UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE MORFOLOGIA E FISIOLOGIA ANIMAL
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIA ANIMAL

LUCIA OLIVEIRA DE MACEDO

Estudo epidemiológico da infecção por nematódeos broncopulmonares em ruminantes no estado de Pernambuco

Recife - 2022

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE MORFOLOGIA E FISIOLOGIA ANIMAL
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIA ANIMAL

LUCIA OLIVEIRA DE MACEDO

Estudo epidemiológico da infecção por nematódeos broncopulmonares em ruminantes no estado de Pernambuco

Tese apresentada ao Programa de Biociência Animal da Universidade Federal Rural de Pernambuco, como pré-requisito parcial para obtenção do grau de Doutora em Biociência Animal.

Orientador: Prof. Dr. Rafael Antonio do Nascimento Ramos

Recife - 2022

Dados Internacionais de Catalogação na Publicação
Universidade Federal Rural de Pernambuco
Sistema Integrado de Bibliotecas
Gerada automaticamente, mediante os dados fornecidos pelo(a) autor(a)

M141e Macedo, Lucia Oliveira
Estudo epidemiológico da infecção por nematódeos broncopulmonares em ruminantes no estado de Pernambuco / Lucia Oliveira Macedo. - 2022.
109 f.

Orientador: Prof. Dr. Rafael Antonio do Nascimento Ramos.
Inclui referências.

Tese (Doutorado) - Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Biociência Animal, Recife, 2022.

1. Protostrongylus rufescens. 2. Dictyocaulus viviparus. 3. Bovinos. 4. Caprinos. 5. Baermann. I. Ramos, Prof. Dr. Rafael Antonio do Nascimento, orient. II. Título

CDD 636.089

Tese apresentada ao Programa de Pós-Graduação em Biociência Animal, como parte dos requisitos necessários à obtenção do grau de Doutora em Biociência Animal, outorgado pela Universidade Federal Rural de Pernambuco, à disposição na Biblioteca Central desta universidade. A transcrição ou utilização de trechos deste trabalho é permitida, desde que respeitadas às normas de ética científica.

Tese elaborada por

LUCIA OLIVEIRA DE MACEDO

Estudo epidemiológico da infecção por nematódeos broncopulmonares em ruminantes no estado de Pernambuco

Aprovada em 24 /11 /2022

BANCA EXAMINADORA

_____
Prof. Dr. Rafael Antonio do Nascimento Ramos (Orientador)
Universidade Federal do Agreste de Pernambuco – UFAPE

_____
Profa. Dra. Gílcia Aparecida de Carvalho
Universidade Federal do Agreste de Pernambuco – UFAPE

_____
Profa. Dra. Márcia Paula de Oliveira Farias
Universidade Federal do Piauí – UFPI

_____
Dra. Carla Lopes de Mendonça
Universidade Federal Rural de Pernambuco – UFRPE

_____
Prof. Dr. Leucio Câmara Alves
Universidade Federal Rural de Pernambuco – UFRPE

LISTA DE FIGURAS

Artigo 1

Artigo 2

Artigo 4

LISTA DE TABELAS

Artigo 2

Artigo 3

Agradecimento

A Deus, autor da vida, que sempre guiou meus passos e me proporcionou as melhores oportunidades.

A meus pais Antonio Macedo e Zuleide Macedo (*in memorian*) por todos os ensinamentos que moldaram o que sou hoje. Agradeço a meus irmãos e sobrinhos por todo apoio, carinho e atenção. Amo muito vocês!

Ao meu orientador Prof. Rafael Ramos por todo apoio durante o desenvolvimento da pesquisa. Sou muito grata por todas as oportunidades proporcionadas, por todo o incentivo e conhecimento que adquiri com você.

À Profa. Gílcia Carvalho por ter me acompanhado durante toda minha vida acadêmica. É um privilégio tê-la durante toda minha trajetória, sempre muito atenciosa e disponível em todos os momentos que precisei de ajuda.

À Profa. Taciana Ramos, Profa. Márcia Farias, Dra. Carla Mendonça, Prof. Luiz Carlos Batista e Prof. Leucio Alves pela disponibilidade em participar da banca de avaliação desta tese. É uma honra ser avaliada por pessoas que tenho como referência na Medicina Veterinária.

A todos os professores do Programa Biociência Animal em especial a Profa. Tatiana Porto e Prof. Pabyton Cadena, por toda atenção e disponibilidade.

Agradeço aos colegas do Laboratório, graduandos, mestrandos, doutorandos, estagiários e funcionários UFAPE, em especial as amigas Renata Brito, Karlla Santos e ao Sr. Jair Rodrigues pela companhia e auxílio no desenvolvimento desta pesquisa.

A todos as pessoas que fazem parte do Departamento de Medicina Veterinária da Universidade de Bari, em particular o Prof. Domenico Otranto por todos os ensinamentos e atenção durante o período de doutorado sanduíche. Minha gratidão, por esta experiência incrível de minha vida profissional e pessoal.

Ao meu grande amigo Marcos Santos, que sempre me apoiou e incentivou em todos os momentos. A amiga Viviane Flores e Lucas Beltrame dos encontros agradáveis que a vida nos proporciona. Meu carinho é enorme por vocês.

A todas as pessoas que direta ou indiretamente contribuíram para esta conquista, minha gratidão.

RESUMO

O presente estudo fornece dados epidemiológicos da infecção por nematódeos broncopulmonares em ruminantes no estado de Pernambuco contemplando os seguintes aspectos: 1) Dados epidemiológicos, morfológicos e moleculares sobre o nematódeo broncopulmonar de uma espécie de *Protostrongylus* em caprinos. Amostras fecais de caprinos (*n* = 217) foram analisadas pela técnica de Baermann e larvas foram detectadas em 18,9% (41/217) das amostras. As larvas apresentaram comprimento médio de 339µm (±52,99µm) e largura média de 18µm (±1,46µm). Morfologicamente eram semelhantes a *Protostrongylus* sp. e molecularmente revelaram forte identidade (98,7%) com sequências homólogas de *Protostrongylus rufescens* disponíveis no GenBank. 2) Visão abrangente dos dados históricos e atuais publicados entre janeiro de 1980 a dezembro de 2020, sobre a infecção por nematódeos broncopulmonares de ruminantes domésticos no Brasil. Neste período foram publicados 24 artigos, sendo em bovinos (*n* = 16), caprinos (*n* = 6), ovinos (*n* = 1) e um estudo (*n* = 1) com caprinos e ovinos. No geral, 12 estudos foram baseados apenas em exame post-mortem, cinco na detecção de espécimes em amostras fecais e sete foram baseados em análise fecal seguida de exame post-mortem. De todos os estudos, 66,7% (n = 16) artigos registraram *Dictyocaulus viviparus*, 4,2% (*n* = 1) *D. filaria*, 8,3% (*n* = 2) *P. rufescens*, 16,7% (*n* = 4) *Muellerius capillaris*, e 4,2% (*n* = 1) co-infecção por *Dictyocaulus filaria* e *M. capillaris*. 3) Prevalência de parasitos pulmonares em ruminantes do semiárido, nordeste do Brasil. Um total de 429 amostras fecais foram coletadas de bovinos (*n* = 219), caprinos (*n* = 122) e ovinos (*n* = 88) e analisadas pela técnica de Baermann. Bovinos e ovinos foram negativos. Larvas de *Protostrongylus* sp. foram detectadas em 8,19% (10/122) dos caprinos. Elas apresentaram comprimento médio de 351µm (±29,06µm) e largura média de 19µm (±1,46µm). Todos os animais positivos eram mantidos em sistema de criação semi-intensivo e não apresentavam sinais clínicos sugestivos da infecção por nematódeos pulmonares. 4) Infecção por nematódeos pulmonares em rebanhos bovinos de corte criados em uma importante área de produção pecuária na região Nordeste do Brasil. De setembro de 2020 a agosto de 2021, foram coletadas mensalmente amostras fecais mensais (*n* = 493) de 46 bovinos de corte. Larvas de nematódeos broncopulmonares foram detectadas em 8,7% (4/46) dos animais. Vinte larvas foram recuperadas, com o número mínimo (*n* = 1) detectado em outubro e dezembro, e o

número máximo (*n* = 13) em novembro. Apresentavam comprimento médio de 363µm (±28,65µm), largura média de 19µm (±1,03µm) e eram morfologicamente semelhantes a *Dictyocaulus* sp.. A infecção por estes nematódeos tem sido relatada nas últimas quatro décadas no Brasil, mas a maioria das informações foi obtida no exame post-mortem. Por fim, relatamos a ocorrência destes parasitos em bovinos e caprinos da região nordeste. Apesar da ausência de sinais clínicos nos animais deste estudo, medidas sanitárias são preconizadas para prevenir a infecção por esses nematódeos e reduzir o impacto econômico que eles podem causar na produção pecuária.

Palavras-chave: *Protostrongylus rufescens*; *Dictyocaulus viviparus*; bovinos; caprinos; Baermann.

ABSTRACT

The present study provides epidemiological data on lungworm infection in ruminants in the state of Pernambuco, including the following aspects: 1) Epidemiological, morphological, and molecular data on the lungworm larva of a species of *Protostrongylus* from goats. Fecal samples from goats (*n* = 217) were analyzed by the Baermann technique and larvae were detected in 18.9% (41/217) of the samples. These larvae had a mean length of 339μm (±52.99μm) and a mean width of 18 μm (±1.46μm). Morphologically they were similar to *Protostrongylus* sp. and molecularly they revealed strong identity (98.7%) with *Protostrongylus rufescens* homologous sequences available in GenBank. 2) To provide a comprehensive overview of historical and current data published between January 1980 and December 2020 on lungworm infection of domestic ruminants in Brazil. In this period, 24 articles were published, being in cattle (*n* = 16), goats (*n* = 6), sheep (*n* = 1) and one study (*n* = 1) with goats and sheep. Overall, 12 studies were based on post-mortem examination only, five on detection of specimens in fecal samples, and seven were based on fecal analysis followed by post-mortem examination. Of all studies, 66.7% (*n* = 16) articles recorded *Dictyocaulus viviparus*, 4.2% (*n* = 1) *Dictyocaulus filaria*, 8.3% (*n* = 2) *P. rufescens*, 16.7% (*n* = 4) *M. capillaris*, and 4.2% (*n* = 1) co-infection by *D. filaria* and *Muellerius capillaris*. 3) To determine the prevalence of lungworms in ruminants from the semi-arid region of northeastern Brazil. A total of 429 fecal samples were collected from cattle (*n* = 219), goats (*n* = 122) and sheep (*n* = 88) and analyzed by the Baermann technique. Cattle and sheep were negative. Larvae of *Protostrongylus* sp. were detected in 8.19% (10/122) of goats. They had an average length of 351μm (±29.06μm) and an average width of 19μm (±1.46μm). All infected goats were raised in a semi-intensive production system and did not exhibit any clinical signs suggestive of the infection by lungworms. 4) Lungworms infection in beef cattle herds reared in an important livestock production area in the northeastern region of Brazil. From September 2020 to August 2021, monthly fecal samples (*n* = 493) were collected from 46 beef cattle. Out of all animals assessed, lungworm larvae were detected in 8.7% (4/46). Animals did not present any clinical sign suggestive of the infection by lungworms parasites. Twenty larvae were retrieved, with the minimal number (*n* = 1) detected in October and December, and the maximum number (*n* = 13) in November. They presented a mean length of 363μm (± 28.65μm), mean width of 19 μm (±1.03μm)

and were morphologically similar to *Dictyocaulus* sp.. Infection by these nematodes has been reported in the last four decades in Brazil, but most of the information was obtained from post-mortem examination. Finally, we report the occurrence of these parasites in cattle and goats in the northeastern region. Despite the absence of clinical signs in the animals in this study, sanitary measures are recommended to prevent infection by these nematodes and reduce the economic impact they can cause in livestock production.

Keywords: *Protostrongylus rufescens*; *Dictyocaulus viviparus*; cattle; goats; Baermann.

SUMÁRIO

1. INTRODUÇÃO

Nematódeos broncopulmonares são parasitos de grande importância econômica, uma vez que acometem vertebrados ungulados e lagomorfos, e são difundidos mundialmente (PANAYOTOVA-PENCHEVA et al., 2018; VEROCAI et al., 2018). Dentre estes representantes, temos as superfamílias Trichostrongyloidea e Metastrongyloidea que compreende diversos agentes transmitidos principalmente por moluscos terrestres (GREWAL et al., 2003; TAYLOR et al., 2016). Os gêneros mais importantes são *Protostrongylus*, *Muellerius* e *Dictyocaulus*. Outros parasitos pulmonares relatados incluem *Varestrongylus*, *Cystocaulus*, *Spiculocaulus* e *Neostrongylus*, porém de menor importância (TAYLOR et al., 2016; VEROCAI et al., 2018).

A transmissão destes parasitos é influenciada pelas condições ambientais que determinam o desenvolvimento desses nematódeos (JENKINS et al., 2006; CABLE et al., 2017). As espécies da superfamília Trichostrongyloidea, apresentam um ciclo de vida direto e a transmissão ocorre com a ingestão de larvas infectantes (L3) presentes nas pastagens (JORGENSEN et al., 1982). Diferentemente dos parasitos da superfamília Metastrongyloidea que apresentam ciclos biológicos indiretos, nos quais as larvas de primeiro estágio (L1) são eliminadas nas fezes dos hospedeiros vertebrados e parasitam gastrópodes terrestres, utilizando-os como hospedeiros intermediários, nos quais ocorre o desenvolvimento das formas infectantes. Os hospedeiros vertebrados se infectam quando ingerem os gastrópodes infectados com L3 (JENKINS et al., 2006; LESAGE et al., 2015).

Diversos gastrópodes terrestres foram identificados como hospedeiros intermediários de metastrongilídeos, desempenhando um papel importante na distribuição e transmissão dos nematódeos broncopulmonares (LESAGE et al., 2015; KUCHBOEV et al., 2012; 2017). Infecções naturais com larvas de protostrongilídeos foram relatadas em diferentes gastrópodes terrestres em pastagens de ruminantes de outros países (GEORGIEV et al., 2003; KUCHBOEV et al., 2012; 2017). No entanto no Brasil, dados sobre o papel destes hospedeiros ainda são desconhecidos (MACEDO et al., 2020). Os estudos no país são direcionados sobretudo ao papel

desses invertebrados como hospedeiros intermediários de nematódeos de importância em cães, gatos e na saúde pública (THIENGO et al., 2008).

Nos pequenos ruminantes, o gênero *Protostrongylus* causa comprometimento pulmonar, levando à oclusão de pequenos bronquíolos, devido ao preenchimento por ovos, larvas, adultos e restos celulares. Por outro lado, as infecções por *Muellerius* spp. caracteriza-se por pequenas lesões focais nodulares e esféricas, de coloração acinzentada (TAYLOR et al., 2016). Já a infecção por *Dictyocaulus* spp., agente causador da bronquite parasitária em bezerros e vacas leiteiras, ocasiona perdas econômicas consideráveis devido à redução da produção de leite, peso corporal ou até mesmo a morte de animais infectados (SCHUNN et al., 2013).

O diagnóstico de parasitos broncopulmonares é pouco utilizado, pois tem sido um desafio convencer veterinários e produtores de ruminantes sobre a importância da detecção desses parasitos, mesmo em animais com infecção subclínica ou assintomáticos (MAY et al., 2018). O método parasitológico apesar de suas limitações, ainda é rotineiramente usado para detecção das larvas de nematódeos broncopulmonares, através da técnica de Baermann (FORRESTER e LANKESTER, 1997; VEROCAI et al., 2020). Os métodos moleculares e sorológicos têm sido considerados ferramentas importantes para a detecção desses parasitos (MORELLI et al., 2020). No método sorológico destaca-se o Ensaio de Imunoadsorção Enzimática (ELISA), embora de difícil acesso a disponibilidade comercial (SEKIYA et al., 2013; McCARTHY et al., 2019).

