, growth retardation and death in extreme cases [1, 15, 32].

Among nematode helminths, the most important and prevalent genera worldwide are those belonging to the superfamily *Trichostrongyloidea*, especially in tropical areas with a semiarid climate. These helminths cause parasitic gastroenteritis in cattle [20, 29, 30]. Among enteric coccidia, the genus *Eimeria* stands out because of its cosmopolitan distribution and parasitism in cattle worldwide, causing high levels of cases of coccidiosis, which can affect up to 100% of young cattle, especially during the first weeks of life [14, 17, 19].

Typically, the frequencies of subclinical cases of worms and coccidiosis are much higher and have the consequence of compromising intestinal physiology and feed conversion. This consistently retards animal growth for indeterminate amounts of time, compared with clinical cases. However, such cases can be quickly diagnosed and treated before death occurs [9, 19].

Antiparasitic drugs have been widely used to control gastrointestinal parasitic infections in animals over recent decades [17]. However, overuse has developed antiparasitic resistance, which has been reported in several regions of the world [3, 13, 31]. Resistant parasite populations hinder effective control in herds: in such situations, the animals develop clinical symptoms of greater severity, and high mortality rates may occur, thus raising the economic losses [1, 29].

Studies relating to the frequency and epidemiology of gastrointestinal parasites in cattle in northeastern Brazil are scarce. Nonetheless, evaluation of the parasite profile and severity of infection is important for development of appropriate management strategies for

parasite control. Therefore, our aim here was to perform an epidemiological survey of gastrointestinal infections by nematodes and coccidia in naturally infected cattle in the semiarid region of the state of Paraíba, Brazil.

Materials and Methods

Study location

This study was carried out in the semiarid region of the state of Paraíba, northeastern Brazil. This state has a total area of 58,584.6 km^2, of which 86.2% (48,788.9 km^2) are located in areas of semiarid climate. These areas are characterized by irregular rainfall that is concentrated in the months of January to May, with average annual rainfall totals of between 250 and 800 mm, maximum temperature of 32 ºC and minimum of 20 ºC, high evaporation rates, average relative humidity of 70% and predominant vegetation of the Caatinga biome [22]. This study was conducted in the period from January to December 2020, in 21 different municipalities belonging to the intermediate regions of Campina Grande, Patos and Sousa-Cajazeiras. One farm per municipality was sampled (Figure 1).

Samples collected and analysis processing

Twenty-one cattle farms with histories of gastrointestinal parasitism were used. The herds on the farms visited ranged in size from 36 to 650 animals. To standardize the collections, between 36 and 40 animals were sampled per farm, totaling 832 cattle of both sexes, various breeds and ages ranging from three months to nine years.

Fecal samples were individually collected directly from the rectal ampulla with the aid of procedure gloves lubricated with glycerin. The samples were immediately transported to the Veterinary Parasitology Laboratory, Instituto Federal de Educação, Ciência e Tecnologia da Paraíba, Campus Sousa, where they were analyzed using the flotation technique, as described by Gordon and Whitlock [16]. Through this, the levels of parasitism by nematodes (in terms of egg count per gram of stool, EPG) and by coccidia (in terms of oocyst count per gram of stool, OoPG) were determined.

Samples positive for EPG on the same farm were pooled. The pools were used to perform coprocultures as described by Roberts and O'Sullivan [35], in order to identify the specific nematode genus. To assess the levels of infection by helminths and coccids, the

following classification was used: low (> 0 ≤ 300 EPG; > 0 ≤ 1000 OoPG); medium (> 300 ≤ 1000 EPG; > 1000 ≤ 3000 OoPG); and high (> 1000 EPG; > 3000 OoPG).

Epidemiological questionnaire

On the farms that we visited, a structured epidemiological questionnaire was applied to collect information on variables that might explain occurrences of gastrointestinal parasite infections, including animal sex (male or female), age of cattle (≤ 12 months or > 12 months), animal score, farming system (extensive, intensive or semi-intensive), herd type (milk, beef or mixed), average daily milk production, calf shelter, period and amount of annual deworming, farm area and number of animals in the herd.

Statistical analysis

The data collected from the epidemiological questionnaires were used for analyses on factors associated with nematode and coccidian infections. The analyses were done in two stages: univariate and multivariate. In the univariate analysis, each independent variable was correlated with the dependent variable (positivity for infection), and those that presented P ≤ 0.20, according to the chi-square or Fisher's exact test [41], were entered into multivariate analysis using multiple logistic regression [21]. A significance level of 5% was adopted for the multivariate analyses. All the analyses were performed using SPSS 20.0 for Windows.

Results

The frequency of gastrointestinal parasitic infections in cattle in the semiarid region of Paraíba was 96.5% (803/832), among which 59.6% (496/832) of the cattle were positive for nematode infections and 36.9% (307/832) were positive for coccidia infections, respectively (Table 1). Coinfections by nematodes and coccidia were observed in 26.7% (222/832) of the animals.

Among the 21 farms analyzed, 95.2% (20/21) and 100% (21/21) had at least one animal positive for helminth infections and for coccidian infections, respectively. The frequencies of animals positive for nematodes on the same farm ranged from 0 to 86%, while for coccidia, the frequencies ranged from 5 to 67.5%.

On 20 farms, at least one animal was EPG positive. Thus, 20 coprocultures were performed to identify third-stage larvae. The presence of five genera of gastrointestinal nematodes was observed: predominantly *Haemonchus spp.* (78.8%), followed by *Trichostrongylus* spp. (12.1%), *Oesophagostomum* sp. (7.7%), *Cooperia* spp. (1.1%) and *Strongyloides* sp. (0.3%), without any relevant changes in frequency orders among the genera (Table 2).

The levels of infection by nematodes and coccidia in the cattle are presented in Table 3. For both groups of parasites, a low level of infection ($> 0 \leq 300$ EPG; $> 0 \leq 1000$ OoPG) was observed most frequently.

The information obtained from the epidemiological questionnaires was evaluated regarding the characteristics of the farms and the management performed on the cattle. It was observed that semi-intensive management was the system most used (76.2%; 16/21), followed by extensive (14.3%; 3/21) and intensive (9.5%; 2/21). Dairy farming was the most frequent type of farming on the farms studied (47.6%; 10/21), followed by mixed farming (38.1%; 8/21) and beef farming (14.3%; 3/21). The number of cattle per farm ranged from 45 to 650 animals and the average daily milk production ranged from zero in herds that were considered to be beef herds to 1,750 liters in herds that were aimed at dairy production.

According to the farmers, anthelmintics were administered to the entire herd at least once a year, mainly during the rainy season (January to May), on 76.2% (16/21) of the farms. On the other 23.8% (5/21), this was done only when verminosis was suspected, and it was administered individually. A clinical picture of diarrhea among the cattle, as isolated occurrences or in the form of an outbreak, was known among the farmers. It was commonly seen especially among calves on dairy farms and such occurrences were claimed to possibly cause the death of sick animals. Acquisition of animals was a common practice reported by the owners, but no quarantine of newly acquired cattle was implemented.

Table 4 shows the categories that presented statistically significant differences ($p \leq 0.20$) regarding nematode and/or coccidian infections in the univariate analysis. Subsequently, in the multivariate analysis, the relevant risk factors for nematode infection were fitness for slaughter and extensive rearing system; and for coccidia, the risk factors were age ≤ 12 months and body score between 1 and 2 (Table 5).

Discussion

In the semiarid region of northeastern Brazil, the frequency of cattle infected by gastrointestinal parasites was found to be high (96.5%), with rates of 59.6% for nematode infections and 36.9% for coccidia. Moreover, 100% of the farms had parasitized animals. High occurrence rates (99%) for gastrointestinal parasites was also previously reported among dairy cows and calves in the semiarid region of Bahia, northeastern Brazil, with rates of parasitism by helminths and coccidia in cattle of 66% and 33%, respectively [10]. In Colombia, a prevalence of 50.5% was observed among the cattle examined [32]. On the other hand, in tropical regions of Sri Lanka, Gunathilaka *et al.* [17] found a prevalence rate of gastrointestinal parasites among cattle of 11.6%. Those authors pointed out that the prevalence of infections and clinical cases may vary depending on the area, environmental factors and sanitary management measures used.

Low levels of infection (OPG $\leq$ 300; OoPG $\leq$ 1000) were observed most frequently with regard to both nematode and coccidian infections. Gunathilaka *et al.* [17], on the other hand, found parasite infection levels in cattle and buffaloes that exceeded EPG $\geq$ 500 and OoPG $\geq$ 5000, which, according to Gupta *et al.* [18] and Kashyap *et al.* [24], are considered to be very high infection levels. Despite the low level of infection found in most animals in the present study, it should be pointed out that some parasite species, such as *Haemonchus placei* and *Eimeria bovis* cause more severe damage regardless of the level of infection [11, 39]. The numbers of nematode eggs and oocysts eliminated by animals also varies depending on the species of the parasite, the susceptibility level of the host, the health status of the animal and the immune status of the animal [36, 40]. The severity of gastrointestinal parasitic infections, on the other hand, may be related to the vulnerability of animals to internal parasites and also to the non-responsive immunity of the host [17].