Apesar da importância econômica e patogenicidade dos parasitos broncopulmonares, o conhecimento é limitado sobre a distribuição, seus ciclos biológicos, incluindo hospedeiros definitivos e intermediários, que são dados cruciais que devem ser integrados na profilaxia dos nematódeos broncopulmonares (KUCHBOEV et al., 2017). Poucos estudos têm sido descritos no Brasil, com predominância de relatos da infecção por *Dictyocaulus viviparus* infectando bovinos (MOLENTO et al., 2006; HENKER et al., 2017; CEZARO et al., 2018), seguido por relatos de *Muellerius capillaris* e *Protostrongylus rufescens* em pequenos ruminantes (DUARTE e MIRANDA, 1984; SALES et al., 2017; MACEDO et al., 2020). Por esta razão, é importante investigar a presença e conhecer aspectos epidemiológicos e moleculares dos nematódeos broncopulmonares em ruminantes no estado de Pernambuco.

2. REVISÃO DE LITERATURA

2.1 Nematódeos broncopulmonares de ruminantes

Os nematódeos broncopulmonares são parasitos patogênicos entre ruminantes domésticos e selvagens (CASSINI et al., 2015; KOWAL et al., 2016; HABTE e SIMENEH, 2019). Dentre os principais representantes destacam-se os das superfamílias Trichostrongyloidea e Metastrongyloidea (TAYLOR et al., 2016). Apesar da importância desses nematódeos, por muito tempo esses parasitos foram negligenciados, quanto a biogeografia, evolução e hospedeiros (VEROCAI et al., 2018). A ocorrência de determinadas espécies depende de complexos processos evolutivos e ecológicos que ocorreram ao longo do tempo. As flutuações climáticas, por exemplo, estão entre os principais fatores que moldaram a biodiversidade e serviram como determinantes das associações parasito-hospedeiro (CABLE et al., 2017).

Os tricostrongilídeos incluindo o gênero *Dictyocaulus*, encontram-se representados por nematódeos da família Dictyocaulidae, que acomete o sistema respiratório de hospedeiros ungulados, localizando-se na traqueia e nos brônquios (PANUSKA, 2006). Causa a dictiocaulose, caracterizada como uma bronquite, com infecção potencialmente fatal em indivíduos fortemente infectados (TAYLOR et al., 2016). De importância para ruminantes domésticos encontramos três espécies: *Dictyocaulus viviparus*, que tem como hospedeiros bovinos, búfalos, veados e camelos (MAHMOOD et al., 2014; PYZIEL et al., 2017; SAZMAND e JOACHIM, 2017), *Dictyocaulus eckerti,* encontrado em bovinos e veados (*roer deer,* gamo, veado-vermelho) (JOHNSON et al., 2003) e nos caprinos e ovinos *Dictyocaulus filaria*, assim como também em camelídeos (PANUSKA, 2006; SAZMAND e JOACHIM, 2017).

A fauna dos metastrongilídeos inclui a maior parte dos gêneros e espécies de parasitos broncopulmonares dos ruminantes domésticos e selvagens em todo o mundo (CASSINI et al., 2015; KOWAL et al., 2016; ASMARE et al., 2018). A família Protostrongylidae inclui gêneros e espécies, divididos em diferentes subfamílias, entre estas as subfamílias Protostrongylinae, Muelleriinae, Varestrongylinae, que apresentam espécies de parasitos estritamente broncopulmonares, com nematódeos

adultos encontrados nos brônquios, bronquíolos ou parênquima pulmonar (CARRENO e HOBERD, 1999).

Pequenos ruminantes domésticos (caprinos e ovinos) podem ser infectados principalmente por espécies pertencentes as subfamílias Protostrongylinae, e Muelleriinae representado por *Protostrongylus rufescens, Muellerius capillaris* e *Cystocaulus ocreatus* (BOEV, 1975; PANAYOTOVA-PENCHEVA, 2011).

2.2 Morfologia

De uma forma geral, os espécimes adultos (machos e fêmeas) de *Dictyocaulus* apresentam o corpo filiforme. Os machos possuem bolsa copuladora pequena e arredondada, espículos curtos e gubernáculo presente, medindo de 4,0 – 8,0 cm. As fêmeas têm a vulva próxima a metade do comprimento do corpo, medindo de 6,0 – 10,0 cm de comprimento (TAYLOR et al., 2016). As larvas de primeiro estágio (L1) de *D. filaria* têm de 550-580 µm, possuem uma protuberância cuticular característica na extremidade anterior*. D. viviparus* as L1 têm 390-450 µm, com as células intestinais contendo inúmeros grânulos de cromatina. Não há protuberância arredondada na extremidade anterior (SOULSBY, 1968).

Os espécimes adultos de *Protostrongylus* sp. são avermelhados e delgados. Os machos possuem bolsa copuladora bem desenvolvida, porém pequena, espículos quase retos e gubernáculo possui prolongamento em suas extremidades distais, medindo de 24,3 – 30,0 mm. Nas fêmeas, a vulva fica próximo a cauda e medem 28,0 – 40,0 mm (PANAYOTOVA-PENCHEVA, 2011). As L1 têm 320 – 400 µm, apresentam a cauda alongada, ligeiramente ondulada e ausência de uma espinha dorsal na inserção da ponta da cauda (SOULSBY, 1968).

Os adultos de *Muellerius* sp. são vermelho-acinzentados, delgados e filiformes. As fêmeas medem de 17,0 – 23,0 mm e os machos medem de 12,0 – 16,0 mm possuem a cauda enrolada em forma de espiral e bolsa muito pequena e dobrada para dentro. Os espículos ramificam em uma parte mais curta e fina e outra parte mais longa e espessa com a presença de numerosas pontas semelhantes a dentes em uma de suas superfícies (PANAYOTOVA-PENCHEVA, 2011). As L1 têm de 300 – 400 µm,

possuem a cauda em forma de S e um pequeno espinho adjacente a extremidade (MORGAN, 1929).

2.3 Epidemiologia

A epidemiologia da infecção por nematódeos broncopulmonares relaciona-se com diferentes fatores ligados as espécies de parasitos envolvidos, hospedeiros e ao ambiente (REGASSA et al., 2010; BORJI et al., 2012; BEKELE e SHIBBIRU, 2017; HABTE e SIMENEH, 2019). A transmissão destes nematódeos é influenciada pela época do ano, quantidade de parasitos presentes nas pastagens e situação imunológica dos animais. Falhas no fornecimento adequado de colostro, estresse no desmame e baixo escore corporal, são fatores que contribuem para a ocorrência da infecção por estes nematódeos (YIMER e DESIE, 2016; TOLOSSA, 2019).

Nos bovinos infectados por *D. viviparus,* os que geralmente desenvolvem sinais clínicos são os bezerros de primeira pastagem. No entanto, frequentemente nas últimas décadas, surtos entre animais adultos foram relatados (HOLZHAUER et al., 2011; SCHUNN et al., 2013; HENKER et al., 2017). A infecção por *D. filaria* em caprinos e ovinos pode acometer todas as idades, entretanto animais de 4 a 6 meses de idade são os mais gravemente afetados (TEWODROS et al., 2015; TOLOSSA, 2019). Assim como, as infecções por *P. rufescens* e *M. capilaris* acomete todas as idades e envolve a complexidade da existência de hospedeiros intermediários adequados (PANUSKA, 2006; HABTE e SIMENEH, 2019).

A prevalência da infecção por nematódeos broncopulmonares é significativamente maior em animais jovens e mantidos em sistema de criação extensivo, o que favorece maior contato com pastagens contaminadas (TOLOSSA, 2019). Acredita-se que os caprinos são mais suscetíveis aos nematódeos broncopulmonares quando comparado a ovinos, devido aos diferentes hábitos alimentares desses animais. Além disso, caprinos demoram mais para desenvolver uma resposta imune (HOSTE et al., 2010).

A ocorrência desses parasitos é diretamente influenciada pelas condições ambientais que determinam o desenvolvimento e a oportunidade de transmissão (HOBERG e BROOKS, 2015; CABLE et al., 2017). As larvas L3 de *D. viviparus*

movem-se das fezes para a pastagem e, eventualmente, usam a esporulação do fungo *Pilobolus* para atingir áreas mais distantes (JORGENSEN et al., 1982). Já os metastrongilídeos têm um ciclo de vida indireto, usando gastrópodes como hospedeiros intermediários (KUCHBOEV et al., 2017). Considerando os diferentes aspectos ambientais e estratégias para a dispersão e desenvolvimento desses nematódeos, as diferenças climáticas (por exemplo, precipitação, umidade e temperatura) favorecem a heterogeneidade na distribuição desses parasitos broncopulmonares (JENKINS et al., 2006; VALENTE et al., 2020).

2.4 Distribuição geográfica

Apesar dos poucos dados sobre a distribuição geográfica dos nematódeos broncopulmonares, foram relatados em diferentes continentes, como nas Américas (WAPENAAR et al., 2007; HENKER et al., 2017; MACEDO et al., 2020), Europa (KOWAL et al., 2016), África (BEKELE e SHIBBIRU, 2017; ASMARE et al., 2018) e Ásia (LAT-LAT et al., 2007). No Brasil, a maioria dos casos de infecções broncopulmonares foram descritas nas regiões Sul e Sudeste do Brasil. Com evidente predominância de infecções em rebanhos bovinos (MOLENTO et al., 2006; HENKER et al., 2017; CEZARO et al., 2018), mas pouco se sabe sobre a ocorrência de parasitos broncopulmonares em rebanhos de caprinos e ovinos.

A prevalência de infecções por *D. viviparus* entre bovinos brasileiros variou de 9,1% até 100% em rebanho onde ocorreram surtos. Descritas nas regiões sul e sudeste do país, conforme a Tabela 1. Por outro lado, são escassas as informações da infecção por *D. filaria,* encontramos apenas um relato com infecção mista com *M. capillaris* em ovinos no Rio Grande do Sul, região Sudeste (GONÇALVES et al., 1980) e outro estudo com caprinos e ovinos no Maranhão, região Nordeste (SALES et al., 2017).

Tabela 1. Distribuição da infecção por *D. viviparus* em bovinos do Brasil.

Região / Estado	Município	% Animais positivos	Referência
SUDESTE			
Rio de Janeiro	Cantagalo	20,0	DUARTE et al. (1982)
Minas Gerais	Coronel Pacheco	95,8	FURLONG et al. (1985)
São Paulo	São Carlos	13,9	OLIVEIRA (1988)
Minas Gerais	Belo Horizonte	100,0	LIMA et al. (1995)
São Paulo	Botucatu	10,5	GONÇALVES et al. (2000)
São Paulo	Jaboticabal	16,7	BORGES et al. (2001)
São Paulo	São Paulo	9,1	LANDIM et al. (2001)
São Paulo	Botucatu e Manduri	19,0	CEZARO et al. (2016)
São Paulo	Botucatu e Manduri	37,9	CEZARO et al. (2018)
SUL			
Rio Grande do Sul	Santa Maria	13,3	SILVA et al. (2005)
Rio Grande do Sul	Santa Maria	100,0	MOLENTO et al. (2006)
Santa Catarina	Arabutã	100,0	SILVA et al. (2016)
Santa Catarina	Arabutã	100,0	SILVA et al. (2017)
Santa Catarina	Arabutã	71,9	HENKER et al. (2017)
Santa Catarina	Arabutã	100,0	PEREIRA et al. (2017)
Paraná	Londrina	55,6	SCHADE et al. (2020)

São escassos os estudos com infecções por outros parasitos broncopulmonares, como *P. rufescens* e *M. capillaris,* no Brasil encontramos alguns relatos nas regiões Sul, Sudeste e Nordeste, principalmente infectando caprinos, conforme tabela 2. Sabe-se que a distribuição destes parasitos é influenciada pelo

complexo ciclo de vida envolvendo gastrópodes como hospedeiros intermediários (PANUSKA, 2006; KUCHBOEV et al., 2017; TOLOSSA, 2019).

Tabela 2. Distribuição da infecção por *P. rufescens* e *M. capillaris* em caprinos do Brasil.

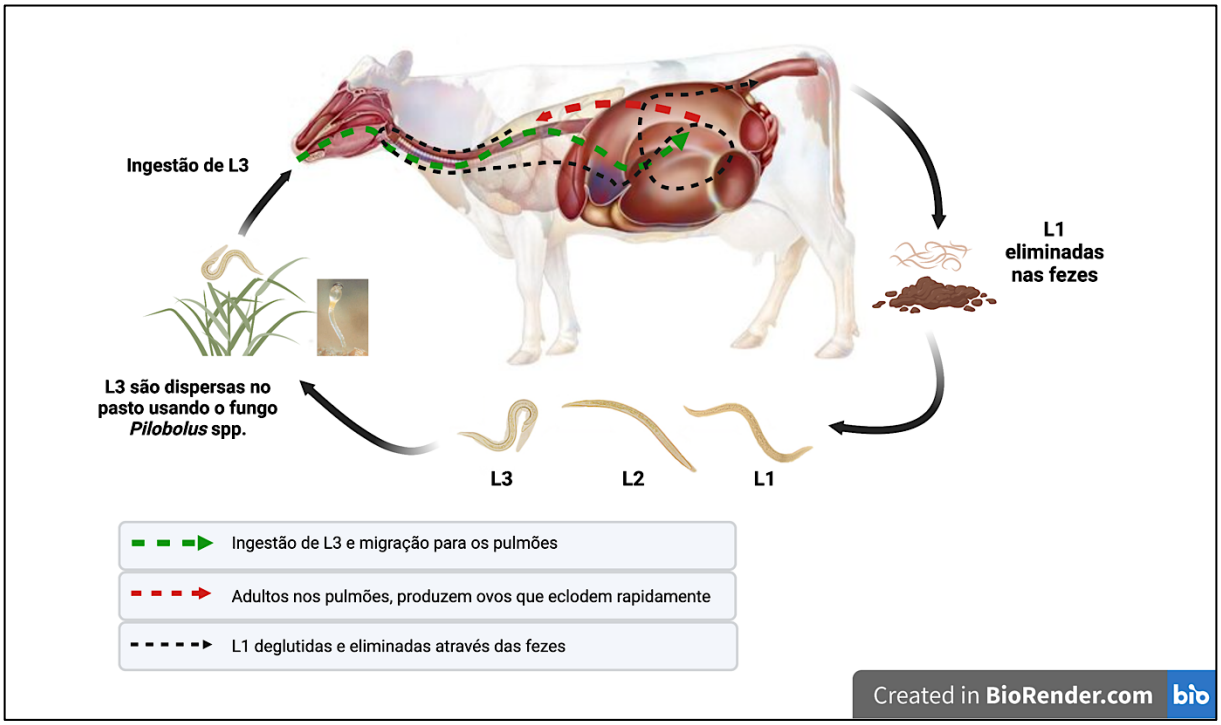
Região / Estado	Parasito	Município	% Animais positivos	Referência
SUDESTE				
Rio de Janeiro	*P. rufescens*	São Gonçalo	100,0	DUARTE e MIRANDA (1984)
Minas Gerais	*M. capillaris*	Contagem, Betim e Ribeirão das Neves	4,0	MACHADO e LIMA (1988)
SUL				
Rio Grande do Sul	*M. capillaris*	Porto Alegre, Canoas e Gravataí	4,3	CARDOSO e OLIVEIRA (1993)
Rio Grande do Sul	*M. capillaris*	Santa Maria	2,1	ROSA et al. (2013)
Rio Grande do Sul	*M. capillaris*	Porto Alegre	1,2	BASSUINO et al. (2018)
NORDESTE				
Pernambuco	*P. rufescens*	Paranatama	18,9	MACEDO et al. (2020)

2.5 Ciclo biológico

O ciclo de vida dos parasitos broncopulmonares em ruminantes pode ser direto ou indireto. As espécies de tricostrongilídeos apresentam um ciclo de vida direto, ao contrário dos metastrongilídeos que tem um ciclo indireto em que necessitam de um hospedeiro intermediário, o que torna um ciclo mais complexo (ADEM, 2016; KUCHBOEV et al., 2017; TOLOSSA, 2019).

As espécies de *Dictyocaulus* tem um ciclo biológico direto (**Figura 1**) e a transmissão ocorre com a ingestão de larvas infectantes (L3) que são dispersas nas pastagens, muitas vezes através da esporulação do fungo *Pilobolus* (JORGENSEN et al., 1982). Após ingestão as larvas penetram nas paredes da mucosa gastrointestinal e através do sistema linfático migram para os pulmões, até atingirem os estágios adultos e iniciar a reprodução (SOLIMAN, 1953). As fêmeas produzem ovos embrionados, que eclodem rapidamente após oviposição nas vias aéreas ou após ser tossido e deglutido. As larvas de primeiro estágio (L1) rompem os alvéolos e atingem a árvore brônquica, migram até a traqueia e são eliminadas com o muco respiratório sendo então deglutidas e então atingem o esôfago, e são eliminadas através das fezes dos hospedeiros, para o ambiente. Na pastagem o desenvolvimento de L1 a L3, geralmente concluído em uma semana com condições favoráveis de temperatura (JORGENSEN, 1980). O rápido desenvolvimento dos estágios larvais dos estágios L1 e L2 é auxiliado pela manutenção de um alto nível de lipídios dentro do corpo larval, de forma que as larvas não se alimentam no estágio pré-infeccioso (CROLL, 1973).

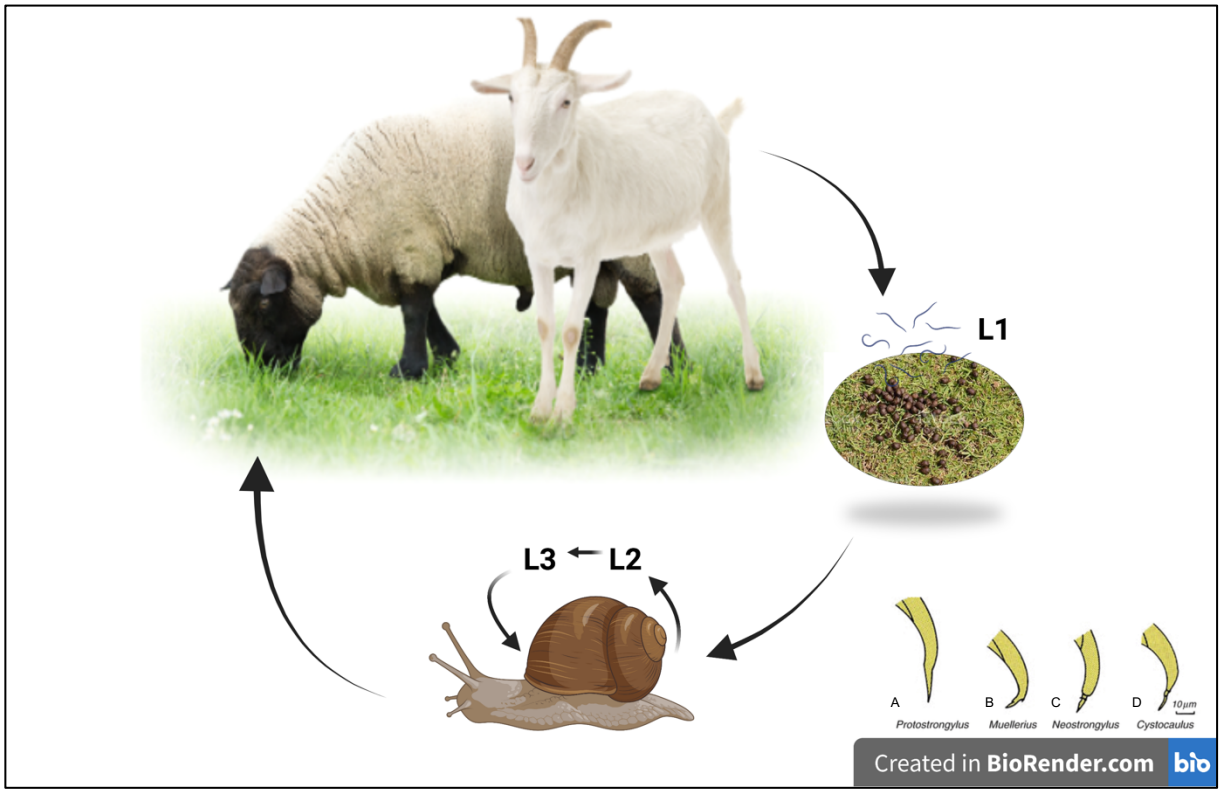
Figura 1. Ciclo biológico de *D. viviparus.*



O ciclo de vida das espécies de metastrongilídeos, por exemplo, dos gêneros *Protostrongylus* e *Muellerius,* possuem fêmeas ovovivíparas e através das fezes de seus hospedeiros eliminam as L1 (TAYLOR et al., 2016). Estas larvas penetram o pé do gastrópode, hospedeiro intermediário e desenvolve-se para L3 em um período de

21 a 30 dias (SAMSON 1985; LESAGE et al., 2015). Os ruminantes se infectam pela ingestão desses gastrópodes e no processo de digestão as L3 são liberadas e migram para o pulmão através do sistema linfático, ocorrendo muda dos estágios nos linfonodos mesentéricos e nos pulmões (TAYLOR et al., 2016).

Figura 2. Ciclo biológico das principais espécies metastrongilídeos que afetam caprinos e ovinos, que são diferenciados pelo tamanho e características das



extremidades caudais das L1s (A - *Protostrongylus rufescens*, B - *Muellerius capillaris*, C - *Neostrongylus linearis*, D - *Cystocaulus ocreatus*).

2.5.1 Hospedeiro intermediário

Uma variedade de moluscos terrestres podem ser hospedeiros intermediários de metastrongilídeos tanto de interesse médico quanto de importância veterinária (ANDERSON, 2000; SPRATT, 2015). Estudos com a identificação desses hospedeiros foram realizados, principalmente, os que envolvem infecção nos humanos (OLIVEIRA et al., 2015; PENAGOS-TABARES et al., 2019; BEZERRA-SANTOS et al., 2020). Os hospedeiros intermediários de espécies envolvidas em doenças que afetam o sistema pulmonar de ruminantes domésticos e responsáveis

por perdas econômicas, também têm sido estudados (ROGERSON et. al., 2008; KUCHBOEV et al., 2012; 2017).

Infecções naturais com larvas de protostrongilídeos foram relatadas em diferentes gastrópodes terrestres em pastagens de ruminantes da Europa (GEORGIEV e GIORGIEV, 2002; GEORGIEV et al., 2003), África (KUCHBOEV et al., 2012; 2017), Asia (LAHMAR et al., 1990), América de Norte (ROGERSON et al., 2008). Moluscos dos gêneros *Helicella, Xeropicta*, *Vallonia, Pupila, Pseudonapaeus*, *Leucozonella* desempenham um papel importante na distribuição e transmissão destes nematódeos (GREWAL et al., 2003; GEORGIEV et al., 2003; KUCHBOEV et al., 2017).

Atualmente, cerca de 590 espécies de gastrópodes terrestres são conhecidas no Brasil (SALGADO e COELHO, 2003). Estudos concentram-se no papel desses invertebrados como hospedeiros intermediários de nematódeos de importância em cães, gatos e na saúde pública (THIENGO et al., 2008). Por exemplo, os espécimes *Bradybaena similares, Subulina octona*, *Sarasinula marginata* e *Achatina fulica* foram encontrados naturalmente infectados (CALDEIRA et al., 2007; THIENGO et al., 2010; OLIVEIRA et al., 2015). No entanto, em ruminantes, dados sobre o papel destes hospedeiros ainda são desconhecidos (MACEDO et al., 2020).

A identificação e posterior controle desses gastrópodes pode ser a chave para interromper a dispersão desses patógenos em todo o mundo, porém as ações antrópicas (por exemplo, desmatamento, mudanças climáticas e intenso comércio entre os países) facilitaram a dispersão desses invertebrados e, consequentemente, os patógenos por eles transmitidos (KAFLE et al., 2018).

2.6 Patogenia e sinais clínicos

As características patogênicas nas infecções por nematódeos broncopulmonares depende do estágio da doença, carga parasitária e imunidade do hospedeiro. Anorexia, diminuição da produção, dispneia, taquipneia e tosse são frequentemente observados e pode ocasionar a morte de animais infectados (WAPENAAR et al., 2007).

A infecção por *Dictyocaulus* pode resultar em pneumonia parasitária e bronquite. Três fases ocorrem no desenvolvimento da doença. Primeiro, larvas são ingeridas e migram para os pulmões, ocorre de 1 a 7 dias após a infecção, nessa fase não há sinais clínicos ou condições patológicas significativas. Segundo, no período pré-patente, de 7 a 25 dias, as larvas chegam ao tecido pulmonar e desenvolve pequenos focos de pneumonia e bronquiolite eosinofílica, que podem aparecer grosseiramente como pequenas áreas multifocais de atelectasia lobular e edema pulmonar, responsável pelos primeiros sinais clínicos de taquipneia e tosse. Terceiro, na fase patente, de 25 a 55 dias, helmintos adultos se desenvolvem nos brônquios e induzem bronquite eosinofílica a mucopurulenta, resultando em atelectasia, enfisema e pneumonia (PANUSKA, 2006; NASHIRUDDULLAH et al., 2007; PANCIERA e CONFER, 2010).

Embora os metastrongilídeos sejam menos patogênicos, esses parasitos podem causar dificuldade respiratória, perda de peso e secreção muco nasal (MANSFIELD et al., 1993). O gênero *Protostrongylus* os adultos são encontrados nos bronquíolos, onde provocam irritação e desenvolvem áreas de inflamação, resultando em pequenos focos de pneumonia lobular (TAYLOR et al., 2016). Além disso, a formação de granulomas e pneumonia eosinofílica crônica foi observada no exame post-mortem (PANAYOTOVA-PENCHEVA e ALEXANDROV, 2010).

M. capillaris é encontrado no parênquima pulmonar, onde forma lesões nodulares, firmes e cinzenta. Em adição a infecção por esse parasito pode estar associado também a uma reação exsudativa (BERRAG et al., 1997; PANAYOTOVA-PENCHEVA E ALEXANDROV, 2010). A infecção é mais patogênica em cabras, onde contagens de larvas fecais podem estar associadas a sinais respiratórios (SAUERLANDER, 1988). Infecções intensas podem resultar em pneumonia intersticial, broncopneumonia ou pleurite fibrinosa. Assim como, pode desencadear infecções secundária, com pneumonia bacteriana (NIMMO, 1979; PANUSKA, 2006).

2.7 Diagnóstico

De modo geral, o diagnóstico de nematódeos brocopulmonares é pouco utilizado, pois tem sido um desafio convencer veterinários e produtores de ruminantes

sobre a importância da detecção desses parasitos, mesmo em animais com infecção subclínica ou assintomáticos (MAY et al., 2018). Uma maior atenção é dada a outras doenças parasitárias, por exemplo, a hemoncose. Muitos estudos sobre infecções por esses parasitos são de exames post-mortem. No entanto, deve ser enfatizado que existem testes de diagnóstico *in vivo* relativamente simples que podem ser implementados (VEROCAI et al., 2020).

Há muito tempo, a técnica de Baermann tem sido empregada para detecção e diferenciação de larvas broncopulmonares e ainda é rotineiramente usada para a confirmação da infecção, pela detecção de L1 nas fezes dos animais (FORRESTER e LANKESTER, 1997; VEROCAI et al., 2020). Essa técnica tem sido considerada o padrão ouro para detecção de L1 e apresenta alta sensibilidade (100%) para detecção de infecção primária em animais jovens (EYSKER, 1997). No entanto, em animais adultos, a sensibilidade provou ser baixa (7,5%), provavelmente devido a reinfecções por estes parasitos, que resultam na ausência ou baixa eliminação de larvas nas fezes (MAY et al., 2018; LURIER et al., 2018). Além disso, requer profissional treinado para realizar a detecção e identificação larval correta, evitando erros de identificação com larvas de tricostrongilídeos gastrointestinais (VIÑA et al., 2013; VEROCAI et al., 2020).

Nos últimos anos, novas ferramentas diagnósticas foram desenvolvidas para avaliação de parasitos em amostras fecais. Por exemplo, as técnicas FLOTAC e Mini-FLOTAC surgiram como técnicas promissoras e confiáveis na detecção de estágios larvais de parasitos em fezes (CRINGOLI et al., 2010; BARDA et al., 2013). Em particular, ambas as técnicas foram empregadas para a detecção de L1 de parasitos broncopulmonares, especialmente aquelas responsáveis pela infecção em cães e gatos (GAGLIO et al., 2008; IANNIELLO et al., 2020). Embora utilizadas em ruminantes, ambas as técnicas necessitam de uma avaliação mais completa para melhor estabelecer valores de sensibilidade em animais de diferentes idades e distintas soluções utilizadas para cada parasito (BAUER et al., 2010; RINALDI et al., 2010).

O lavado broncoalveolar também é considerado uma opção para detecção de L1 de nematódeos broncopulmonares. Apesar da baixa sensibilidade (24,7%), este método pode obter dois dados importantes como a detecção dos parasitos e a resposta imune local desenvolvida pelo hospedeiro contra esses nematódeos

(HAGBERG et al., 2005; HOLMGREN et al., 2014; LURIER et al., 2018). Mesmo assim, seu uso tem sido mais restrito para esse fim em ruminantes.

As técnicas clássicas baseadas em microscopia, usadas rotineiramente para diagnosticar esses nematódeos em ruminantes, apresentam limitações. Com isso, as ferramentas sorológicas e moleculares têm sido consideradas ferramentas importantes para a detecção desses parasitos (MORELLI et al., 2020).

Apesar da dificuldade de encontrar ferramentas sorológicas comercialmente disponíveis para detecção destes parasitos, o método sorológico, representado principalmente por teste ELISA, pode detectar anticorpos específicos contra *D. viviparus* em bovinos adultos, representando uma alternativa para testes em larga escala (SEKIYA et al., 2013; McCARTHY et al., 2019). Isso inclui testes ELISA que têm especificidade e sensibilidade de 100% e 97,5%, respectivamente (FIEDOR et al., 2009). No entanto, há apenas um curto período em que animais reinfectados podem ser diagnosticados como positivos, o que representa uma limitação para seu uso em ruminantes adultos (McCARTHY et al., 2019).