In the coprocultures, the genus *Haemonchus* spp. (78.8%) was more frequent, as also observed in other studies evaluating bovine gastrointestinal nematodes [37, 33]. This nematode probably acquires resistance faster due to its high biotic potential and high genetic variability, thus harboring the allele that causes decreased susceptibility to a drug [5, 8]. According to Borges *et al.* [6] and Santos *et al.* [38], the species considered most relevant in cattle is *H. placei*, because it causes gastric hemorrhagic lesions due to its high capacity for hematophagy [39]. Higher prevalences for the order *Strongylida* were also found in predominantly dairy cattle in northeastern Colombia (17.9%) and in the state of Minas Gerais, Brazil (47.2%), by Pinilla Leon *et al.* [32] and Henriques *et al.* [20], respectively.

A high frequency of enteric coccidia of the genus *Eimeria* spp. was observed in cattle in the present study (36.9%). However, lower prevalence was found by Pinilla Leon *et al.* [32] in northeastern Colombia (17.4%). Li *et al.* [28], in a systematic review study with meta-analysis in mainland China, found that the prevalence of *Eimeria* spp. in cattle ranged from 4.6% to 87.5% and recommended prevention strategies for detecting coccidia in calves in intensive livestock models in that region. A high prevalence of *Eimeria* spp. (72.07%) in cattle was also diagnosed by Hamid *et al.* [19], in nine provinces in Indonesia, mainly in calves, and the farmers were found to have been unaware of coccidiosis. Thus, it was suggested that an outreach program should be set up between farmers and veterinarians in order to ensure effective and efficient actions to prevent or cure the disease.

The dairy, mixed and beef production types observed among the farms studied here provide a significant source of income for farmers in the Brazilian semiarid region. Gastrointestinal parasitism among cattle is a limiting factor with regard to both dairy and beef production [12]. However, analysis on the factors associated with nematode infections revealed that beef animals were more susceptible (odds ratio = 2.99), as was the extensive farming system (odds ratio = 3.8). Probably because beef cattle are more simply managed, they spend most of their time in the extensive farming system, with little or no anthelmintic treatment. According to Almeida *et al.* [1], the extensive system is the main form of cattle rearing in Brazil, and this leads to frequent infections by parasites present in the pastures.

Age < 12 months was found to be an associated factor for enteric coccidian infection (odds ratio = 2.9). According to Bilal *et al.* [4] and Hamid *et al.* [19], the susceptibility of calves to coccidian infections is associated with their lower immunity, compared with the immunocompetence of adult cattle. It is also likely that poor physical condition among calf pens or confinements, high stocking rates and high humidity and temperature contribute to contamination of these environments. According to Gunathilaka *et al.* [17], high humidity and moderate temperature facilitate survival and sporulation of oocysts. One important finding may be that at the time when the calves' rumen begins to become functional and the calves start to ingest feed at the trough, they might lose out in competing for space with larger calves. Thus, they might tend to only ingest food scraps that fall to the floor, thereby increasing fecal-oral contamination by oocysts [25].

Another factor associated with enteric coccidian infections was cattle body scores between 1 and 2 (odds ratio = 5.2). This situation indicates decreased feed conversion as a consequence of damage to the intestine, which leads to decreased growth rates and weight gains [23]. Similar results were found by Bacha and Haftu [2] in Ethiopia, in which the body

condition of cattle had a strong relationship with the presence of gastrointestinal parasites. Likewise, Regasa *et al.* [34] found that infection by different species of *Eimeria* spp. in cattle, especially calves, was also associated with poor body condition among the animals. Furthermore, according to Kumar *et al.* [27], the type of diet and the availability of vitamins, minerals and other nutrients are directly related to the susceptibility of the animal to parasites.

Limited knowledge among farmers regarding management practices and animal health contribute to higher levels of nematode and coccidian infections in cattle [29]. Controlling these infections through good management practices can increase the profit margin of livestock activity in the semiarid region of Paraíba.

Conclusion

It was concluded that the frequency of presence of gastrointestinal nematodes and coccidia in cattle herds in the semiarid region of Paraiba, Brazil, is high, especially at low levels of infection. *Haemonchus* spp. and *Eimeria* spp. were the most frequently found nematode and coccidian genera, respectively. Beef cattle and the extensive breeding system were the factors associated with nematode infections; and age $\leq$ 12 months and body score between 1 and 2 were the factors associated with coccidian infections. Adoption of health management appropriate to the reality of each herd and availability of periodic technical assistance from a trained veterinarian can contribute to reduction of parasite rates and, consequently, improve animal performance, thereby generating greater profitability for cattle farmers.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lídio Ricardo Bezerra de Melo, Luana Carneiro de Sousa, Clarisse Silva Menezes de Oliveira, Brendo Andrade de Lima, Ana Luzia Peixoto da Silva, Estefany Ferreira de Lima, Thais Ferreira Feitosa, Vinícius Longo Ribeiro Vilela. The first draft of the manuscript was written by Lídio Ricardo Bezerra de Melo, Luana Carneiro de Sousa, Thais Ferreira Feitosa, Vinícius Longo Ribeiro Vilela; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Declarations

Conflict of Interest The authors declare that they did not have any conflict of interest relevant to the content of this article.

Ethical Approval This study was approved by the Ethics Committee for the Use of Animals of the Instituto Federal da Paraíba under protocol number 23000.000663.2020-81.

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Figure Caption

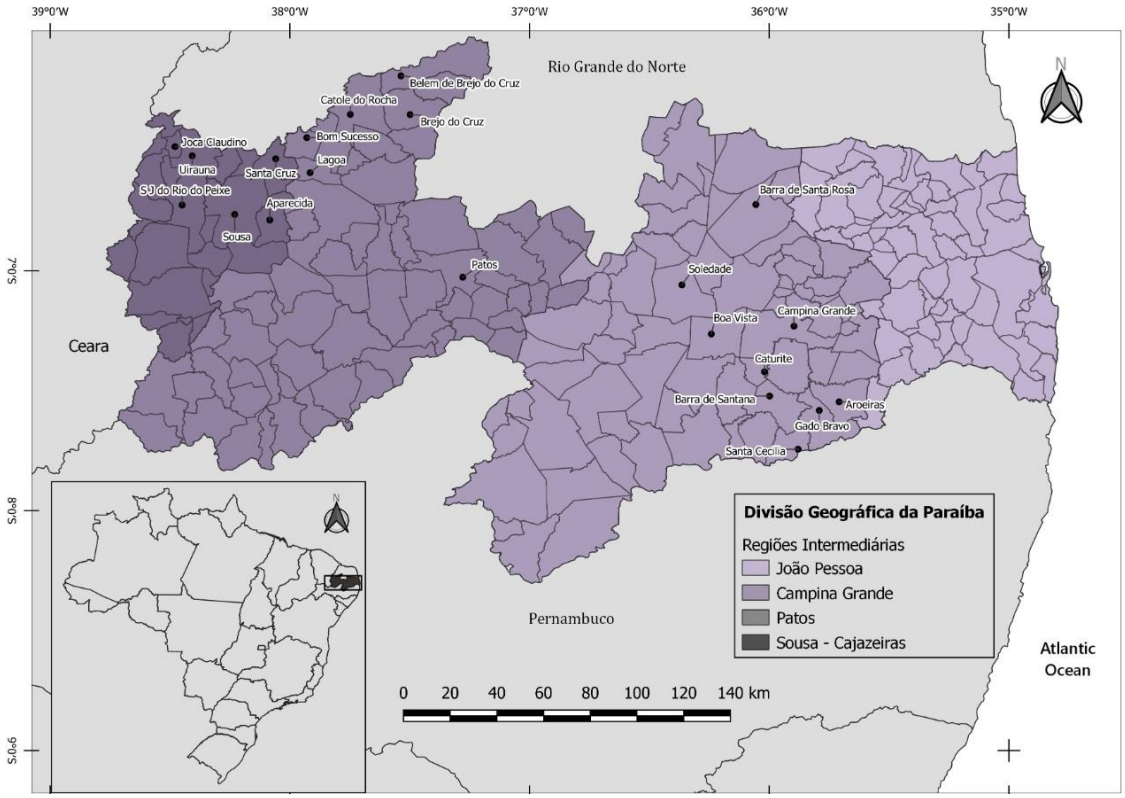

Figure. 1 Geographical distribution of the 21 municipalities visited for the epidemiological survey of gastrointestinal infections by nematodes and coccidia in cattle in the semiarid region of Paraíba, northeastern Brazil.

Table 1 Frequencies and percentages of cattle positive for nematodes and coccidia on farms in 21 municipalities in the semiarid region of Paraíba, northeastern Brazil.