Levando-se em consideração também a dificuldade de diferenciar as larvas de primeiro estágio de espécies de protostrongilídeos, que são morfologicamente semelhantes entre os diversos gêneros (KAFLE et al., 2017; Verocai et al., 2020). Ferramentas moleculares empregando marcadores genéticos de DNA ribossômico (r) ITS2 e marcadores de DNA mitocondrial podem apoiar estudos na diferenciação das espécies, no desenvolvimento de testes diagnósticos aprimorados e vacinas (CARRENO et al., 2009; GASSER et al., 2012). Recentemente, o uso de tecnologias de sequenciamento de última geração revolucionou o diagnóstico de bactérias e infecções virais, mas ainda é raramente usado para detecção de parasitos (AVRAMENKO et al., 2015). Considerando a diversidade de parasitos broncopulmonares que infectam ruminantes, esse tipo de abordagem pode ser considerada uma ferramenta promissora. Recentemente, parasitos gastrointestinais de ruminantes domésticos foram avaliados por estas técnicas (AVRAMENKO et al., 2015; AVRAMENKO et al., 2017), portanto, o uso desta tecnologia também pode ser implementado para os nematódeos broncopulmonares.

3. REFERÊNCIAS

ADEM, J. Lungworm infection of small ruminant in Ethiopia. **Advances in Life Science and Technology**, v. 43, p. 12–22, 2016.

ANDERSON, R.C. **Nematode parasites of vertebrates: their development and transmission**. 2ª ed. Wallingford: UK, CABI Publishing 650 p. 2000.

ASMARE, K., SIBHAT, B., HAILE, A., SHEFERAW, D., ARAGAW, K., ABERA, M., ABEBE, R., WIELAND, B. Lungworm infection in small ruminants in Ethiopia: Systematic review and meta-analysis. **Veterinary Parasitology: Regional Studies and Reports**, v. 14, p. 63–70, 2018.

AVRAMENKO, R.W., REDMAN, E.M., LEWIS, R., BICHUETTE, M.A., PALMEIRA, B.M., YAZWINSKI, T.A., GILLEARD, J.S. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. **International Journal for Parasitology**, v. 47, n. 13, p. 893–902, 2017.

AVRAMENKO, R.W., REDMAN, E.M., LEWIS, R., YAZWINSKI, T.A., WASMUTH, J.D., GILLEARD, J.S. Exploring the Gastrointestinal "Nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. **PloS One**, v. 10, n. 12, p. 1–18, 2015.

BARDA, B., ZEPHERINE, H., RINALDI, L., CRINGOLI, G., BURIONI, R., CLEMENTI, M., ALBONICO, M. Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. **Parasites & Vectors**, v. 6, n. 1, 1–6, 2013.

BASSUINO, D.M., KONRADT, G., BIANCHI, M.V., SNEL, G.G.M., SONNE, L., PAVARINI, S.P., DRIEMEIER D. Causes of death in goats in Rio Grande do Sul state, Brazil: analysis of 322 cases (2000-2016). **Pesquisa Veterinária Brasileira**, v. 38, n. 11, p. 2080–2087, 2018.

BAUER, B.U., POMROY, W.E., GUEYDON, J., GANNAC, S., SCOTT, I., PFISTER, K. Comparison of the FLOTAC technique with the McMaster method and the Baermann technique to determine counts of *Dictyocaulus eckerti* L1 and strongylid eggs in faeces of red deer (*Cervus elaphus*). **Parasitology Research**, v. 107, n. 3, p. 555–560, 2010.

BEKELE, T., SHIBBIRU, T. Prevalence of ovine lungworm and associated risk factors in and around Debre Berhan town, Ethiopia. **International Journal of Veterinary Health Science & Research**, v. 5, n. 6, p. 190–195, 2017.

BERRAG, B., RHALEM, A., SAHIBI, H., DORCHIES, P., CABARET, J. Bronchoalveolar cellular responses of goats following infections with *Muellerius capillaris* (Protostrongylidae, Nematoda). **Veterinary Immunology and Immunopathology**, v. 58, n. 1, p. 77–88, 1997.

BEZERRA-SANTOS, M.A., MENDOZA-ROLDAN, J.A., ABRAMO, F., LIA, R.P., TARALLO, V.D., SALANT, H., BRIANTI, E., BANETH, G., OTRANTO, D. Transmammary transmission of *Troglostrongylus brevior* feline lungworm: a lesson from our gardens. **Veterinary Parasitology**, v. 285, p. 1–7, 2020.

BOEV, S., 1975. **Protostrongylids. Fundamentals of Nematology** Helminthological Laboratory, Academy of Sciences of the USSR, Moscow, Washington, D.C., and Amerind Publishing Co., New Delhi, v. 25, 338p. 1984.

BORGES, F.A., SILVEIRA, D.M., GRAMINHA, E.B.N., CASTAGNOLLI, K.C., SOARES, V.E., NASCIEMENTO, A.A., COSTA, A.J. Fauna helmintológica de bovinos da região de Jaboticabal, Estado de São Paulo, Brasil. **Semina: Ciências Agrárias**, v. 22, n. 1, p. 49–53, 2001.

BORJI, H., AZIZZADEH, M., EBRAHIMI, M., ASADPOUR, M. Study on small ruminant lungworms and associated risk factors in northeastern Iran. **Asian Pacific Journal of Tropical Medicine**, v. 5, n. 11, p. 853–856, 2012.

CABLE, J., BARBER, I., BOAG, B., ELLISON, A.R., MORGAN, E.R., MURRAY, K., PASCOE, E.L., SAIT, S.M., WILSON, A.J., BOOTH, M. Global change, parasite transmission and disease control: lessons from ecology. **Philosophical transactions of the Royal Society of London. Series B, Biological sciences,** v. 372, n. 1719, p. 1–17, 2017.

CALDEIRA, R. L., MENDONÇA, C. L., GOVEIA, C. O., LENZI, H. L., GRAEFF-TEIXEIRA, C., LIMA, W. S., MOTA, E. M., PECORA, I. L., MEDEIROS, A. M., CARVALHO, O. First record of molluscs naturally infected with *Angiostrongylus cantonensis* (Chen, 1935) (Nematoda: Metastrongylidae) in Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 102, n. 7, p. 887–889, 2007.

CARDOSO, J.I.S., OLIVEIRA, C.M.B. Fauna parasitária de caprinos na grande Porto Alegre. **Revista Brasileira de Parasitologia Veterinária**, v. 2, n. 1, p. 57–60, 1993.

CARRENO, R.A., DIEZ-BAÑOS, N., HIDALGO-ARGÜELLO, M., NADLER, S.A. Characterization of *Dictyocaulus* species (Nematoda: Trichostrongyloidea) from three species of wild ruminants in northwestern Spain. **The Journal of Parasitology**, v. 95, n. 4, p. 966–970, 2009.

CARRENO, R.A., HOBERG, E.P. Evolutionary relationships among the Protostrongylidae (Nematoda: Metastrongyloidea) as inferred from morphological characters, with consideration of parasite-host coevolution. **Journal of Parasitology** v. 85, n. 1, p. 638–648, 1999.

CASSINI, R., PÁRRAGA, M.A., SIGNORINI, M., FRANGIPANE DI REGALBONO, A., STURARO, E., ROSSI, L., RAMANZIN, M. Lungworms in Alpine ibex (*Capra ibex*) in the eastern Alps, Italy: An ecological approach. **Veterinary Parasitology**, v. 214, n. (1-2), p. 132–138, 2015.

CEZARO, M.C., NEVES, J.H., CURY, J.R.L.M., DALANEZI, F.M., OLIVEIRA, R.M., FERREIRA, J.C.P., NETO, V.A.K., SCHMIDT, E.M.S. Gastrointestinal and pulmonary nematodes in calves naturally infected in the cities of Botucatu and Manduri, in the Brazilian state of São Paulo. **Brazilian Journal of Veterinary Research and Animal Science**, v. 38, p. 1286–1292, 2018.

CEZARO, M.C., TVARIJONAVICIUTE, A., TECLES, F., CÉRON, J.J., ECKERSALL, D.P., FERREIRA, J.C., SCHMIDT, E.M. Changes in biochemical analytes in calves infected by nematode parasites in field conditions. **Veterinary Parasitology**, v. 219, p. 1–6, 2016.

CRINGOLI, G., RINALDI, L., MAURELLI, M.P., UTZINGER, J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. **Nature Protocols**, v. 5, n. 3, p. 503–515, 2010.

CROLL N.A. Feeding shown to be unnecessary in preinfective larvae of *Dictyocaulus viviparus*. **International Journal for Parasitology**, v. 3, n. 4, p. 571–572, 1973.

DUARTE, M.J.F., GOMES, P.A.C., SANT'ANNA, D.B. Prevalência e intensidade de infecção helmíntica em bezerros de Cantagalo, RJ, Brasil. **Pesquisa Agropecuária Brasileira**, v. 17, n. 10, p. 1521–1524, 1982.

DUARTE, M.J.F., MIRANDA, I.M. Ocorrência de *Protostrongylus rufescens* (Nematoda: Protostrongylidae) em caprino do Estado do Rio de Janeiro. **Pesquisa Agropecuária Brasileira,** v. 19, n. 5, p. 647–649, 1984.

EYSKER M. The sensitivity of the Baermann method for the diagnosis of primary *Dictyocaulus viviparus* infections in calves. **Veterinary Parasitology**, v. 69, n. 1-2, p. 89–93, 1997.

FIEDOR, C., STRUBE, C., FORBES, A., BUSCHBAUM, S., KLEWER, A. M., VON SAMSON-HIMMELSTJERNA, G., SCHNIEDER, T. Evaluation of a milk ELISA for the serodiagnosis of *Dictyocaulus viviparus* in dairy cows. **Veterinary Parasitology**, v. 166, n. 3-4, p. 255–261, 2009.

FORRESTER, S.G., LANKESTER, M.W. Extracting protostrongylid nematode larvae from ungulate feces. **Journal of Wildlife Diseases**, v. 33, n. 3, p. 511–516, 1997.

FURLONG, J., NOVAS, J.C.V., CARDOSO FILHO, J.B. Parasitose dos bovinos na região da mata de Minas Gerais. **Pesquisa Agropecuária Brasileira,** v. 20, n. 12, p. 1409–1413, 1985.

GAGLIO, G., CRINGOLI, G., RINALDI, L., BRIANTI, E., GIANNETTO, S. Use of the FLOTAC technique for the diagnosis of *Aelurostrongylus abstrusus* in the cat. **Parasitology Research**, v. 103, n. 5, p. 1055–1057, 2008.

GASSER, R.B., JABBAR, A., MOHANDAS, N., HÖGLUND, J., HALL, R.S., LITTLEWOOD, D. T., JEX, A.R. Assessment of the genetic relationship between *Dictyocaulus* species from *Bos taurus* and *Cervus elaphus* using complete mitochondrial genomic datasets. **Parasites & Vectors**, v. 5, n. 241, 1–11, 2012.

GEORGIEV, D.M, KOSTADINOVA, A., GEORGIEV, B.B. Land snails in the transmission of protostrongylids on pastures in Southern Bulgaria: variability of infection levels related to environmental factors. **Acta Parasitologica**, v. 48, n. 3, p. 208–217, 2003.

GEORGIEV, D.M., GEORGIEV, B.B. Terrestrial gastropods as intermediate hosts of protostrongylid nematodes in pastures for sheep and goats in the region of Stara Zagora, Bulgaria. **Acta Zoologica Bulgarica**, v. 54, p. 47-54, 2002.

GONÇALVES, P.C., SANTOS, O.P., UENO, H. *Muellerius capillaris* em ovinos: ocorrência e identificação de adultos. **Pesquisa Agropecuária Brasileira**, v. 15, p. 137–141, 1980.

GONÇALVES, R.C., LISBÔA, J.A.N., SOUZA, M.V., ALMEIDA, C.T., KUCHEMBUCK, M.R.G., CHIACCHIO, S.B. Aspectos clínicos e epidemiológicos da broncopneumonia dos bezerros em Botucatu, SP. **Revista Brasileira de Ciência Veterinária**, v. 7, n. 3, p. 144–147, 2000.

GREWAL, P.S., GREWAL, S.K., TAN, L., ADAMS, B.J. Parasitism of molluscs by nematodes: types of associations and evolutionary trends. **Journal of Nematology**, v. 35, n. 2, p. 146–156, 2003.

HABTE, D., SIMENEH, A. Prevalence, associated risk factors and species identification of lung worm infection in sheep in Dangla district, Western Amhara, North West Ethiopia. **International Journal of Veterinary and Science Research**, v. 5, n. 1, p. 76–85, 2019.

HAGBERG, M., WATTRANG, E., NISKANEN, R., TRÅVÉN, M., HÖGLUND, J., LUNDÉN, A. Mononuclear cell subsets in bronchoalveolar lavage fluid during *Dictyocaulus viviparus* infection of calves: a potential role for gamma/delta TCR-expressing cells in airway immune responses? **Parasite Immunology**, v. 27, n. 5, p. 151–161, 2005.

HENKER, L.C., SCHWERTZ, C.I., LUCCA, N.J., PIVA, M.M., GIACOMIN, P., GRIS, A., RHODEN, L.A., NORBURY, L.J., DA SILVA, A.S., ROSA, R.A., MENDES, R.E. Dictyocaulosis in dairy cows in Brazil: an epidemiological, clinical-pathological and therapeutic approach. **Acta Parasitologica**, v. 62, n. 1, p. 129–132, 2017.

HOBERG, E.P., BROOKS, D.R. Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. **Philosophical transactions of the Royal Society of London. Series B, Biological sciences**, v. 370, n. 1665, 1–7, 2015.

HOLMGREN, S., HAGBERG GUSTAVSSON, M., LUNDÉN, A., WATTRANG, E. Cytokine mRNA expression in bronchoalveolar lavage cells during *Dictyocaulus viviparus* infection in calves. **Parasite Immunology**, v. 36, n. 2, p. 78–86, 2014.

HOLZHAUER, M., VAN SCHAIK, G., SAATKAMP, H.W., PLOEGER, H.W. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. **Veterinary Record**, v. 169, n. 494, p. 1–5, 2011.

HOSTE, H., SOTIRAKI, S., LANDAU, S. Y., JACKSON, F., BEVERIDGE, I. Goat-nematode interactions: think differently. **Trends in Parasitology**, v. 26, n. 8, p. 376–381, 2010.

IANNIELLO, D., PEPE, P., ALVES, L. C., CIUCA, L., MAURELLI, M. P., AMADESI, A., BOSCO, A., MUSELLA, V., CRINGOLI, G., RINALDI, L. Why Use the Mini-FLOTAC to detect metastrongyloid larvae in dogs and cats? **Acta Parasitologica**, v. 65, n. 2, p. 546–549, 2020.

JENKINS, E.J., VEITCH AM, KUTZ, S.J., HOBERG, E.P., POLLEY, L. Climate change and the epidemiology of protostrongylid nematodes in northern ecosystems: *Parelaphostrongylus odocoilei* and *Protostrongylus stilesi* in Dall's sheep (*Ovis* d. *dalli*). **Parasitology**, v. 132, n. 3, p. 387–401, 2006.

JOHNSON, M., MACKINTOSH, C.G., LABES, R.E., TAYLOR, M.J., WHARTON, D.A. *Dictyocaulus* species: cross infection between cattle and red deer. **New Zealand Veterinary Journal**, v. 51, n. 2, p. 93–98, 2003.

JORGENSEN, R.J. Epidemiology of bovine dictyocaulosis in Denmark. **Veterinary Parasitology**, v. 7, p. 153–167, 1980.

JORGENSEN, R.J., RONNE, H., HELSTED, C., ISKANDER, A.R. Spread of infective *Dictyocaulus viviparus* larvae in pasture and to grazing cattle: experimental evidence of the role of Pilobolus fungi. **Veterinary Parasitology,** v. 10, p. 331–339, 1982.

JORGENSEN, R.J., RONNE, H., HELSTED, C., ISKANDER, A.R. Spread of infective *Dictyocaulus viviparus* larvae in pasture and to grazing cattle: experimental evidence of the role of *Pilobolus* fungi. **Veterinary Parasitology**, v. 10, n. 4, p. 331–339, 1980.

KAFLE, P., LEJEUNE, M., VEROCAI, G.G., HOBERG, E.P., KUTZ, S.J. Morphological and morphometric differentiation of dorsal-spined first stage larvae of lungworms (Nematoda: Protostrongylidae) infecting muskoxen (*Ovibos moschatus*) in the central Canadian Arctic. **International Journal for Parasitology. Parasites and Wildlife**, v. 4, n. 3, p. 283–290, 2015.

KAFLE, P., PEACOCK, S. J., GROND, S., ORSEL, K., KUTZ, S. Temperature-dependent development and freezing survival of protostrongylid nematodes of Arctic ungulates: implications for transmission**. Parasites & Vectors**, v. 11, n. 1, p. 1–12, 2018.

KOWAL, J., KORNAŚ, S., NOSAL, P., BASIAGA, M., WAJDZIK, M., SKALSKA, M., WYROBISZ, A. Lungworm (Nematoda: Protostrongylidae) infection in wild and domestic ruminants from Małopolska region of Poland. **Annals of Parasitology,** v. 62, n. 1, p. 63–66, 2016.

KUCHBOEV, A.E., KARIMOVA, R.R., RUZIEV, B.H., AZIMOV, D.A. Ecological aspects of nematodes of the family Protostrongylidae, parasites of Bovidae. **Scientia Parasitologica**, v. 13, n.1, p. 17–25, 2012.

KUCHBOEV, A.E., KRÜCKEN, J., KARIMOVA, R.R., RUZIEV, B.H., PAZILOV, A. Infection levels of protostrongylid nematodes in definitive caprine and intermediate gastropod hosts from Uzbekistan. **Journal of Helminthology**, v. 91, n. 2, p. 236–243, 2017.

LAHMAR, S., CABARET, J., CHENITI, T. Land snails and periods at high risk for protostrongylid infection on a sheep-grazed pasture of northeast Tunisia. **Veterinary Parasitology**, v. 36, n. 1-2, p. 105–115, 1990.

LANDIM, V.J.C., COSTA, A.J., COSTA, G.H.N., ROCHA, U.F., BARBOSA, O.F., MORAES, F.R. Parasitic nematodes in weaned calves from the Northeast region of São Paulo state, Brazil. **Ars Veterinaria**, v. 17, n. 1, p. 42–50, 2001.

LAT-LAT, H., HASSAN, L., SANI, R.A., SHEIKH-OMAR, A.R., HISHAMFARIZ, M., NG, V. First report of bovine lungworm disease in South-East Asia. **Tropical Biomedicine**, v. 24, n. 1, p. 77–81, 2007.

LESAGE, C., PATRELLE, C., VRIGNAUD, S., DECORS, A., FERTÉ, H., JOUET, D. Intermediate hosts of *Protostrongylus pulmonalis* (Frölich, 1802) and P. oryctolagi Baboš, 1955 under natural conditions in France. **Parasites & Vectors**, v. 8, n. 104, p.1–9, 2015.

LIMA, J.D., MUNIZ, P., LIMA W.S., GONÇALVES, L.C.B., SILVA, A.C., GUIMARÃES, A.M. Eficácia de Doramectin contra nematódeos gastrintestinais e pulmonares de

bovinos naturalmente infectados de Minas Gerais**. Revista Brasileira de Parasitologia Veterinária**, v. 4, n. 1, p. 49–52, 1995.

LURIER, T., DELIGNETTE-MULLER, M. L., RANNOU, B., STRUBE, C., ARCANGIOLI, M.A., BOURGOIN, G. Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach. **Preventive Veterinary Medicine**, v. 154, p. 124–131, 2018.

LURIER, T., DELIGNETTE-MULLER, M. L., RANNOU, B., STRUBE, C., ARCANGIOLI, M. A., BOURGOIN, G. Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach. **Preventive veterinary medicine**, v. 154, p. 124–131, 2018.

MACEDO, L.O., MIRANDA, L.F., OLIVEIRA, J.C.P., RAMOS, C.A., VEROCAI, G.G., CARVALHO, G.A., ALVES, L.C., RAMOS, R.A.N. *Protostrongylus rufescens* in goats: Morphological and molecular characterization. **Small Ruminants Research**, v. 182, p. 11–14, 2020.

MACHADO, T.M.M., LIMA, W.S. *Muellerius capillaris* (MUELLER, 1889) em caprinos no Estado de Minas Gerais. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 1, p. 69–70, 1988.

MAHMOOD, F., KHAN, A., HUSSAIN, R., ANJUM, M.S. Prevalence and pathology of *Dictyocaulus viviparous* infection in cattle and buffaloes. **The Journal of Animal & Plant Sciences**, v. 24, n. 3, p. 743–748, 2014.

MANSFIELD, W., BARKEMA, H. W., EYSKER, M., O'HANDLEY, R. M. An outbreak of dictyocaulosis in lactating cows on a dairy farm. **Journal of the American Veterinary Medical Association**, v. 231, n. 11, p. 1715–1718, 2007.

MAY, K., BRÜGEMANN, K., KÖNIG, S., STRUBE, C. The effect of patent *Dictyocaulus viviparus* (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs. **Parasites & Vectors**, v. 11, n. 1, p. 1–11, 2018.

McCARTHY, C., HÖGLUND, J., CHRISTLEY, R., JUREMALM, M., KOZLOVA, I., SMITH, R., VAN DIJK, J. A novel pooled milk test strategy for the herd level diagnosis of *Dictyocaulus viviparus*. **Veterinary Parasitology**, v. 1, p. 1–7, 2019.

MOLENTO, M.B., DEPNER, R.A., MELLO, M. H. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing-season calves. **Veterinary Parasitology**, v. 141, n.3-4, p. 373–376, 2006.

MORELLI, S., DIAKOU, A., DI CESARE, A., SCHNYDER, M., COLOMBO, M., STRUBE, C., DIMZAS, D., LATINO, R., TRAVERSA, D. Feline lungworms in Greece: copromicroscopic, molecular and serological study. **Parasitology Research**, v. 119, n. 9, p. 2877–2883, 2020.

MORGAN, D.O. On the morphology and biology of a larval stage of *Muellerius capillaris* (Mueller, 1889) Cameron, 1927; a lungworm of sheep and goats. **Journal of Helminthology**, v. 7, n. 03, p. 153–160, 1929.

NASHIRUDDULLAH, N., DARZI, M. M., SHAHARDAR, R. A., KAMIL, S. A., MIR, M. S., MIR, M. Pathology of spontaneous *Dictyocaulus* sp. infection in hangul (*Cervus elaphus hanglu*), sheep and goat. **Journal of Veterinary Parasitology**, v. 21, n. 1, p. 37-40, 2007.

NIMMO, J.S. Case report: six cases of verminous pneumonia (*Muellerius* sp.) in goats. **The Canadian Veterinary Journal**, v. 20, n. 2, p. 49–52, 1979.

OLIVEIRA, A.P., GENTIL, E. R., MALDONADO, JÚNIOR, A., TORRES E.J.L., THIENGO, S.C. *Angiostrongylus cantonensis* infection in molluscs in the municipality of São Gonçalo, a metropolitan area of Rio de Janeiro, Brazil: role of the invasive species *Achatina fulica* in parasite transmission dynamics. **Memórias do Instituto Oswaldo Cruz**, v. 110, n. 6, p. 739-44, 2015.

OLIVEIRA, G.P. Epidemiologia dos nematódeos gastrintestinais de bovinos leiteiros no município de São Carlos, estado de São Paulo. **Pesquisa Agropecuária Brasileira,** v. 23, n. 2, p. 189–195, 1988.

PANAYOTOVA-PENCHEVA, M.S., ALEXANDROV, M.T. Some pathological features of lungs from domestic and wild ruminants with single and mixed protostrongylid infections. **Veterinary Medicine International**, v. 1, p. 1–9, 2010.

PANAYOTOVA-PENCHEVA, M.S. Species composition and morphology of protostrongylids (Nematoda: Protostrongylidae) in ruminants from Bulgaria. **Parasitology Research**, v. 109, n. 4, p. 1015–1020, 2011.

PANAYOTOVA-PENCHEVA, M.S., DAKOVA, V., TRIFONOVA, A. First report on *Protostrongylus cuniculorum* (Nematoda: Protostrongylidae) in the European brown hare (Lepus europaeus Pallas, 1778 L.) from Bulgaria. **Parasitology Research**, v. 117, n. 11, p. 3391–3397, 2018.

PANCIERA, R.J., CONFER, A.W. Pathogenesis and pathology of bovine pneumonia. **The Veterinary clinics of North America. Food animal practice**, v. 26, n. 2, p. 191–214, 2010.

PANUSKA, C. Lungworms of ruminants. **Veterinary Clinics Food Animal Practice**, v. 22, p. 583–593, 2006.

PENAGOS-TABARES, F., LANGE, M.K., VÉLEZ, J., HIRZMANN, J., GUTIÉRREZ-ARBOLEDA, J., TAUBERT, A., HERMOSILLA, C., CHAPARRO GUTIÉRREZ, J.J. The invasive giant African snail *Lissachatina fulica* as natural intermediate host of *Aelurostrongylus abstrusus*, *Angiostrongylus vasorum*, *Troglostrongylus brevior*, and *Crenosoma vulpis* in Colombia. **PLoS Neglected Tropical Diseases**, v. 13, p. 4, p. 1–18, 2019.

PEREIRA, R.G., SILVA, A.S., MENDES, R.E., BOTTARI, N.B., SCHWERTZ, C.I., LUCCA, N.J., HENKER, L.C., MORSCH, V.M., SCHETINGER, M.R.C., MACHADO, G. Butyrylcholinesterase activity in dairy cows naturally infected by *Dictyocaulus viviparous* and treated with eprinomectin. **Comparative Clinical Pathology**, v. 26, p. 155–158, 2017.

PYZIEL, A.M., LASKOWSKI, Z., DEMIASZKIEWICZ, A.W., HÖGLUND, J. Interrelationships of *Dictyocaulus* spp. in wild ruminants with morphological description of *Dictyocaulus cervi* n. sp. (Nematoda: Trichostrongyloidea) from Red Deer, Cervus elaphus**. The Journal of Parasitology**, v. 103, p. 5, p. 506–518, 2017.

REGASSA, A., TOYEB, M., ABEBE, R., MEGERSA, B., MEKIBIB, B., MEKURIA, S., DEBELA, E., ABUNNA, F. Lungworm infection in small ruminants: prevalence and associated risk factors in Dessie and Kombolcha districts, northeastern Ethiopia. **Veterinary Parasitology**, v. 169, n. 1-2, p. 144–148, 2010.

RINALDI, L., MAURELLI, M. P., MUSELLA, V., SANTANIELLO, A., COLES, G. C., CRINGOLI, G. FLOTAC: an improved method for diagnosis of lungworm infections in sheep. **Veterinary Parasitology**, v. 169, n. 3-4, p. 395–398, 2010.

ROGERSON, J. D., FAIRBANKS, W. S., CORNICELLI, L. Ecology of gastropod and bighorn sheep hosts of lungworm on isolated, semiarid mountain ranges in Utah, USA. **Journal of Wildlife Diseases**, v. 44, n. 1, p. 28–44, 2008.

ROSA, F.B., CAPRIOLI, R.A., SILVA, T.M., GALIZA, G.J.N., BARROS, C.S.L., IRIGOYEN, L.F., FIGHERA, R.A., KOMMERS, G.D. Doenças de caprinos diagnosticadas na região Central no Rio Grande do Sul: 114 casos. **Pesquisa Veterinária Brasileira**, v. 33, n. 2, p. 199–204, 2013.

SALES, D.P., SANTOS, A.C.G., SILVA E SILVA, A.N., GONÇALVES, L.N.R., JÚNIOR, G.S.A., COSTA, V.A. Nota de pesquisa: Ocorrência de helmintose pulmonar em caprinos e ovinos da microrregião da baixada maranhense, estado do Maranhão – Brasil. **Revista Científica da FHO|UNIARARAS**, v. 5, n. 1, p. 1–10, 2017.

SALGADO, N.C., COELHO, A.C.S. Moluscos terrestres do Brasil (Gastrópodes operculados ou não, exclusive Veronicellidae, Milacidae e Limacidae). **Revista Biologia Tropical**, v. 51, n. 3, 149–189, 2003.

SAMSON, J., HOLMES, J.C. The effect of temperature on rates of development of larval *Protostrongylus* spp. (Nematoda: Metastrongyloidea) from bighorn sheep, *Ovis canadensis canadensis*, in the snail *Vallonia pulchella*. **Canadian Journal of Zoology**, v. 63, n. 6, p. 1445–1448, 1985.

SAUERLANDER, R. Experimental infection of sheep and goats with *Muellerius capillaris* (Protostrongylidae, Nematoda). **Zentralblatt fur Veterinarmedizin. Reihe B. Journal of Veterinary Medicine**, v. 35, n. 7, p. 525–548, 1988.

SAZMAND, A., JOACHIM, A. Parasitic diseases of camels in Iran (1931-2017) - a literature review. **Parasite**. v. 24, n. 21, p. 1–15, 2017.

SCHADE, J., ALBERNAZ, R.M., REIS, A.C.F., FONTEQUE, J.H. Dictiocaulose em bovinos no município de Londrina, PR, Brasil. **Veterinária e Zootecnia**, v. 27, 1–6, 2020.

SCHUNN, A.M., CONRATHS, F.J., STAUBACH, C., FRÖHLICH, A., FORBES, A., SCHNIEDER, T., STRUBE C. Lungworm Infections in German dairy cattle herds-seroprevalence and GIS-supported risk factor analysis. **PLoS One**, v. 8, n. 9, 1–10, 2013.

SEKIYA, M., ZINTL, A., DOHERTY, M.L. Bulk milk ELISA and the diagnosis of parasite infections in dairy herds: a review. **Irish Veterinary Journal**, v. 66, n. 1, p. 1–12, 2013.

SILVA, A.D., SILVA, A.S., BALDISSERA, M.D., SCHWERTZ, C.I., BOTTARI, N.B., CARMO, G.M., MACHADO, G., LUCCA, N.J., HENKER, L.C., PIVA, M.M., GIACOMIN, P., MORSCH, V. M., SCHETINGER, M., ROSA, R.A., MENDES, R.E. Oxidative stress in dairy cows naturally infected with the lungworm *Dictyocaulus viviparus* (Nematoda: Trichostrongyloidea). **Journal of Helminthology**, v. 91, 462–469, 2016.

SILVA, A.S., BALDISSERA, M.D., BOTTARI, N.B., DOLESKI, P.H., CARMO, G.M., SCHWERTZ, C.I., LUCCA, N.J., HENKER, L.C., PIVA, M.M., GIACOMIN, P., STEFANI, L.M., LEAL, D., MACHADO, G., MENDES, R.E. Ectonucleotidase and adenosine deaminase as inflammatory marker in dairy cows naturally infected by *Dictyocaulus viviparus*. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 51, p. 9–13, 2017.

SILVA, M.C., BARROS, R.R., GRAÇA, D.L. Surto de dictiocaulose em bovinos no município de Santa Maria, RS, Brasil. **Ciência Rural**, v. 35, n. 3, p. 629–632, 2005.