			Nematodes				Coccidia			
Nº	Locality	No. animals	No. positive	Percentage (%)	OPG Min	OPG Max	No. positive	Percentage (%)	OoPG Min	OoPG Max
1	Uiraúna	40	20	50	0	2150	19	47.5	0	1000
2	Aroeiras	39	17	43.6	0	350	12	30.8	0	300
3	S J do Rio do Peixe	40	29	72.5	0	2500	27	67.5	0	1000
4	Caturité	40	13	32.5	0	800	04	10	0	1000
5	Barra de Stª Rosa	40	27	67.5	0	1900	02	5	0	150
6	Soledade	36	31	86.1	0	950	11	30.5	0	550
7	Lagoa	40	27	67.5	0	350	10	25	0	750
8	Patos	40	21	52.5	0	550	27	67.5	0	53400
9	Bom Sucesso	40	23	57.5	0	1050	08	20	0	1100
10	Campina Grande	39	22	56.4	0	700	07	17.9	0	200
11	Santa Cruz	39	19	48.7	0	400	15	38.5	0	3550
12	Boa Vista	40	33	82.5	0	1550	20	50	0	850
13	Gado Bravo	40	34	85	0	650	26	65	0	1500
14	Barra de Santana	40	26	65	0	1650	14	35	0	3000
15	Brejo do Cruz	40	20	50	0	2450	23	57.5	0	3850
16	Joca Claudino	40	22	55	0	500	13	32.5	0	1500
17	Catolé do Rocha	40	27	67.5	0	2900	09	22.5	0	450
18	B de Brejo do Cruz	40	27	67.5	0	1550	23	57.5	0	1700
19	Sousa	40	30	75	0	1700	05	12.5	0	150
20	Aparecida	39	28	71.8	0	4900	25	64.1	0	8750
21	Santa Cecília	40	0	0	0	0	07	17.5	0	1000
	TOTAL	832	496	59.6	-	-	307	36.9	-	-

Table 2 Percentage (%) of gastrointestinal nematode genera in cattle per farm in the semiarid region of Paraíba, Brazil.

Genus	Farm																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Media
H	86.5	85.9	83.7	81.3	74	83.5	74.5	71	69.5	72.5	84	80.8	66.5	82.3	74.8	80.8	85	84.3	87.5	68.8	78.8
T	8.2	9	8	10.8	12.6	1	15	13.2	18.8	22.8	7.4	10.5	21.5	10	11.3	9.2	3.8	11.3	11.7	26	12.1
O	4.8	4	6	4.5	10.3	9	8.2	11.8	11.5	3.7	6.8	8.4	12	7.7	13.6	10	11.2	4.4	0.8	5.2	7.7
C	0	0.8	2	3.4	2.3	6.5	2.3	3	0	0.8	1.3	0	0	0	0	0	0	0	0	0	1.1
S	0.5	0.3	0.3	0	0.8	0	0	1	0.3	0.3	0.5	0.3	0	0	0.3	0	0	0	0	0	0.3

H: *Haemonchus* spp.; T: *Trichostrongylus* spp.; O: *Oesophagostomum* sp.; C: *Cooperia* spp.; S: *Strongyloides* sp.

Table 3 Level of infection in cattle by nematodes and coccidia in the semiarid region of the state of Paraíba, Brazil.

Infection level (EPG)	Nematodes		Infection level (OoPG)	Coccidia	
	Positive	Percentage (%)		Positive	Percentage (%)
Low (> 0 ≤ 300)	359	72.4	Low (> 0 ≤ 1000)	292	95.1
Medium (>300≤ 1000)	107	21.6	Medium (> 1000 ≤ 3000)	11	3.6
High (> 1000)	30	6	High (> 3000)	4	1.3
Total	496	100%	Total	307	100%

Table 4 Univariate analysis on risk factors associated with positivity for nematodes and coccidia in cattle in the semiarid region of the state of Paraíba, Brazil.

Variable/category		Nematodes		Coccidia	
	Number of cattle	Number of positive cattle (%)	*P*	Number of positive cattle (%)	*P*
Gender					
Female	554	302 (54.5)	<0.001*	186 (33.6)	0.005*
Male	278	194 (69.8)		121 (43.5)	
Age					
≤ 12 month	313	205 (65.5)	0.007*	161 (51.4)	<0.001*
> 12 month	519	291 (56.1)		146 (28.1)	
Score					
1-2	479	278 (58)	0.279	225 (47)	<0.001*
>2	353	218 (61.7)		82 (23.2)	
Herd type					
Dairy	399	214 (53.6)	<0.001*	150 (37.6)	<0.001*
Mixed	313	198 (63.2)		140 (44.7)	
Meat	120	84 (70)		17 (14.1)	
Dairy Production					
None	40	27 (67.5)		2 (5)	
Up to 50 gallons	156	108 (69.2)	0.007*	45 (28.8)	<0.001*
51 to 100 gallons	158	101 (63.9)		72 (45.5)	
101 to 200 gallons	79	45 (56.9)		29 (36.7)	
> 200 gallons	399	215 (53.8)		159 (39.8)	
Rearing system					
Semi-intensive	632	384 (60.7)	<0.001*	274 (43.3)	<0.001*
Extensive	120	84 (70)		17 (14.1)	
Intensive	80	27 (33.7)		16 (20)	
Calf housing					
Yes	633	373 (58.9)	0.469	256 (40.4)	<0.001*
No	199	123 (61.8)		51 (25.6)	

* Variables that showed p value ≤ 0.20 according to (x^2) test or Fisher's exact test.

Table 5 Multivariate analysis on factors associated with nematode and coccidian infection in cattle in the semiarid region of the state of Paraíba, Brazil.

Factors associated with infection	Odds ratio	95% CI	*p* value
Nematodes			
Beef cattle	2.99	0.99 –8.98	0.050
Extensive farming system	3.8	1.6–9.1	0.003
Coccidia			
Age ≤ 12 months	2.9	1.77–3.85	0.002
Body score between 1 and 2	5.2	1.6–16.9	0.007

CI: confidence interval.

CAPÍTULO III

Eimeria spp. infecting cattle in the semiarid region of Brazil

Manucrito submetido ao periódico “Parasitology”
ISSN: 1469-8161; Fator de impacto: 3.234; Qualis A2

Eimeria spp. infecting cattle in the semiarid region of Brazil

Eimeria spp. infectando bovinos no semiárido do Brasil

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ABSTRACT

Our aim was to describe the diversity of *Eimeria* species in cattle herds in the semiarid region of the state of Paraíba, northeastern Brazil. We visited 20 farms to obtain fecal samples from 40 randomly selected cattle on each farm. These individually collected samples were subjected to analysis using the centrifugation-floatation technique in sucrose solution. Coccidia were viewed under optical microscope with 40X and 100X objectives, for species identification, through microphotography and micrometric measurements of oocysts and their sporocysts. From each positive animal, 20 coccidia were photographed and measured. Infection by coccidia was detected in 17.12% (137/800) of the samples analyzed. All the farms had at least one animal that was positive for coccidia (100%; 20/20). In total, 2740 coccidia were photographed and measured. The species detected, in descending order of frequency, were: *Eimeria bovis* (35.10%; 962/2740); *Eimeria canadensis* (17.48%; 479/2740); *Eimeria auburnensis* (14.70%; 403/2740); *Eimeria ellipsoidalis* (9.70%; 266/2740); *Eimeria zuernii* (7.22%; 198/2740); *Eimeria brasiliensis* (4.56%; 125/2740); *Eimeria bukidnonensis* (3.97%; 109/2740); *Eimeria illinoisensis* (2.91%; 80/2740); *Eimeria wyomingensis* (1.42%; 39/2740); *Eimeria alabamensis* (1.27%; 35/2740); *Eimeria cylindrica* (0.76%; 21/2740); *Eimeria pellita* (0.54%; 15/2740); *Eimeria ildefonsoi* (0.21%; 6/2740); and *Eimeria subspherica* (0.07%; 2/2740). It was concluded that cattle in the semiarid region of Brazil were parasitized by 14 species of *Eimeria*. It is believed that the sanitary management

employed, as well as the system used for raising these animals, is the crucial point that leads to high rates of infection in this region.

Keywords: Coccidia, eimeriosis, oocysts, ruminants.

RESUMO

Descreveu-se a diversidade de espécies de *Eimeria* spp. nos rebanhos bovinos do Semiárido do Estado da Paraíba, Nordeste do Brasil. A pesquisa foi realizada em 20 fazendas, utilizando 40 bovinos por fazenda. Após as coletas individuais, as amostras de fezes foram analisadas pela técnica de centrífugo-flutuação em solução de sacarose. Os coccídios foram visualizados por microscópio óptico com objetiva de 40X e 100X, acoplado a um microcomputador, para identificação específica por microfotografia e mensurações micrométricas. De cada animal positivo, foram fotografados e mensurados 20 oocistos e seus respectivos esporocistos. A presença de *Eimeria* spp. foi detectada em 17,12% (137/800) das amostras analisadas. Os animais apresentaram parasitismo por até sete espécies distintas. Todas as propriedades apresentaram ao menos um animal positivo para *Eimeria* spp. (100%; 20/20). No total, foram fotografados e mensurados 2740 coccídios, de 14 espécies distintas, cujas ocorrências, em ordem decrescente, foram: *Eimeria bovis* (35,10%; 962/2740), seguida de *Eimeria canadensis* (17,48%; 479/2740), *Eimeria auburnensis* (14,70%; 403/2740), *Eimeria ellipsoidallis* (9,70%; 266/2740), *Eimeria zuernii* (7,22%; 198/2740), *Eimeria brasiliensis* (4,56%; 125/2740), *Eimeria bukidnonensis* (3,97%; 109/2740), *Eimeria illinoisensis* (2,91%; 80/2740), *Eimeria wyomingensis* (1,42%; 39/2740), *Eimeria alabamensis* (1,27%; 35/2740), *Eimeria cylindrica* (0,76%; 21/2740), *Eimeria pellita* (0,54%; 15/2740), *Eimeria ildefonsois* (0,21%; 6/2740) e *Eimeria subspherica* (0,07%; 2/2740). Concluiu-se que os bovinos do Semiárido do Brasil foram parasitados por 14 espécies de *Eimeria*, e acredita-se que o manejo sanitário empregado, bem como o sistema de criação desses animais, seja o ponto crucial para os altos índices de infecção nesta região.