SOLIMAN K. N. Migration route of *Dictyocaulus viviparus* and *D. filaria* infective larvae to the lungs. **Journal of Comparative Pathology**, v. 63, n. 2, p. 75–84, 1953.

SOULSBY, E.J.L. **Helminths, arthropods and protozoa of domesticated animals (Veterinary Helminthology & Entomology).** 6ª ed., Baltimore, The Williams and Wilkins Company, 824p. 1968.

SPRATT, D.M. Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: A review. **International Journal for Parasitology: Parasites and wildlife**, v. 4, n. 2, p. 178–189, 2015.

TAYLOR, M. A, COOP, R. L, WALL, R.L**. Parasitologia Veterinária.** 3ª ed., Rio de Janeiro: Guanabara Koogan, 742p. 2016.

TEWODROS, A.E. A Review on: Lungworm Infection in Small Ruminants. **World Journal of Pharmaceutical and Life Sciences**, v. 1, n. 3, p. 149-159, 2015.

THIENGO, S.C., FERNANDEZ, M.A., TORRES, E.J., COELHO, P.M., LANFREDI, R.M. First record of a nematode Metastrongyloidea (*Aelurostrongylus abstrusus*

larvae) in *Achatina* (*Lissachatina*) *fulica* (Mollusca, Achatinidae) in Brazil. **Journal of Invertebrate Pathology**, v. 98, n. 1, p. 34–39, 2008.

THIENGO, S.C., MALDONADO, A., MOTA, E.M., TORRES, E.J., CALDEIRA, R., CARVALHO, O.S., OLIVEIRA, A.P., SIMÕES, R.O., FERNANDEZ, M.A., LANFREDI, R.M. The giant African snail *Achatina fulica* as natural intermediate host of *Angiostrongylus cantonensis* in Pernambuco, northeast Brazil. **Acta Tropica**, v. 115, n. 3, p. 194–199, 2010.

TOLOSSA, Y.H. Lungworms infection of domestic ruminants with particular to Ethiopia: A review. **International Journal of Advanced Research in Biological Sciences**, v. 9, n. 6, p. 89–103, 2019.

VALENTE, R., ROBLES, M., DIAZ, J.I. Gastropods as intermediate hosts of *Angiostrongylus* spp. in the Americas: bioecological characteristics and geographical distribution. **Memórias do Instituto Oswaldo Cruz**, v. 115, p. 1–9, 2020.

VALENTE, R., ROBLES, M., DIAZ, J.I. Gastropods as intermediate hosts of *Angiostrongylus* spp. in the Americas: bioecological characteristics and geographical distribution. **Memórias do Instituto Oswaldo Cruz**, v. 115, n. e200236, p. 1–9, 2020.

VEROCAI, G.G., CHAUDHRY, U.N., LEJEUNE, M. Diagnostic methods for detecting internal parasites of livestock. **The Veterinary clinics of North America. Food Animal Practice**, v. 36, n. 1, p. 125–143, 2020.

VEROCAI, G.G., KUTZ, S.J., HOBERG, E.P. Historical biogeography among species of *Varestrongylus lungworms* (Nematoda: Protostrongylidae) in ungulates: episodic expansion and host colonization linking Eurasia and North America. **Parasitology Research**, v. 117, p. 7, p. 2125–2137, 2018.

VIÑA, M., PANADERO, R., DÍAZ, P., FERNÁNDEZ, G., PÉREZ, A., DÍEZ-BAÑOS, P., MORRONDO, P., LÓPEZ, C.M. Evaluation of the use of pooled fecal samples for the diagnosis of protostrongylid infections in sheep. **Veterinary Parasitology**, v. 197, n. 1-2, p. 231–234, 2013.

WAPENAAR, W., BARKEMA, H.W., EYSKER, M., O'HANDLEY, R.M. An outbreak of dictyocaulosis in lactating cows on a dairy farm. **Journal of the American Veterinary Medical Association**, v. 231, n. 11, p. 1715–1718, 2007.

YIMER, A., DESIE, A. Epidemiological studies on ovine lungworm species in Northern Ethiopia. **Journal of Veterinary Science & Technology**, v. 7, n. 6 p. 1–4, 2016.

4. OBJETIVOS

4.1 Geral

Avaliar a prevalência da infecção por nematódeos broncopulmonares, além de obter informações sobre a biologia destes agentes em ruminantes do estado Pernambuco, Brasil.

4.2 Específicos

Relatar a ocorrência de nematódeos broncopulmonares utilizando métodos integrados de diagnóstico clássico e molecular em ruminantes na Microrregião de Garanhuns, Pernambuco.

Fornecer uma visão abrangente de dados históricos e atuais publicados sobre a infecção por nematódeos pulmonares em bovinos, caprinos e ovinos do Brasil.

Determinar a prevalência da infecção por *Protostrongylus* sp. em ruminantes na Microrregião de Garanhuns, Pernambuco.

Relatar a ocorrência de *Dictyocaulus* sp. em rebanho bovino da Zona da Mata, estado de Pernambuco.

5. CAPÍTULOS

5.1 Artigo 1

(Artigo publicado no periódico Small Ruminant Research, v. 182, p. 11-14, 2020)

Protostrongylus rufescens in goats: Morphological and molecular characterization

Abstract

Gastropod-borne nematodes (GBN) of veterinary concern are mainly represented by lungworms that may infect several mammalian host species. Although recognized as important nematodes throughout the world, these invertebrates have been little studied in South America. The aim of this study was to provide epidemiological, morphological and molecular data on the lungworm larva of a species of *Protostrongylus* from goats. In March 2019 a study for detection of gastrointestinal nematodes of goats was conducted on the municipality of Paranatama, state of Pernambuco, northeaster Brazil. Faecal samples (n=217) were analysed through the modified Gordon and Whitlock technique (data not shown), and larvae were detected using the Baermann technique. The sample size was estimated based on the goat population of the study area. Larvae were detected in 18.9 % (41/217) of the analysed samples, and presented a mean length of 339 μm (±52.99 μm) and mean width of 18 μm (±1.46 μm). Morphologically they were similar to Protostrongylus sp. Sequencing of the 18 rRNA gene and BLASTn search revealed strong identity (98.7 %) with homologous sequences of *Protostrongylus rufescens* available from GenBank. Finally, this study provides new and relevant epidemiological, morphological and molecular data on *P. rufescens* on goats. Further studies on this nematode are needed in order to elucidate biological aspects such as the seasonal dynamics of infection and intermediate hosts involved in its life cycle.

Keywords: *Protostrongylus rufescens*; Lungworm; Small ruminants; Molecular identification.

1. Introduction

Gastropod-borne nematodes (GBN) have acquired a great importance over the last years due to their ability to infect a wide range of animals, including humans (Colella et al., 2015; Deak et al., 2017). It is believed that millions of people in several parts of the world are at risk
of GBN infections (Lu et al., 2018). In this context, the gastropod control may be the key to interrupt the dispersion of these pathogens throughout the world, however the anthropic actions (e.g., deforestation, climatic changes and intense trade between countries) have facilitated the dispersion of these invertebrates and consequently the pathogens for them transmitted (Kafle et al., 2018). Some of these GBN have a zoonotic potential, such as *Angiostrongylus cantonensis*, the causative agent of eosinophilic meningitis (Romero-Alegría et al., 2014; Barratt et al., 2016).

In veterinary medicine, GBN are represented mainly by lungworms within the superfamily Metastrongyloidea, which may infect a variety of animal species, including ruminants (Kuchboev et al., 2012; Asmare et al., 2018). Lungworm infections in domestic and wild ruminants have been well characterized in Europe (Kowal et al., 2016) and North America (Wapenaar et al., 2007; Verocai et al., 2018a, b), but in the South America these nematodes remain neglected for a long time. Most likely, more attention has been paid to gastrointestinal nematodes due to their capacity to cause disease, and substantial economic losses to producers. Although considered less virulent, these metastrongyloids may cause weight loss, muco-nasal secretion and difficulty respiratory (Mansfield et al., 1993). In addition, the formation of granulomas

and chronic eosinophilic pneumonia has been observed at post-mortem examination (Panayotova-Pencheva and Alexandrov, 2010).

Amongst the species that infect goats, *Protostrongylus rufescens* is considered one important GBN reported worldwide (Panayotova-Pencheva and Alexandrov, 2010; Borji et al., 2012; Jabbar et al., 2013; Asmare et al., 2018). This metastrongyloid parasitizes bronchioles and alveoli where females lay eggs. The first-stage larvae (L1) hatch within the lungs, are coughed and swallowed, and are shed in faeces (Kuchboev et al., 2017). The off definitive host cycle is featured by the development from L1 to third-infective stage larvae (L3) within gastropods (Lesage et al., 2015). Many genera of terrestrial gastropods (e.g., *Xeropicta*, *Vallonia*, *Agriolimax*, *Helicella* and *Helix*) have been incriminated as intermediate hosts of this nematode and play an important role on its dispersion (Grewal et al., 2003; Kuchboev et al., 2017).

In Brazil, there has been only a single report of *Protostrongylus* infection in goats (Duarte and Miranda, 1984). Therefore, the aim of this study was to report the occurrence of *P. rufescens* using integrated classical and molecular diagnostic methods, and provide epidemiological data on the lungworm larva of a species of *Protostrongylus* from goats.

2. Material and methods

2.1. Lungworm larvae detection and ethical aspects

On March 2019 a study for detection of gastrointestinal nematodes of goats (*Capra hircus*) used for meat production was conducted in the municipality of Paranatama (8°55′15″S, 36°39′29″ W), state of Pernambuco, northeaster Brazil. This area is featured by a semi-arid climate with mean annual temperature of 22 °C (from

17 °C to 30 °C), mean rainfall of 147mm (from 25mm to 295 mm) and relative air humidity of 90 %.

Faecal samples of 217 animals [92 young (younger than 6 months) and 125 adults (older than 6 months] from 15 different farms were analysed through the modified Gordon and Whitlock technique (data not shown) (Gordon and Whitlock, 1939). This sample size (n=217) was estimated based on the goat population (n=2,000) of the study area. In addition, an estimated prevalence of 50 %, confidence level of 95 % and statistical error of 5 % were considered (Thrusfield, 2004). Some larvae were detected on samples of five animals. Afterwards, in order to obtain more larvae for the proper identification, all samples were analysed through the Baermann technique (Forrester and Lankester, 1997).

The Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal Rural de Pernambuco approved all procedures herein carried out (protocol number: 07/2017).

2.2. Fecal and morphological analysis

Larvae isolated using Baermann were morphologically analysed, with special attention given to features of their anterior and posterior extremities (Boev, 1975). Photomicrographs were taken using a digital camera (CMOS-5.0) mounted directly on the microscope (New Optics NO 226). The software TCapture 4.3 was used for the image acquisition and measurements.

In order to confirm the morphological identification, larvae were individually isolated using a 10 µL micropipette and stored in plastic vials containing phosphate buffered saline (PBS) at - 20 °C until molecular processing.

2.3. Molecular analyses

Genomic DNA from individual larvae was extracted using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, USA) in accordance with the manufacturer's instructions.

The samples were subjected to PCR reaction with primers 1096F (5'-GGTAATTCTGGAGCTAATAC-3') and 1912R (5'-TTTACGGTCAGAACTAGGG-3') (Holterman et al. 2006), which delimit DNA fragments of approximately 850 *bp* of the 18S *rRNA* gene, common for various nematode species. The amplifications were visualized after 1% agarose gel electrophoresis in UV transilluminator. Then, the amplified fragments were purified using ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems by Thermo Fisher Scientific - BR) and sequenced in both directions using the Sanger method in automatic sequencer ABI 3130 (Applied Biosystems). The chromatograms were analysed using BioEdit v.7.2.5 software (Hall, 1999) and consensus sequences were submitted to BLASTn search (Altschul et al., 1990) to determine the sequence identity, based on comparisons with orthologous sequences available of *Protostrongylus* in the GenBank database.

2.4. Data analyses

Descriptive statistical analysis was performed to obtain relative and absolute frequency of protostrongylids infection in goats. In addition, the Lilliefors test was used to verify the normality of the data. The chi-square (x^2) test with Yates correction was used to assess the difference of positivity between male and females, and between young and old animals. The significance level was taken to be 5%. The BioEstat software, version 5.3, was used to perform the statistical analyses (Ayres et al., 2007).

3. Results

Protostrongylid larvae were detected in 18.9 % (41/217) of all the assessed goat faecal samples. In particular, 19.4 % (35/180) of females and 16.2 % (6/37) of males scored positive (p=0.648; χ2=0.209). Conversely, in 29.3 % (27/92) of young and 11.2 % (14/125) of adult animals Protostrongylid larvae were retrieved (p=0.000; χ2=11.390). All animals were kept in the semi-intensive production system and did not present any clinical manifestation related to the infection by lungworms.

Larvae (n=30) presented a mean length of 339 µm (±52.99 µm) and mean width of 18 µm (±1.46 µm). Morphologically, they were similar to *Protostrongylus* sp. (Fig. 1). The tip of the tail was typical of Protostrongylinae, consisting of an elongate, thin, pointed, and slightly undulating process; and the absence of a dorsal-spine at the insertion of the tip of the tail (typical of the protostrongylid subfamilies Muelleriinae, Varestrongylinae, and Elaphostrongylinae). In addition, the presence of small granules was observed in the intestine.

The PCR amplification resulted in fragment of 856 bp of the 18S rRNA gene. After sequencing and BLASTn search significant identity of 98.7 % was observed between the consensus sequence obtained in the present study and the only *P. rufescens* DNA sequence available from Genbank database. The consensus sequence was deposited at Genbank under accession number MK802542.

4. Discussion

This study provides for the first time epidemiological, morphological and molecular data of larvae species of *Protostrongylus* from goats in northeaster Brazil.

In this country, data about this parasite are limited to a one single report from the southeast (Duarte and Miranda, 1984), and target prevalence studies are non-existent. The overall positivity (18.9 %; 41/217) herein observed in live animals was an unexpected finding since most of cases of detection of lungworms in small ruminants are achieved at post-mortem examination (Kuchboev et al., 2017). Previous studies in Ethiopia, have demonstrated a frequency of protostrongylid infection varying from 33.8 % (Addis et al., 2011) to 46.7 % (Terefe et al., 2013) but assessed always at post-mortem examination. In fact, the detection of larvae in these animals has occurred less frequently than other lungworms in other animal species, such as carnivores.

In the present study, females and males were equally affected (p=0.648; χ^2=0.209), however young animals were the more affected than adults (p=0.000; χ^2=11.390). Considering that all animals were maintained in the same breeding system (i.e., semi-intensive system), this difference between young and adults may be attributed to the immunological status of these goatlings. In fact, it is believed that only young animals in their first grazing are clinically affected and old animals acquire a strong immunity (Adem, 2016), which may explain the absence of clinical sings and the reduced number of infected animals. The breeding system has been considered an important key in the epidemiology of lungworms, since some species such as *P. rufescens* require an intermediate host (e.g., snails) to complete its biological life cycle (Yimer and Desie, 2016; Kuchboev et al., 2017).

Occasional findings of lungworm infections in goats represent an important update on this nematode species in Brazil, opening new possibilities for its diagnosis in small ruminants. Although, other lungworms species (e.g., *Muellerius capillaris* and *Dictyocaulus filaria*) have already been reported in Brazil (Machado and Lima, 1988; Sales et al., 2017) data are scant. Most likely, the scarcity of reports may be related to

the minor pathogenic relevance of these parasites when compared with gastrointestinal nematodes and, subsequently, analysis of fecal samples using the Baermann technique are not routine. In addition, the complexity of the life cycle of these parasites (except for *D. filaria*) that involves gastropods as intermediate hosts may be an important hindrance for the establishment of these parasitoses in some regions.