Palavras-chave: Coccídios, eimeriose, oocistos, ruminantes.

INTRODUCTION

Cattle farming occupies a prominent place in the worldwide agricultural scenario, and Brazil is one of the largest milk producers and meat exporters (Brasil, 2021). In the northeastern region of Brazil, beef cattle products and byproducts are used in food and commerce, thus generating stability and development (Carneiro, 2019). In the state of Paraíba, which is within this region of Brazil, cattle raising is also a viable activity that provides one of the main sources of animal protein for human consumption (IBGE, 2020). However, there are some obstacles to herd productivity. Among these, parasitism by enteric protozoa is responsible for diarrhea, weight loss and decreased meat and milk production, and may even lead to animal mortality in severe cases (Daugschies and Najdrowski, 2005; Dubey, 2019; Lopez-Osorio *et al.*, 2020).

The main protozoa of veterinary medical interest belong to the phylum Apicomplexa. These are characterized by obligate intracellular parasitism that causes disease and destroys the host cells (Dubey, 2019). The genus *Eimeria* belongs to the class Sporozoasida, family Eimeriidae, and is transmitted by fecal-oral contamination. It develops in the epithelial cells of the digestive tract, where it causes an enteritis called eimeriosis or coccidiosis (Florião *et al.*, 2016; Martins *et al.*, 2020).

This disease becomes important because of the losses resulting from mortality among young animals and because of the reduced performance of those that recover from the infection, due to their lower food consumption and consequently diminished weight development (Abebe *et al.*, 2008). Adult animals are mostly asymptomatic hosts, but serve as sources of infection for young animals, which are more susceptible to infections and may present gastrointestinal disorders and growth retardation (Daugschies and Najdrowski, 2005; Hillesheim and Freitas, 2016).

Several species of *Eimeria* are known to parasitize cattle. Some of them, such as *Eimeria zuernii* and *Eimeria bovis*, are classified as more pathogenic (Bangoura and Daugschies, 2007). Animals parasitized by these species have clinical signs associated with bloody diarrhea, dehydration, anorexia and weight loss; depending on the severity of infection, these animals may die (Cardoso *et al.*, 2017).

Eimeria alabamensis and *Eimeria auburnensis* have been reported in outbreaks of moderately pathogenic clinical coccidiosis (Hillesheim and Freitas, 2016). *Eimeria ellipsoidalis* has been described as an occasional cause of diarrhea (Mielke *et al.*, 1993). *Eimeria brasiliensis*, *Eimeria bukidnonensis*, *Eimeria canadensis*, *Eimeria cylindrica* and

Eimeria ildefonsoi have been characterized as presenting low pathogenicity, such that they are manifested subclinically (Lima, 2004; Daugschies and Najdrowski, 2005; Das *et al*., 2015; Florião *et al*., 2016; Hillesheim and Freitas, 2016).

Therefore, identification of the various species of *Eimeria* spp. becomes very important. This enables focused disease control and prevention measures and favors adequate administration of medicines and disinfection of animal facilities in conventional production systems (Daugschies and Najdrowski, 2005), especially under conditions of higher stocking rates (Lima, 2004).

Considering the scarcity of information and the economic losses caused by coccidiosis, the objective of this study was to describe the diversity of species of *Eimeria* infecting cattle herds in northeastern Brazil.

MATERIAL AND METHODS

Experimental design

Between January and December 2020, fecal sample collections were carried out on 20 farms that had cattle herds of more than 40 animals that were raised within a semi-intensive system. These farms were located in municipalities in the state of Paraíba that lie within the intermediate regions of Campina Grande, Patos and Sousa-Cajazeiras, which all have a semi-arid climate (Figure 1). The average annual precipitation in the state of Paraíba is between 250-800 mm. The rainfall is irregular and usually concentrated in the months of March to May. The maximum temperature is 32°C and the minimum is 20°C; the evaporation rates are high and the relative air humidity is close to 70%. The vegetation is predominantly that of the Caatinga biome (INMET, 2010).

Forty animals were randomly sampled from each of the 20 farms (which were all dairy farms), without distinction regarding their breed, sex or age, thus totaling 800 sampled animals.

Sample collection and processing for analysis

Feces were collected directly from the rectal ampulla of the animals with the aid of clean plastic bags. The animals were identified according to their number, sex, age and farm. The samples were stored in isothermal boxes and were sent to the Veterinary Parasitology Laboratory (VPL) of the Veterinary Hospital of the Federal Institute of Paraíba (IFPB), Sousa, PB, Brazil, for further laboratory analysis.

To investigate the enteric protozoa, the centrifugation-floatation technique in sucrose solution was used, as originally described by Sheather (1923) and as modified by Duszynski and Wilber (1997). Through this, any presence of oocysts of *Eimeria* spp. was determined. Then, the feces of the positive animals were diluted in an aqueous solution of 2.5% potassium dichromate ($K_2Cr_2O_7$), at a ratio of one-sixth feces to five-sixths solution. This mixture was placed in Petri dishes and left in a B.O.D. chamber at 28 ºC and relative humidity > 80% for 15 days, to await oocyst sporulation (Gonçalves, 2008).

After this sporulation period, centrifugation-floatation was performed in a new sucrose solution. From this, a drop of the surface material was removed, placed on a slide under a coverslip, and the oocysts were viewed using a MAX-300 microscope with 40X and 100X objectives. This was coupled to a microcomputer through the MvImage® software, in the same way as described by Araújo *et al.* (2020).

From each positive sample, 20 intact sporulated oocysts of the genus *Eimeria* were photographed and measured in terms of the maximum, average and minimum diameters, and the morphometric index (MI) of these oocysts and sporocysts was calculated. In addition, the thickness of the oocyst wall was measured and the presence or absence of internal morphological structures was noted. To make morphological identifications of *Eimeria* species, the reference keys for sporulated oocysts described by Levine and Ivens (1967), Levine (1985), Duszynski and Wilber (1997), Berto *et al.* (2014) and Florião *et al.* (2016) were used.

Statistical analysis

The mean diameter, lower limit, upper limit, standard deviation and coefficient of variation (CV) of the oocysts of *Eimeria* spp. and their sporocysts were evaluated using the Microsoft Office Excel 2010® software.

RESULTS

Oocysts of *Eimeria* spp. were found on 100% (20/20) of the farms visited. The presence of oocysts was detected in 17.12% (137/800) of the samples. It was found that 10.21% (14/137) of the cattle were parasitized by only one *Eimeria* species; 27% (37/137) were parasitized by two different species; 23.35% (32/137) had three species; 16.78% (23/137) were parasitized by four species; 14.59% (20/137) were parasitized by five species; 4.37% (6/137) were parasitized by six species; and 3.64% (5/137) were parasitized by seven different species of *Eimeria*.

Based on the morphological characteristics of 2,740 intact sporulated oocysts, 14 species of the genus *Eimeria* were identified, which are listed here in descending order of frequency of occurrence: *E. bovis* Züblin, 1998; *E. canadensis* Bruce, 1921; *E. auburnensis* Cristenses and Poeter, 1939; *E. ellipsoidalis* Becker and Frye, 1929; *E. zuernii* Rivolta, 1878; *E. brasiliensis* Torres and Ramos, 1939; *E. bukidnonensis* Tubangui, 1939; *E. illinoisensis* Levine and Ivens, 1967; *E. wyomingensis* Huizinga and Winger, 1942; *E. alabamensis* Christenses, 1941; *E. cylindrica* Wilson, 1931; *E. pellita* Supperer, 1952; *E. ildefonsoi* Torres and Ramos, 1939; and *E. subspherica* Christenses, 1941.

The morphological characteristics and respective occurrence rates of the *Eimeria* species obtained in this study are described in Table 1. The percentages of occurrence and the mean values for the length, width, standard deviation, morphometric index, coefficient of variation and number of oocysts and sporocysts found in these *Eimeria* species are described in Table 2.

Eimeria bovis (Figure 1A): Ovoid oocysts with a smooth or slightly rough wall, two layers, micropyle in inner layer, micropyle cap absent. 962 sporulated oocysts were measured: 24 to 32 (mean: 28) µm in length by 17 to 23 (20) µm in width, with a morphometric index of 1.40 µm. The oocysts contained four sporocysts, ranging from ovoid to cylindrical or elongated; sporocyst residues dispersed, polar granule present. 962 sporocysts were measured: 8 to 19 (13) µm by 4 to 8 (6) µm. The occurrence rate found was 35.1% (962/2740), among the oocysts evaluated.

Eimeria canadensis (Figure 1B): Oocysts ovoid or ellipsoid, micropyle broad and prominent, absence of micropyle cap, double wall. 479 oocysts were measured: 32 to 38 (35) µm by 19 to 26 (22) µm, with a morphometric index of 1.59 µm. Polar granule absent, sporocyst residue present, sporocysts ranging from elongated to ellipsoidal, and measuring 10 to 22 (16) µm in length by 6 to 9 (7) µm in width. Occurrence rate of 17.48% (479/2740).

Eimeria auburnensis (Figure 1C): Oocysts ovoid, micropyle present, micropyle cap absent, double walled. 403 oocysts were measured: 32 to 42 (37) µm in length by 19 to 26 (22) µm in width, with a morphometric index of 1.68 µm. Each oocyst had four sporocysts, ranging in shape from elongated to ovoid and measuring 12 to 21 (16) µm by 5 to 10 (7) µm. Polar granule and sporocyst residue present. Occurrence rate of 14.7% (403/2740).

Eimeria ellipsoidalis (Figure 1D): Ellipsoid oocysts, absence of micropyle, thin wall. The measurements on 266 oocysts ranged from 19 to 26 (22) µm on the major diameter by 13 to 19 (16) µm in minor diameter, with a morphometric index of 1.37 µm. Polar granule

present with elongated and ellipsoid sporocysts measuring between 5 and 16 (10) µm by 4 to 8 (6) µm; sporocyst residue present. Occurrence rate of 9.7% (266/2740).

Eimeria zuernii (Figure 1E): Subspherical or slightly ovoid oocysts, micropyle absent, single wall. 198 oocysts were measured: 15 to 22 (18) µm in length by 13 to 19 (16) µm in width, with a morphometric index of 1.12 µm. Elongated and ovoid sporocysts measuring 4 to 13 (8) µm in length by 4 to 7 (5) µm in width. Polar granule absent, sporocyst residue present. Occurrence rate of 7.22% (198/2740), among the oocysts analyzed.

Eimeria brasiliensis (Figure 1F): Ellipsoidal or slightly ovoid oocysts, presence of clear micropyle, double wall with rough surface. 125 oocysts were measured: 32 to 39 (35) µm by 24 to 28 (26) µm, with a morphometric index of 1.34 µm. Polar granule present in some and absent in others, elongated and ovoid sporocysts measuring 11 to 21 (16) µm by 6 to 8 (7) µm; sporocyst residue present. Occurrence rate of 4.56% (125/2740).

Eimeria bukidnonensis (Figure 1G): Piriform oocysts with micropyle present, micropyle cap absent, striated wall brownish color. 109 oocysts were measured: 36 to 49 (42) µm by 26 to 35 (30) µm, with a morphometric index of 1.40 µm. Polar granule absent, sporocysts elongated and ovoid, measuring 10 to 19 (14) µm in length by 7 to 11 (9) µm in width; sporocyst residue present. Occurrence rate of 3.97% (109/2740).

Eimeria illinoisensis (Figure 1H): Oocysts ellipsoid or ovoid, micropyle absent, smooth wall, polar granule absent. 80 oocysts were measured: 23 to 29 (26) µm in length by 18 to 22 (20) µm in width, with a morphometric index of 1.30 µm. Elongated ovoid sporocysts with a larger diameter between 6 and 17 (11) µm and a smaller diameter between 5 and 7 (6) µm; sporocyst residue present. Occurrence rate of 2.91% (80/2740).

Eimeria wyomingensis (Figure 1I): Oocysts ovoid, micropyle present, micropyle cap absent, smooth wall. 39 oocysts were measured: 38 to 44 (41) µm by 26 to 29 (27) µm, with a morphometric index of 1.51 µm; polar granule absent. Ellipsoid sporocysts, sporocyst residue absent. 39 sporocysts were measured: 12 to 20 (16) µm in length by 6 to 9 (7) µm in width. Occurrence rate of 1.42% (39/2740).

Eimeria alabamensis (Figure 1J): Oocysts ovoid and ellipsoid, micropyle absent, with smooth wall, polar granule present in some oocysts and absent in others. 35 oocysts were measured: 14 to 23 (18) µm in length by 12 to 16 (14) µm in width, with a morphometric index of 1.50 µm. The sporocysts ranged from elongated to ovoid, measuring 7 to 13 (10) µm by 3 to 6 (4) µm, with a tiny Stieda body and absence of sporocyst residue. Occurrence rate of 1.27% (35/2740), among the oocysts evaluated.

Eimeria cylindrica (Figure 1K): Elongated ellipsoidal oocysts, micropyle absent, with thin double layered wall, polar granule present. 21 oocysts were measured: 20 to 23 (21) µm in length by 13 to 17 (15) µm in width, with a morphometric index of 1.40 µm. Elongated ellipsoidal sporocysts with larger diameter measuring between 8 and 12 (10) µm and smaller diameter measuring between 4 and 7 (5) µm; Stieda body and sporocyst residue present. Occurrence rate of 0.76% (21/2740).

Eimeria pellita (Figure 1L): Oocysts ovoid, micropyle present, oocyst coloration brownish, thick and rough wall, polar granule present. 15 oocysts were measured: 36 to 41 (38) µm in length by 25 to 28 (26) µm in width, with a morphometric index of 1.46 µm. Elongated sporocysts measuring between 13 and 19 (15) µm in major diameter by 8 to 11 (9) µm in minor diameter. Occurrence rate of 0.54% (15/2740).

Eimeria ildefonsoi (Figure 1M): Oocysts ovoid or cylindrical with tapered portion, double wall, micropyle present, polar granule absent. Six oocysts were measured: 43 to 44 (43) µm in length by 24 to 25 (24) µm in width, with a morphometric index of 179 µm. Ellipsoidal sporocysts, measuring between 18 and 19 (18) µm in length by 8 to 9 (8) µm in width; sporocyst residue present. Occurrence rate of 0.21% (6/2740).

Eimeria subspherica (Figure 1N): Subspherical and spherical oocysts, double layered wall, micropyle absent, polar granule present in some and absent in others. Two oocysts were measured: between 14 and 16 (15) µm in length by 13 to 16 (14) µm in width, with a morphometric index of 1.07 µm. Ovoid and spherical sporocysts, measuring between 4 and 8 (6) µm in length by 4.0 to 4.4 (4.2) µm in width; sporocyst residue present. Occurrence rate of 0.07% (2/2740).

DISCUSSION

The present study was the first to identify and describe distinct species of *Eimeria* parasitizing cattle in the semiarid region of northeastern Brazil. Because of the high occurrence rates found, along with the presence of highly pathogenic species, we believe that the number of cases of bovine coccidiosis was high. According to Hamid *et al.* (2019) and Dubey (2019), coccidiosis is distributed worldwide, reaching up to 100% of calves in the first weeks of age. Thus, it has a high impact on livestock development and economic results. The high incidence of infections by *Eimeria* spp. is associated with higher prevalence of the subclinical form, which therefore makes it difficult to assess the real economic impact caused by eimeriosis in cattle herds. The most frequent clinical sign is severe and/or hemorrhagic diarrhea; presence of the subclinical form is associated with less pathogenic species, low

environmental pressure from more pathogenic species or an acquired immune response that already exists (Daugschies *et al.*, 2007; Gillhuber *et al.*, 2014).

This investigation also revealed that 14 species of *Eimeria* were present, which can be considered to be a high level of diversity. Lopez-Osorio *et al.* (2020) found similar diversity among cattle in different production systems in Colombia, in which they identified 13 species of *Eimeria*. Seven species of *Eimeria* were also found by Das *et al.* (2015), in dairy cattle in India; and by Florião *et al.* (2016), on an organic dairy cattle farm in Rio de Janeiro, Brazil.

Although the occurrence rate of *Eimeria* spp. observed among the cattle studied here (17.12%; 137/832) can be considered high, it differed from the results found by Hillesheim and Freitas (2016), in the state of Paraná, Brazil, who reported that the prevalence of coccidia was 48.2% (53/110) among the animals evaluated, on family-run farms. The rate in the present study was also lower than the prevalences recorded by Lopez-Osorio *et al.* (2020) in Colombia and by Hastutiek *et al.* (2019) in Indonesia, with 75.5% (1006/1333) and 53.42% (190/357), respectively, in the cattle herds evaluated. We believe that the semi-intensive rearing system used on the farms studied here may have had a relationship with the lower rates of infections found in the present study. Higher levels of coccidia are mainly related to fecal-oral contamination in feedlots (Kimeli *et al.*, 2020). In addition, the high average annual temperatures and low rainfall to which oocysts are subjected in the environment of the semiarid climate may have led to reduction of their survival and consequent reduction of reinfection of the animals.

Notable numbers of species of *Eimeria* were identified parasitizing the same individuals. It can be highlighted that some animals (5/137) had up to seven different species. This demonstrates that there was high potential for coinfection among the cattle in the herds examined. Mixed infections were also observed by Abebe *et al.* (2008) in Ethiopia, ranging from two to eight species per animal. Amid the diversity of *Eimeria* species infecting the same animal, it is difficult to control these parasites through vaccines (Sultana *et al.*, 2014; Dubey, 2019), since the variability of Eimeria spp. is very high. Studies have indicated that the real impact of coinfections by *Eimeria* spp. is still uncertain, considering that clinical signs of diarrhea in calves have been found only in association with single infections by *E. zuernii* or *E. bovis* (Bangoura *et al.*, 2011; Enemark *et al.*, 2013; Lopez-Osorio *et al.*, 2020).