Albeit considered of minor pathogenic relevance, *P. rufescens* may cause gross pulmonary lesions, and fatal pneumonia in high intensity infections in goats, domestic sheep, and mouflons (Panayotova-Pencheva and Alexandrov, 2010). It is believed that the major difficulty for the detection of P. rufescens is due to the low number of L1 excreted on faeces of adult animals, which play an important role on the environmental contamination and consequently the intermediate hosts exposition (Berrag and Urquhart, 1996).

Protostrongylus rufescens use different gastropod species (e.g., *Xeropicta*, *Pseudonapaeus* and *Pupilla*) as intermediate hosts, but studies with these invertebrates has never been conducted in Brazil. From an epidemiological perspective the role of gastropods as intermediate host of some pathogens has a great importance, since recently these invertebrates have spread and exotic species has suppressed the native population in some areas (Lu et al., 2018).

Finally, this study provides important data on *P. rufescens* infection on goats in Brazil. Results from herein presented serve as wellspring for further studies to better understand the dynamics of lungworm infection, and potential impact on production. In addition, open new possibilities to assess the seasonal dynamics, the intermediate hosts involved, and the main risk factors associated to the infection by this neglected parasite of small ruminants.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgements

This article is based on the PhD thesis (Postgraduate Program in Animal Bioscience), developed at the Federal Rural University of Pernambuco, with support from a fellowship from Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

References

Addis, M., Fromsa, A., Ebuy, Y., 2011. Study on the prevalence of lungworm infection in small ruminants in Gondar town, West Ethiopia. Int. J. Anim. Vet. Adv. 10, 1683–1687.

Adem, J., 2016. Lung Worm Infection of Small Ruminant in Ethiopia. Adv. Lif. Sci. Tech. 43, 12–22.

Altschul, S.F, Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. Res. 215, 403–410.

Abebe, R., Melesse, M., Mekuria, S., 2016. Lungworm Infection in small ruminants in and Around Wolaita Soddo Town, Southern Ethiopia. J. Vet. Sci. and Tech. 7, 1–5.

Asmare, K., Sibhat, B., Haile, A., Sheferaw, D., Aragaw, K., Abera, M., Abebe, R., Wieland, B., 2018. Lungworm infection in small ruminants in Ethiopia: Systematic review and meta-analysis. Vet. Parasitol. Reg. Stud. Reports. 14, 63–70.

Ayres, M., Ayres Junior, Ayres, D.L., Santos, A.S., 2007. Bioestat 5.0 - Aplicações estatísticas nas áreas das ciências biomédicas. ONG Mamiraua, Belém, PA. 364p.

Barratt, J., Chan, D., Sandaradura, I., Malik, R, Spielman, D., Lee, R., Marriott, D., Harkness, J., Ellis, J., Stark, D., 2016. *Angiostrongylus cantonensis*: A review of its distribution, molecular biology and clinical significance as a human pathogen. Parasitol. 143, 1087–1118.

Berrag, B., Urquhart, G.M., 1996. Epidemiological aspects of lungworm infections of goats in Morocco. Vet. Parasitol. 61, 81–8.

Boev, S., 1975. Protostrongylids. Fundamentals of nematology. v. 25. Helminthological Laboratory, Academy of Sciences of the USSR, Moscow, Washington, D.C., and Amerind Publishing Co., New Delhi, 1984, 338 p.

Borji, H., Azizzadeh, M., Ebrahimi, M., Asadpour, M., 2012. Study on small ruminant lungworms and associated risk factors in northeastern Iran. Asian. Pac. J. Trop. Dis. 1, 853–856.

Colella, V., Giannelli, A., Brianti, E., Ramos, R.A.N., Cantacessi, C., Dantas-Torres, F., Otranto, D., 2015. Feline lungworms unlock a novel mode of parasite transmission. Sci. Reports. 5, 1–6.

Deak, G., Gherman, C.M., Ionică, A.M., Vezendan, A.D., D'Amico, G., Matei, I.A., Daskalaki, A.A., Marian, I., Damian, A., Cozma, V., Mihalca, A.D., 2017. *Angiostrongylus vasorum* in Romania: an extensive survey in red foxes, *Vulpes vulpes*. Parasit. Vectors. 10, 1–6.

Duarte, M.J.F., Miranda, I.M., 1984. Ocorrência de *Protostrongylus rufescens* (Nematoda: Protostrongylidae) em caprino do Estado do Rio de Janeiro. Braz. J. Vet. Parasitol. 19, 647–649.

Forrester, S.G., Lankester, M.W., 1997. Extracting protostrongylid nematode larvae from ungulate feces. J. Wildl. Dis. 33, 511–516.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Sci. Ind. Res. 12, 50–52.

Grewal, P.S., Grewal, S.K., Tan, L., Adams, B.J., 2003. Parasitism of Molluscs by Nematodes: Types of associations and evolutionary trends. Int. J. Nematol. 35, 146–156.

Hall, T.A., 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol. Biol. Evol. 23, 1792–1800.

Jabbar, A., Mohandas, N., Jex, A.R., Gasser, R.B., 2013. The mitochondrial genome of *Protostrongylus rufescens* – implications for population and systematic studies. Parasit. Vectors. 6. 1–9.

Kafle, P., Stephanie, J.P., Grond, S., Orsel, K., Kutz, S., 2018. Temperature-dependent development and freezing survival of protostrongylid nematodes of Arctic ungulates: implications for transmission. Parasit. Vectors. 11, 1–12.

Kowal, J., Kornas, S., Nosal, P., Basiaga, M., Wajdzik, M., Skalska, M., Wyrobisz, A., 2016. Lungworm (Nematoda: Protostrongylidae) infection in wild and domestic ruminants from Małopolska region of Poland. Ann. Parasitol. 62, 63–66.

Kuchboev, A.E., Karimova, R.R., Ruziev, B.H., Azimov, D.A., 2012. Ecological aspects of nematodes of the family Protostrongylidae, parasites of Bovidae. Sci Parasitol. 13, 17–25.

Kuchboev, A.E., Krucken, J., Karimova, R.R., Ruziev, B.H., Pazilov, A., 2017. Infection levels of protostrongylid nematodes in definitive caprine and intermediate gastropod hosts from Uzbekistan. J. Helminthol. 91, 236–243.

Lesage, C., Patrelle, C., Vrignaud, S., Decors, A., Ferté, H., Jouet, D., 2015. Intermediate hosts of *Protostrongylus pulmonalis* (Frölich, 1802) and *P. oryctolagi* Baboš, 1955 under natural conditions in France. Parasit Vectors. 8, 1–9.

Lu, X.T., Gu, Q.Y., Limpanont, Y., Song, L.G., Wu, Z.D., Okanurak, K., Lv, Z.Y., 2018. Snail-borne parasitic diseases: an update on global epidemiological distribution, transmission interruption and control methods. Infect. Dis. Poverty. 7, 1–16.

Machado, T.M.M., Lima, W.S., 1988. *Muellerius capillaris* (Mueller, 1889) em caprinos no Estado de Minas Gerais. Braz. J. Vet. Anim. Sci. 40, 69–70.

Mansfield, L.S., Gamble, H.R., Baker, J.S., Lichtenfels, J.R., 1993. Lungworm infection in a sheep flock in Maryland. J. Am. Vet. Med. Assoc. 202, 601–606.

Panayotova-Pencheva, M.S., Alexandrov, M.T., 2010. Some pathological features of lungs from domestic and wild ruminants with single and mixed protostrongylid infections. Vet. Med. Int. 1, 1–9.

Romero-Alegría, A., Belhassen-García, M., Velasco-Tirado, V., Garcia-Mingo, A., Alvela-Suárez, L., Pardo-Lledias, J., Sáncheez, M.C., 2014. *Angiostrongylus costaricensis*: Systematic Review of Case Reports. Adv. Infect Dis. 4, 36–41.

Sales, D.P., Santos, A.C.G., Silva and Silva, A.N., Gonçalves, L.N.R., Júnior, G.S.A., Costa, V.A., 2017. Research note: Occurrence of pulmonary helminthiasis in

goats and sheep from the microregion of maranhense lowlands, brazilian state of Maranhão. Rev. Cient. FHO UNIARARAS. 5, 53–62.

Terefe, Y., Tafess, K., Fekadie, G., Kebede, N., 2013. Prevalence of lungworm infection in small ruminants in North Gondar zone, Amhara National Regional State, Ethiopia. J. Parasitol. Vector Biol. 5, 40–45.

Verocai, G.G., Kutz, S.J., Hoberg, E.P., 2018a. Historical biogeography among species of *Varestrongylus* lungworms (Nematoda: Protostrongylidae) in ungulates: episodic expansion and host colonization linking Eurasia and North America. Parasitol. Res. 117, 2125–2137.

Verocai, G.G., Kutz, S.J., Hoberg, E.P. 2018b. *Varestrongylus* (Nematoda: Protostrongylidae), lungworms of ungulates: a phylogenetic framework based on comparative morphology. Parasitol. Res. 117, 2075–2083.

Wapenaar, W., Barkema, H.W., Eysker, M., O'Handley, R.M., 2007. An outbreak of dictyocaulosis in lactating cows on a dairy farm. J. Am. Vet. Med. Assoc. 231, 1715–1718.

Yimer, A., Desie, A., 2016. Epidemiological studies on ovine lungworm species in Northern Ethiopia. J. Vet. Sci. Technol. 7, 1–4.

Figure caption

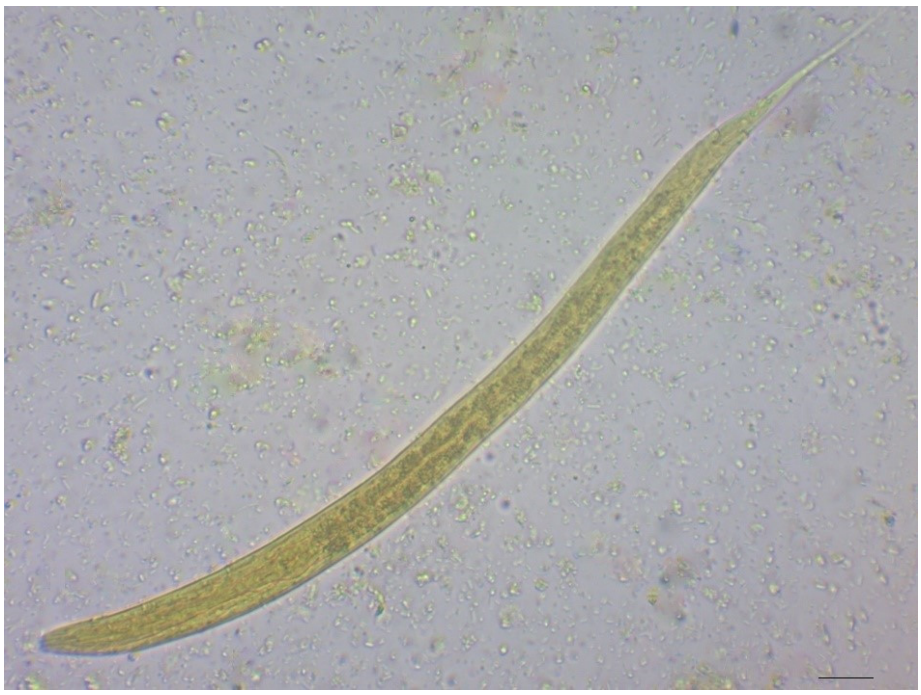


Figure 1. First-stage larva (L1) of *Protostrongylus* rufescens detected in faeces of goats scale-bar = 10 µm).

5.2 Artigo 2

(Artigo publicado no periódico Veterinary Parasitology: Regional Studies and Reports, v. 26. p. 1 - 7, 2021)

Lungworms in ruminants from Brazil: A retrospective epidemiological study over four decades

Abstract

Lungworms such as *Dictyocaulus* spp. in cattle and small ruminants, and *Muellerius capillaris* and *Protostrongylus rufescens* in small ruminants are important pathogens, causing respiratory disease in these livestock species. Despite their veterinary importance, lungworms of livestock have been poorly studied in certain regions of the world, including Brazil. Therefore, much of their epidemiology and economic impacts on production remain unknown. This article aims to provide a comprehensive overview of the historical and current data published on lungworm infection of domestic ruminants in Brazil. This review consisted of a comprehensive search of technical and scientific publications between January 1980 to December 2020, using online sources such as PubMed, Google Scholar and Scielo. Twenty-four articles published over the last 40 years reporting lungworms exclusively in cattle (*n* = 16), goats (*n* = 6) and sheep (*n* = 1) in Brazil were included. In addition, a study (*n* = 1) with both goats and sheep were also utilized. Overall, 12 studies were based only on post-mortem examination, five in the detection of specimens in fecal samples, and seven were based on fecal analysis followed by post-mortem examination. Out of all studies, 66.7% (*n* = 16) articles registered *D. viviparus*, 4.2% (*n* = 1) *D. filaria*, 8.3% (*n* = 2) *P. rufescens,* 16.7% (*n* = 4) *M. capillaris*, and 4.2% (*n* = 1) co-infection by *D. filaria* and *M. capillaris*. The existence of suitable environmental conditions, as well as intermediate and definitive

hosts in Brazil contribute for the survival and development of these nematode species. The majority of the reports of lungworms originate from the Southern and Southeastern regions of the country, whose mild temperatures likely contribute to their occurrence. Finally, lungworms of ruminants have been reported over the past four decades in Brazil, but most of the information was obtained at post-mortem examination. Therefore, further studies to investigate epidemiological aspects in different hosts and regions of the country are needed.

Keywords: Cattle, goats, sheep, nematodes.

1. Introduction

Although known for a long time, there has been an increased interest on lungworm infections in ruminants among veterinarians and researchers over the last decades (Pyziel et al., 2018; Verocai et al., 2020a). Several species belonging to the superfamily Metastrongyloidea (e.g., *Muellerius capillaris* and *Protostrongylus rufescens*) and Trichostrongyloidea (e.g., *Dictyocaulus viviparus* and *Dictyocaulus filaria*) may infect domestic livestock, in particular cattle and small ruminants (Panuska, 2006; Mangiola et al., 2014; McNulty et al., 2016). In fact, these nematodes get more attention in Europe (Cassini et al., 2015; Kowal et al., 2016), Africa (Bekele and Shibbiru, 2017; Asmare et al., 2018) and North America (Wapennar et al., 2007), but have been neglected in South America. Aside from their pathogenic relevance, it has been demonstrated that lungworm infections cause considerable economic losses in the livestock industry, especially due to the reduction in milk production. It is believed that the estimated costs may range from 159 to 167 € per cow during an outbreak (Holzhauer et al., 2011). As studied in cattle, subclinical infections can also cause

significant production losses. In one study, a reduction of 1.62 kg/cow/day of milk was found in dairy cows that were infected with *D. viviparus* (May et al., 2018). Another research detected a reduction ranging from 1.01–1.68 kg/cow/day of milk production from infected cows over the summer months (Dank et al., 2015).