Eimeria bovis was the most frequently found species (35.1%), and it stood out as the most pathogenic species among the more than 20 species of *Eimeria* already described in cattle (Daugschies and Najdrowski, 2005; Bangoura and Daugschies, 2007; Deplazes *et al.*, 2016). According to Hermosilla *et al.* (2012) and Lopez-Osorio *et al.* (2020), the clinical

conditions caused by these species give rise to enteric infections that result in severe hemorrhagic typhlocolitis, clinically characterized by hemorrhagic and catarrhal diarrhea.

The other two most frequent species were *E. canadensis* (17.48%) and *E. auburnensis* (14.70%). This finding differed from the sequence observed by Vidal *et al.* (2013), who found that the species *E. ellipsoidalis* (39.7%), *E. alabamensis* (18.4%) and *E. bovis* (12.1%) predominated in calves in the state of Rio de Janeiro, in southeastern Brazil; and also differed from the findings of Hillesheim and Freitas (2016), in calves in Paraná, southern Brazil, where *E. bovis*, *E. auburnensis* and *E. alabamensis* were the most frequently encountered species, with rates of 23.6%, 11.8% and 9.1%, respectively. These species caused outbreaks of clinical coccidiosis of considerable pathogenicity.

Eimeria canadensis, *E. ellipsoidalis*, *E. brasiliensis*, *E. bukidnonensis*, *E. illinoisensis*, *E. wyomingensis*, *E. alabamensis*, *E. cylindrica*, *E. pellita*, *E. ildefonsoi* and *E. subspherica*, which were also described in the present study, are considered to be highly prevalent worldwide (Eckert *et al.*, 1995; Florião *et al.*, 2016). Even in the absence of clinical disease, cattle can be severely affected due to damage inflicted on intestinal tissue, thereby compromising the digestive process and general homeostasis, with adverse effects on wellbeing and animal performance (Daugshies and Nadjrowsky 2005).

One important morphological tool that helps to differentiate the species of the genus Eimeria is the morphometric index (MI). This consists of dividing the largest diameter by the smallest. Oocysts may vary in size but their MIs show a rectilinear trend that reflects the volumetric shape of the sporulated oocysts. This index is more precise for comparison between species than the average of dimensions, and also for comparing intraspecific variation (Long and Joyner, 1984; Vidal *et al.*, 2013; Araújo *et al.*, 2020).

Molecular tools are already being studied and are available for identifying oocysts of coccidia in cattle (Hastutiek *et al.*, 2022). However, Lopez-Osorio *et al.* (2020) and Vidal et *al.* (2013) demonstrated that oocyst morphology is still an appropriate and reliable method for differentiating coccidia in epidemiological surveys. This was also shown by Araújo *et al.* (2020), through identifying species of coccidia in pigs in the semiarid region of northeastern Brazil.

Nonetheless, oocysts and/or sporocysts with MIs greater than 1.1 should always be described carefully. These may have a variety of shapes, such as ellipsoidal, ovoid or pear-shaped, i.e. they form a so-called "ellipsoidal complex". On the other hand, spherical oocysts usually have an MI of 1.0, while subspherical oocysts have MIs between 1.0 and 1.1 (Levine, 1985; Berto *et al.*, 2014).

Our measurements of the length and width (DM and dm) of the oocysts and sporocysts showed CVs below 25%. According to Siqueira and Tibúrcio (2011), CVs below 10% are considered low, while those above 30% are considered very high. In the current study, the CVs of oocyst length and width were lower than those of sporocysts, i.e. the oocysts were more homogeneous, thus only showing low to medium dispersion.

CONCLUSION

The diversity of *Eimeria* species is high among cattle in the semiarid region of northeastern Brazil. *E. bovis*, which is considered to be the most pathogenic species, showed the highest frequency of occurrence. Multiple infections by up to seven species were found in the animals. The semi-intensive rearing system and the semiarid conditions to which oocysts are exposed may have been limiting factors regarding their survival. These conditions may therefore have acted towards reduction of reinfection among the animals, since, despite the high rate of occurrence of infections that was found, these values were lower than those reported from studies conducted in other locations.

Declarations

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflict of interest

The authors declare that they did not have any conflict of interest relevant to the content of this article.

Ethical standards

The activities involved in this research were approved by our institution's Ethics Committee for Animal Use (CEUA/IFPB), under protocol number 23000.000663.2020-81.

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Legends of the figures.

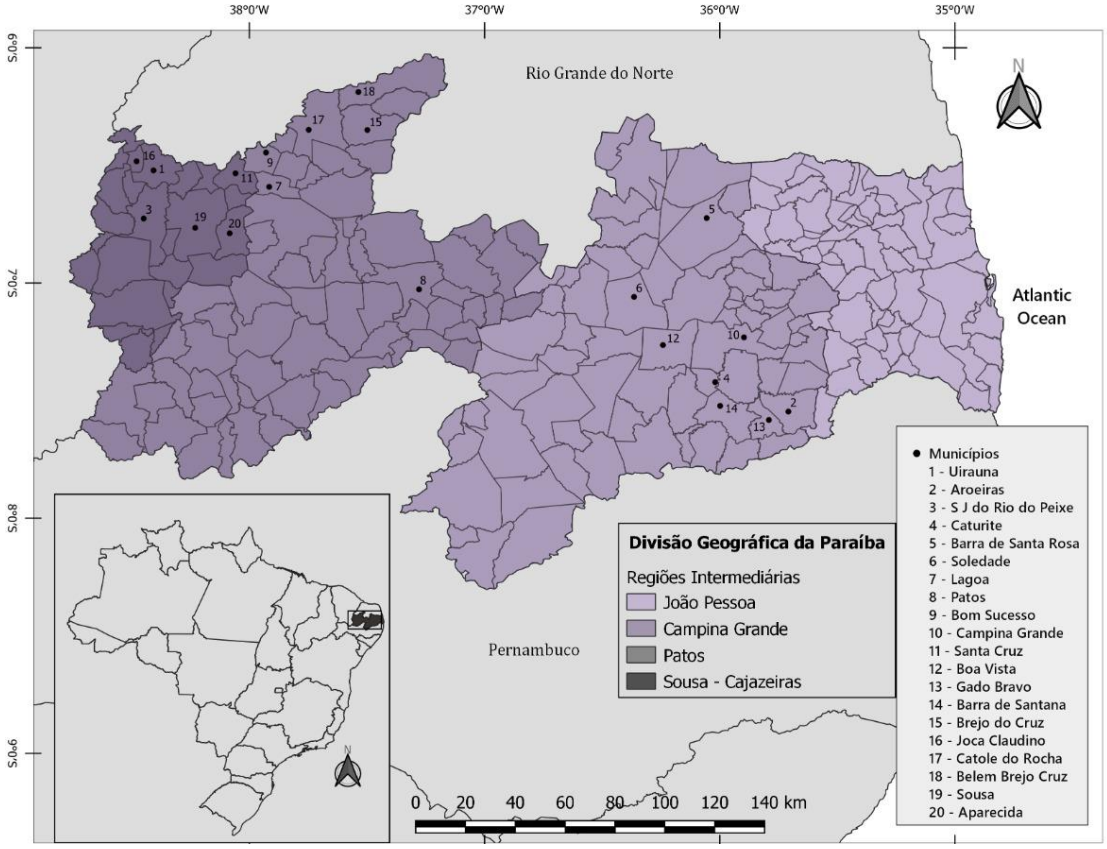

Figure 1. Geographical distribution of municipalities in the semiarid region of Paraíba, northeastern Brazil, in which farms were visited to collect bovine feces.

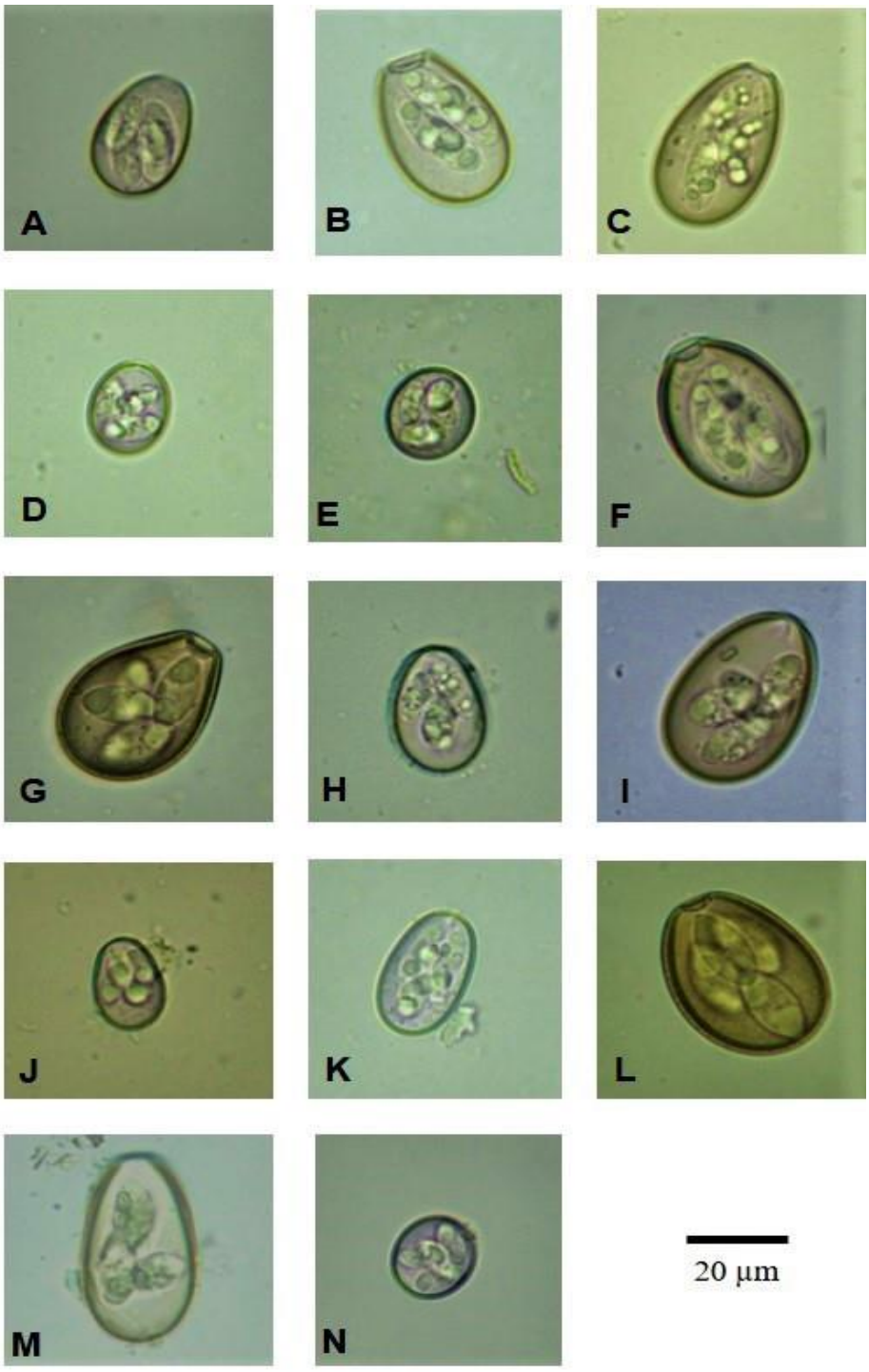

Figure 2. Photomicrographs of oocysts of *Eimeria* spp. in cattle in the semiarid region of Paraíba, Northeastern Brazil. **A**: *Eimeria bovis;* **B:** *Eimeria canadensis*; **C:** *Eimeria auburnensis*; **D:** *Eimeria ellipsoidalis*; **E:** *Eimeria zuernii*; **F:** *Eimeria brasiliensis*; **G:** *Eimeria bukidnonensis*; **H:** *Eimeria illinoisensis*; **I:** *Eimeria wyomingensis*; **J:** *Eimeria alabamensis*; **K:** *Eimeria cylindrica*; **L:** *Eimeria pellita*; **M:** *Eimeria ildefonsoi*; **N:** *Eimeria subspherica.*

Table 1. Occurrence and morphology of oocysts and sporocysts of *Eimeria* spp. in cattle in the semiarid region of northeastern Brazil.

Coccidia	Oocysts				Esporocysts					
	Shape	**Polar granule**	**Outer layer**	**Micropyle**	**Shape**	**Polar granule**	**Stieda body**	**Residuum**	**Nº of cattle**	**Occurrence**
E. bovis	Ovoid	Present	Smooth	Present	Ovoid and cylindrical or elongated	Present	Present	Present	102	35.10%
E. Canadensis	Ovoid or Ellipsoidal	Absent	Bi-layered	Present	Elongated and ellipsoidal	Absent	Present	Present	77	17.48%
E. auburnensis	Ovoid	Present	Bi-layered	Present	Elongated and ovoid	Present	Present	Present	71	14.70%
E. ellipsoidalis	Ellipsoidal	Present	Thin	Absent	Elongated and ellipsoidal	Present	Present	Present	51	9.70%
E. zuernii	Spherical	Absent	Single	Absent	Elongated and ovoid	Absent	Present	Present	45	7.22%
E. brasiliensis	Ellipsoidal or Ovoid	Optative	Bi-layered	Present	Elongated ellipsoid	Optative	Present	Present	16	4.56%

E. bukidnonensis	Piriform	Absent	Striated	Present	Elongated and ovoid	Absent	Present	Present	19	3.97%
E. illinoisensis	Ellipsoidal or Ovoid	Absent	Smooth	Absent	Ovoid elongated	Absent	Present	Present	18	2.91%
wyomingensis	Ovoid	Absent	Smooth	Present	Ellipsoid	Absent	Present	Absent	13	1.42%
E. alabamensis	Ovoid or Ellipsoidal	Optative	Smooth	Absent	Elongated and ovoid	Optative	Present	Absent	16	1.27%
E. cylindrical	Ellipsoidal elongated	Present	Thin	Absent	Elongated ellipsoid	Present	Present	Present	13	0.76%
E. pellita	Ovoid	Present	Thick	Present	Elongated	Present	Present	Present	4	0.54%
E. ildefonsoi	Ovoid or cylindrical	Absent	Bi-layered	Present	Ellipsoid	Absent	Present	Present	2	0.21%
E. subspherica	subspherical or Spherical	Optative	Bi-layered	Absent	Ovoid and spheric	Optative	Present	Present	2	0.07%

Table 2. Micrometric measurements of oocysts and sporocysts of *Eimeria* spp. infecting cattle in the semiarid region of Northeast Brazil.

Coccidia	Oocysts								Sporocysts							
	Length (µm)	SD	CV (%)	Width (µm)	SD	CV (%)	SI	N	Length (µm)	SD	CV (%)	Width (µm)	SD	CV (%)	SI	N
E. bovis	28(24-32)	2.12	7.57	20(17-23)	1.38	6.9	1.40	962	13(8-19)	2.08	16	6(4-8)	0.84	14	2.16	962
E. canadensis	35(32-38)	1.55	4.43	22(19-26)	1.09	4.95	1.59	479	16(10-22)	2.12	13.5	7(6-9)	0.89	12.71	2.28	479
E. auburnensis	37(32-42)	2.41	6.51	22(19-26)	1.34	6.09	1.68	403	16(12-21)	2.15	13.44	7(5-10)	0.90	12.86	2.28	403
E. ellipsoidalis	22(19-26)	1.68	7.64	16(13-19)	1.42	8.88	1.37	266	10(5-16	1.93	19.3	6(4-8)	0.83	13.83	1.66	266
E. zuernii	18(15-22)	1.35	7.5	16(13-19)	1.52	9.5	1.12	198	8(4-13)	1.52	19	5(4-7)	0.70	14	1.6	198
E. brasiliensis	35(32-39)	1.56	4.46	26(24-28)	1.07	4.12	1.34	125	16(11-21)	2.57	16.06	7(6-8)	0.70	10	2.28	125
E. bukidnonensis	42(36-49)	3.45	8.21	30(26-35)	2.84	9.47	1.40	109	14(10-19)	2.06	14.71	9(7-11)	0.95	10.56	1.55	109
E. illinoisensis	26(23-29)	1.60	6.15	20(18-22)	1.02	5.1	1.30	80	11(6-17)	2.38	21.64	6(5-7)	0.74	12.33	1.83	80
E. wyomingensis	41(38-44)	1.53	3.73	27(26-29)	0.64	2.37	1.51	39	16(12-20)	3.05	19.06	7(6- 9)	0.78	11.14	2.28	39
E. alabamensis	18(14-23)	3.04	16.89	14(12-16)	1.50	10.71	1.28	35	10(7-13)	1.51	15.1	4(3-6)	0.66	16.5	2.5	35
E. cylindrica	21(20-23)	1.13	5.38	15(13-17)	0.94	6.33	1.40	21	10(8-12)	1.18	11.8	5(4-7)	0.76	15.2	2	21
E. pellita	38(36-41)	2.40	6.32	26(25-28)	1.02	3.92	1.46	15	15(13-19)	1.66	11.07	9(8-11)	0.85	9.44	1.66	15
E. ildefonsoi	43(43-44)	0.16	0.37	24(24-25)	0.04	0.17	1.79	6	18(18-19)	0.45	2.5	8(8-9)	0.32	4	2.25	6
E. subspherica	15(14-16)	1.69	11.27	14(13-16)	2.54	18.14	1.07	2	6(4-8)	3.39	56.5	4.2(4-4.4)	0.35	8.33	1.42	2

SD: Standard deviation; CV: coefficient of variation; SI: shape index; N: number of oocysts or sporocysts measured.

CONCLUSÃO GERAL

A partir dos resultados obtidos no presente trabalho de tese é possível inferir que:

A resistência anti-helmíntica por nematódeos gastrintestinais é elevada em rebanhos bovinos do semiárido da Paraíba, Nordeste do Brasil. Assim como é elevada a multirresistência observada em quase todas as fazendas avaliadas, principalmente aos fármacos ivermectina, albendazol e closantel. O levamisole foi o fármaco que apresentou o menor nível de resistência. Diante disto, sugere-se a revisão do manejo sanitário nos rebanhos do semiárido, evitando-se vermifugações em massa sem critérios técnicos. Devendo-se realizar o trânsito de animais apenas após diagnóstico parasitológico, principalmente em animais provenientes de propriedades com histórico de problemas no controle de verminoses e que utilizam pastos consorciados entre bovinos e pequenos ruminantes.

É elevada a frequência de coccídios e nematóides gastrintestinais em rebanhos bovinos no Semiárido da Paraíba. A adoção de um manejo sanitário adequado para a realidade de cada rebanho e a assistência técnica periódica por um médico veterinário capacitado contribui para a diminuição dos índices parasitários e melhor desempenho dos animais, gerando maior lucratividade aos pecuaristas.

Em relação às 14 espécies de *Eimeria* sp., que foram identificadas parasitando bovinos no Semiárido do Nordeste do Brasil, concluiu-se que a diversidade é alta, sendo *E. bovis* considerada a *Eimeria* mais patogênica e a que apresentou maior ocorrência. Infecções múltiplas por até sete espécies foram encontradas nos animais. O sistema de criação semi-intensivo e as condições de semiárido aos quais os oocistos estão expostos podem ser fatores limitantes de sua sobrevivência e atuarem na redução da reinfecção dos animais, uma vez que, apesar de ter sido encontrado alto percentual de ocorrência de infecções, esses valores foram inferiores aos relatados em estudos de outras localidades.

ANEXOS

ANEXO 1

FICHA DE CAMPO											
Prop/Faz			Endereço:							Data:	
Fármaco	Ord	Ani	Sexo	Idade	Prenhês	Kg	Sinais clínicos	OPGD0	OOPG	OPGD10	OOPG
Lev	1										
Lev	2										
Lev	3										
Lev	4										
Lev	5										
Lev	6										
Lev	7										
Lev	8										
Lev	9										
Lev	10										
MÉDIA DO OPG											
RCOF %											
Iver	1										
Iver	2										
Iver	3										
Iver	4										
Iver	5										
Iver	6										
Iver	7										
Iver	8										
Iver	9										
Iver	10										
MÉDIA DO OPG											
RCOF %											
Alb	1										
Alb	2										
Alb	3										
Alb	4										
Alb	5										
Alb	6										
Alb	7										
Alb	8										
Alb	9										

Alb	10										
MÉDIA DO OPG											
RCOF %											
Clos	1										
Clos	2										
Clos	3										
Clos	4										
Clos	5										
Clos	6										
Clos	7										
Clos	8										
Clos	9										
Clos	10										
MÉDIA DO OPG											
RCOF %											

ANEXO 2

QUESTIONÁRIO EPIDEMIOLÓGICO

Data: _____/_____/_____

Município:_____

Nome da Propriedade: _____

Microrregião: _____

Lat. _____ Long._____

Tipo de Exploração:

(1) Leite (2) Corte (3) Misto

média de litros/dia_____

Número de bovinos:_____

Sistema de Criação:

(1) Extensivo (2) Semi-extensivo (3) Intensivo

Estratégia de utilização de anti-helmíntico:

(1) Entrada e saída das águas (0) Não (1) Sim

(2) Somente durante a época seca (0) Não (1) Sim

(3) Entrada/Saída das águas e durante a seca

(4) Somente durante as águas

(5) Somente diante do quadro clínico característico

(6) Mensalmente

(7) Outro esquema. Qual? Defina: _____

(8) Não utiliza

Em caso de resposta da opção 5 faz-se outra pergunta. Aplicação de anti-helmínticos diante dos seguintes sinais clínicos:

Pelos arrepiados

(0) Não (1) Sim

Papeira

(0) Não (1) Sim

Diarréia

(0) Não (1) Sim

Emaciação (magro)

(0) Não (1) Sim

Anorexia (perda de apetite)

(0) Não (1) Sim

Tosse (0) Não (1) Sim

Corrimento nasal (0) Não (1) Sim

Não ocorre nenhum dos sinais acima (0) Não (1) Sim

Não utiliza anti-helmíntico (0) Não (1) Sim

Conhecimento sobre:

Controle estratégico (0) Não (1) Sim

Controle tático (0) Não (1) Sim

Controle curativo (0) Não (1) Sim

Controle supressivo (0) Não (1) Sim

Princípio ativo utilizado:

Ivermectina (0) Não (1) Sim

Albendazol (0) Não (1) Sim

Levamisol (0) Não (1) Sim

Closantel (0) Não (1) Sim

Disofenol (0) Não (1) Sim

Moxidectina (0) Não (1) Sim

Outro: _____

Grupo animal para vermifugação:

(1) Somente bezerros mamando

(2) Todos os animais após desmame

(3) Somente os clinicamente demonstrados

(4) Toda a população da propriedade

(5) novilhas e vacas em lactação

(6) Não utiliza

Período de vermifugação:

(1) Logo após o parto (2) Uma vez ao ano (3) Duas vezes ao ano

(4) Mais de três vezes ao ano (5) Não utiliza

Rotação de princípio ativo:

(1) Após 1 ano utilizando o mesmo princípio (2) Rotaciona a cada vermifugação

(3) Rotaciona de acordo com o preço (4) Esporadicamente

(5) Não rotaciona

Manejo de rebanho/pastagens:

Abrigo coletivo para os animais (0) Não (1) Sim

Abrigo separado para os bezerros (0) Não (1) Sim

Piquete separado para fêmeas e machos (0) Não (1) Sim

Maternidade (0) Não (1) Sim

Problema parasitário na propriedade em ordem de importância:

Moscas (0) Não (1) Sim

Carrapato (0) Não (1) Sim

Miíases (0) Não (1) Sim

Verminose (0) Não (1) Sim

Piolhos (0) Não (1) Sim

Diarréia (0) Não (1) Sim

Acontece aborto na propriedade (0) Não (1) Sim

Se sim, com que freqüência_____

ENTREVISTA ESTRUTURADA

Área total da propriedade:

(1) Menos de 10 ha (2) De 10 a 25 ha (3) De 26 a 50 ha

(4) De 51 a 100 ha (5) Mais de 100 ha

Grau de escolaridade do entrevistado:

(1) Analfabeto (2) 1º grau incompleto (3) 1º grau completo

(4) 2º grau incompleto (5) 2º grau completo (6) 3º grau

Idade do entrevistado:

(1) Menos de 20 anos (2) De 21 a 30 anos (3) De 31 a 40 anos

(4) De 41 a 50 anos (5) Acima de 50 anos

Participação em entidades associativas:

(1) Cooperativas (2) Sindicato dos trabalhadores Rurais (3) Sindicato Rural

(4) Associação dos produtores (5) Nenhuma (6) Laticínios

(7) Escolheu duas ou mais (8) Internet

Qual a melhor forma de receber informações sobre a sanidade dos animais?

(1) Reuniões (2) Materais impressos (3) Palestras

(4) Rádio (5) Jornal (6) Televisão

(7) Não tem acesso (8) Escolheu duas ou mais

A quem recorre, de preferência, quando ocorrem doenças nos animais?

(1) Vizinho (2) Prático (3) Medicação por conta própria

(4) Médico Veterinário (5) Ninguém

Número de bovinos:_____

(1) Menos de 10 cabeças (2) De 11 a 40 cabeças (3) De 41 a 60 cabeças

(4) De 61 a 100 cabeças (5) Mais de 100 cabeças

Demais obervações e constatações:_____

_____

_____

_____

ANEXO III

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nome da Pesquisa: Avaliação da resistência anti-helmíntica e diversidade de parasitos gastrintestinais em bovinos no semiárido da Paraíba, Nordeste do Brasil.
Pesquisador responsável: Prof. Dr. Vinícius Longo Ribeiro Vilela
Informações sobre a pesquisa: Será realizado estudo sobre a resistência anti-helmíntica dos nematóides e a diversidade dos parasitas gastrintestinais de bovinos no semiárido da Paraíba.
Objetivo da pesquisa: Determinar a real eficácia anti-helmíntica dos principais fármacos utilizados no rebanho, os níveis de infecção e a frequência dos nematóides e enteroprotozoários que parasitam os bovinos e causam perdas produtivas.
A sua participação é muito importante, pois contribuirá para a efetivação dos objetivos propostos.

_____
Pesquisador responsável

Eu, _____, portador(a) de RG _____, abaixo assinado, tendo recebido as informações acima, e ciente dos meus direitos abaixo relacionados, de acordo com o item IV da Resolução nº 196/96 do Conselho Nacional de Saúde que regulamenta as pesquisas envolvendo seres humanos, concordo em participar da pesquisa.
a) A garantia de receber todos os esclarecimentos sobre as perguntas do questionário antes e durante o transcurso da pesquisa, podendo afastar-me a qualquer momento se assim o desejar, bem como está assegurado o absoluto sigilo das informações obtidas.
b) A segurança plena de que não serei identificado mantendo o caráter oficial da informação, assim como, está assegurada que a pesquisa não acarretará nenhum prejuízo individual ou coletivo.
c) A segurança de que não terei nenhum tipo de despesa material ou financeira durante o desenvolvimento da pesquisa, bem como, esta pesquisa não causará nenhum tipo de risco, dano físico ou mesmo constrangimento moral e ético ao entrevistado.
d) A garantia de que toda e qualquer responsabilidade nas diferentes fases da pesquisa é dos pesquisadores, bem como, fica assegurado poderá haver divulgação dos resultados finais em órgãos de divulgação científica em que a mesma seja aceita.
e) A garantia de que todo o material resultante será utilizado exclusivamente para a construção da pesquisa e ficarão sob a guarda dos pesquisadores, podendo ser requisitado pelo entrevistado em qualquer momento.
Tenho ciência do exposto acima e desejo participar da pesquisa.

Local e data _____

_____
Assinatura do(a) entrevistado(a