The pathogenic impact of these parasites depends on their species, location in the hosts' respiratory tract, number of larvae ingested (infective dose), and the immune system of animals (Bekele and Shibbiru, 2017). For example, *D. viviparus* is observed mainly in calves at first grazing season (Schunn et al., 2013) causing subclinical or clinical disease characterized by respiratory signs such as coughing and dyspnea, but may also lead to death (May et al., 2018). On the other hand, infections caused by *M. capillaris* and *P. rufescens* in small ruminants may go unnoticed because these are often asymptomatic (Panuska, 2006; Habte and Simeneh, 2019). However, there have been reports of decreases in productivity and clinical signs associated with small ruminant infection, including weight loss, mucosal secretion and respiratory problems (Mansfield et al., 1993).

The geographic distribution of lungworms of livestock is greatly influenced by environmental conditions, which directly impact development of larval stages, and opportunities for transmission (Hoberg and Brooks, 2015; Cable et al., 2017). *Dictyocaulus* species have a direct life cycle, with the first-stage larvae (L1) developing into the second and the infective third-stage larvae (L3) on the pasture. The larvae of *D. viviparus* move from the feces to the pasture, and eventually use the sporulation of the fungus *Pilobolus* to reach areas farther away (Jorgensen et al., 1982). In contrast, all metastrongyloids infecting small ruminants have an indirect life cycle, using gastropods as intermediate hosts. Within the gastropods, the L1 develop into the infective L3 (Kuchboev et al., 2017). Considering the different environmental aspects

and strategies for the development and dispersion of these nematodes, the climatic differences (e.g., rainfall, humidity and temperature) observed throughout Brazil favor the heterogeneity in the distribution of these lungworms across the country (Valente et al., 2020).

Indeed, most of cases of lungworm infections in Brazil were described in the Southern and Southeastern regions. The infection of cattle with *D. viviparus* is evident (Molento et al., 2006; Henker et al., 2017; Cezaro et al., 2018), but little is known about the occurrence of lungworms in small ruminants. Overall, the reports of these parasites in goats and sheep are limited to *D. filaria* and *M. capillaris* in sheep (Gonçalves et al., 1980; Sales et al., 2017), and *D. filaria, M. capillaris*, and *P. rufescens* in goats (Gonçalves et al., 1980; Duarte and Miranda, 1984; Machado and Lima, 1988; Cardoso and Oliveira, 1993; Rosa et al., 2013; Sales et al., 2017; Bassuino et al., 2018; Macedo et al., 2020).

It is known that epidemiological characteristics of these nematodes are highly affected by different environmental conditions and management practices (Panuska, 2006; Adem, 2016). In this sense, it is important to note that Brazil is a very large country with many different biomes, which confers a wide heterogeneity on the distribution of parasites in domestic and wild animals. Retrospective epidemiological studies may be useful to better characterize areas of occurrence of nematodes that have been poorly investigated, providing a valuable framework for veterinarians and researchers. Therefore, the aim of this article was to provide a comprehensive overview of the historical and current data published on lungworm infection in domestic ruminants in Brazil. Distribution of data according to temperature conditions has been graphically represented (Supplementary File S1). Additionally, epidemiological

aspects and diagnostic methods have been extensively discussed to better understand these differences across the country.

2. Methods

2.1. Review procedures and map construction

This review consisted of a comprehensive search of technical and scientific reports published from January 1980 to December 2020, using online sources such as PubMed, Google Scholar and Scielo. Keywords, including “ruminants”, “small ruminants”, “lungworms”, “parasitic pneumonia”, “verminous pneumonia”, “sheep”, “goat”, “bovine” and “Brazil” were combined for search articles. All articles written in English or Portuguese were included in this review.

Articles that described natural infection by lungworms in cattle, sheep and goats, epidemiology, case reports and outbreaks were considered in this study. Finally, they were screened to assess their originality, time of publication, aim of the study, technique employed for diagnosis and reliability in the presentation of results.

Two maps were constructed for represent graphically data of distribution and temperature throughout Brazil; they were processed on QGIS 3.10.14 A Coruña. The first one image shows the distribution of parasites and was constructed using as parameter the estimative of Kernel density, Quartic model and radius of 100 km from the central point (Fig. 1). Data were categorized in very low, low, middle, high and very high, based on the number of municipalities in which lungworm parasites were diagnosed in each region.

The second map was made using a captured image from National Institute for Space Research and processed with raster calculator and false-color single band rendering (Supplementary File S1).

3. Results and discussion

A total of 24 peer-reviewed articles reporting lungworms in domestic ruminants (cattle, goats and sheep) were published over the last 40 years in Brazil. Of these, 87.5% (21/24) were Original Research Articles and 12.5% (3/24) Short Communications. Of these studies, 91.6% (22/24) aimed to report the occurrence of lungworms, and only 8.4% (2/24) focused on assessing the anthelmintic efficacy in naturally infected animals.

Of all reports, 66.7% (16/24) registered *D. viviparus*, 4.2% (1/24) *D. filaria,* 8.3% (2/24) *P. rufescens*, 16.7% (4/24) *M. capillaris* and 4.2% (1/24) co-infection by *D. filaria* and *M. capillaris.* The majority of studies were concentrated in the Southeastern and South of Brazil with 45.8% (11/24) of reports in both regions, followed by the Northeastern region with 8.3% (2/24) (Tables 1 and 2). Fig. 1 illustrates the Kernel map with graphical representation of distribution of lungworms in domestic ruminants from Brazil.

From 1980 to 1999, only 8 articles (33.3%) reported lungworm infections in ruminant livestock. Interestingly, from 2000 to 2020 the number of articles nearly doubled, with 16 reports (66.7%) distributed across 8 states and 24 municipalities. It is important to highlight that most of these parasites were diagnosed through the retrieval of adult specimens (54.2%; 13/24) at post-mortem examination, followed by the association of post-mortem examination and the Baermann technique (25.0%; 6/24),

and finally detected only by means of the Baermann technique (20.8%; 5/24) in live animals.

The overall distribution of lungworms infecting ruminants in Brazil according to the mean temperature is graphically represented in the Supplementary File S1.

3.1. Cattle lungworm

Sixteen articles were found reporting cattle lungworm infections, 93.7% (15/16) of which were Original Research Papers and 6.3% (1/16) Short Communication. These reports came from 11 municipalities, distributed in the Southern (Paraná, Rio Grande do Sul and Santa Catarina) and Southeastern (Minas Gerais, Rio de Janeiro and São Paulo) regions of the country. In total, 706 individual animals were assessed for infection through analysis of feces and/or at post-mortem examination. Altogether, these studies detected 37.5% (264/706) of cattle as positive for *D. viviparus*. The combination of the Baermann technique followed by post-mortem examination detected the highest percentage of infections (50.4%; 133/264), followed by post-mortem examination alone (37.1%; 98/264), and ante-mortem diagnosis using the Baermann technique (12.5%; 33/264).

The lack of information in the other regions of the country does not necessarily mean that there is no infection with *D. viviparus*. Most likely, its known distribution is biased by the larger concentration of researchers in agricultural sciences in the Southern and Southeastern regions of Brazil, which has steadily grown in the recent past (Sidone et al., 2016). Notwithstanding, these two regions comprise the majority of the country's cattle population (50.2% of total Brazilian herd - IBGE) and climatic conditions (e.g., mild temperature and high rainfall mean) in these regions are

important factors that may contribute for the occurrence of lungworms in these animals (Henker et al., 2017; Cezaro et al., 2018).

It is believed that the distribution of *D. viviparus* in cattle is much more widespread, and number of infected animals is certainly higher than that presented here. For example, in the Northeastern region there are numerous personal communications from veterinarians reporting clinical cases of respiratory disease suggestive of lungworm infection in cattle. Therefore, more complete information about the distribution of *D. viviparus* depends of new studies, especially in regions where information is meager such as the Northern and Northeastern of Brazil (Schunn et al., 2013). It is important to note that cases of *D. viviparus* infection have not been reported in the Midwestern region of Brazil. This area has been recognized as one of the main of beef cattle producing regions in the world, and therefore, their veterinary and sanitary management needs to be stricter (Millen et al., 2011). Possibly, the absence of reports of *D. viviparus* may be attributed also to the climatic conditions, which is extremely dry in some periods of the year (Zvinorova et al., 2016).

Most of records of *D. viviparus* infections (87.5%) were detected through post-mortem examination. Undoubtedly, the low sensitivity of some diagnostic tools on detection of lungworm larvae in feces is an obstacle to better characterize the occurrence and prevalence of these nematodes in a given region (Charlier et al., 2016; Lurier et al., 2018). The dynamics of lungworm infection in cattle is highly dependent on climate and seasonality, and therefore, it is highly variable across space and time (Ploeger, 2002; McCarthy and Van Dijk, 2020). In fact, the prevalence of *D. viviparus* is likely underestimated worldwide, as well as the economic losses associated to the impact of infection on animal health and livestock production (Dank et al., 2015; May et al., 2018).

3.2. Goats and sheep lungworms

Eight peer-reviewed articles have been published on small ruminant lungworms, including 75.0% (6/8) original research articles and 25.0% (2/8) short communications. Lungworm infections were reported from 15 municipalities distributed in the Southern (Rio Grande do Sul), Southeastern (Minas Gerais and Rio de Janeiro) and Northeastern (Maranhão and Pernambuco) regions of Brazil (Table 2). Overall, a total of 2270 individual animals were assessed through analysis of feces by Baermann and/or at post-mortem examination. These studies detected lungworms in 6.5% (181/2770) of cases. Most of them were represented by the parasitism by *M. capillaris* (43.1%; 78/181), followed by *D. filaria* (33.7%; 61/181) and *P. rufescens* (23.2%; 42/181). Antemortem diagnosis through the Baermann technique detected the highest percentages of infections (58.0%; 105/181), followed by the Baermann technique together with post-mortem examination (39.2%; 71/181), and finally by post-mortem examination alone (2.8%; 5/181).

Similar to the cattle lungworm, the reported areas of distribution of lungworms of small ruminant species may be influenced by several factors, including climatic condition (e.g., temperature and rainfall) (Tolossa, 2019), and the number of animals present in a given region. In fact, the Southeastern and Southern regions of Brazil concentrate approximately 93.9% of the Brazilian small ruminant herd, which coincides with a larger number of researchers focused on health and production of such animals (Sidone et al., 2016).

However, one must take into account the contrasting life cycle of different lungworm species that infect small ruminants. Similarly to the cattle lungworm, *D. filaria* has a direct life cycle and requires less time to develop into the L3 infective stage

(Habte and Simeneh, 2019). On the other hand, *M. capillaris* and *P. rufescens* present a complex life cycle involving gastropods as intermediate hosts (e.g., *Vallonia*, *Xeropicta* and *Agriolimax*) (Panuska, 2006; Kuchboev et al., 2017; Tolossa, 2019).

In sheep, particularly, there have been only two reports of lungworm infection. Both M. capillaris and D. filaria were detected in the state of Rio Grande do Sul, in the Southern region of Brazil (Gonçalves et al., 1980). More recently, almost four decades later, *D. filaria* was reported from the state of Maranhão, located in the Northeastern part of the country (Sales et al., 2017). Current knowledge about *D. filaria* suggests that this parasite is found in regions of high humidity, which can favor the biology of the parasite and where it can be linked to clinical disease, including coughing, weight loss, weakness, and eventually death (Yimer and Desie, 2016).

3.3. Diagnosis of lungworm infection

Overall, it is believed that lungworm infection in ruminants in Brazil is largely underdiagnosed. This leads to gaps in the knowledge of distribution and epidemiology of these parasites in a country of continental scales, and subsequently suboptimal management practices and control strategies. A major challenge is to convince veterinarians and ruminant producers about the importance of the detection of these parasites even in animals with subclinical or asymptomatic infection (May et al., 2018). Understandably, lungworm infections may rank lower in importance comparing to other parasitic diseases, such as haemonchosis and coccidiosis. The majority of the compiled reports of lungworm infections come from post-mortem examinations, many of which not necessarily linked to clinical disease. Nevertheless, it must be emphasized that there are relatively simple in vivo diagnostic tests that can be implemented (Verocai et al., 2020b). Veterinarians should educate clients and producers that

implementation of such diagnostic tests may be of value as differential diagnosis for other respiratory infections. In addition, diagnostic results will be useful to guide anthelmintic treatment decisions and other strategies of control, in order to reduce the incidence of infection, clinical disease, mortality, and subsequently minimizing potential economic losses.

Most commonly, the confirmation of lungworm infection in vivo is achieved by the detection of L1 in animal feces. For a long time, the Baermann technique has been employed for detection of lungworm larvae and morphological identification of species. While still routinely used in diagnostic laboratories, and relatively inexpensive to perform, processing samples using the Baermann technique can be time consuming (Forrester and Lankester, 1997; Verocai et al., 2020b). Nevertheless, the Baermann technique has been considered the gold standard for the detection of L1 in feces, and presents a high sensitivity (100%) for detection of primary infection in young animals (Eysker, 1997). However, in adult animals the sensitivity has proven to be low (7.5%), most likely due to lungworm reinfections that result in the absence or low larval shedding in feces (May et al., 2018; Lurier et al., 2018). In addition, it is important to note that this method is reliable only using fresh fecal samples (collected and examined on the same day). In stored samples evaluated after 24 h, there is a reduction in the larvae motility, reducing the possibility of detection (Rode and Jørgensen, 1989). Also, it requires well-trained personnel to perform correct larval detection and identification, avoiding misidentifications with trichostrongylid larvae (Vi˜na et al., 2013; Verocai et al., 2020b).

Over the last years, new diagnostic tools have been developed to assessment of parasites in fecal samples. For instance, the FLOTAC and Mini-FLOTAC techniques have emerged as promising and reliable techniques on the detection of diagnostic

stages of parasites in feces (Cringoli et al., 2010; Barda et al., 2013). In particular, both these techniques have been employed for the detection of first-stage larvae of lungworms, especially those responsible for infection in companion animals (e.g., dogs and cats) (Gaglio et al., 2008; Ianniello et al., 2020). Although used in ruminants, especially for the detection of nematodes and protozoans, both FLOTAC and Mini-FLOTAC techniques need a more complete evaluation to better establish values of sensitivity for the detection of L1 of lungworms (Bauer et al., 2010; Rinaldi et al., 2010).

In addition to the previously mentioned techniques, the bronchoalveolar lavage (BAL) is also considered an option for detection of L1 of lungworms. Despite of the low sensitivity (24.7%) this method may obtain two important data such as the detection of lungworms and the local immune response developed by the host against these nematodes (Hagberg et al., 2005; Holmgren et al., 2014; Lurier et al., 2018). Although scarcely used for the sole purpose of detection of lungworm infections, BAL can still be recommended for clinical management of individual animals presenting clinical disease rather than assessing lungworm prevalence in herds.

Based on the limitations presented by the classical microscopy-based techniques routinely used to diagnose these nematodes in ruminants, serological and molecular methods have been considered important tools for detection of lungworm infections (Morelli et al., 2020). Despite the difficulty of finding commercially available serological tests for the detection of lungworms, these methods, particularly represented by ELISA tests, may detect specific antibodies against adult *D. viviparus* in cattle, representing an alternative for large-scale testing elsewhere (Sekiya et al., 2013; McCarthy et al., 2019). These include ELISA tests that have specificity and sensitivity of 100% and 97.5%, respectively (Fiedor et al., 2009), but are not available in Brazil. However, there is only a short period of time in which re-infected animals may

be diagnosed as positive, and therefore posing a limitation for its use in adult ruminants (McCarthy et al., 2019).

Taking also into account the difficulty for species-level identification of L1 of different protostrongylids, which are morphologically similar to various genera (Kafle et al., 2017; Verocai et al., 2020b), the use of molecular tools has increased, especially for research purposes. Molecular diagnostic tests targeting ITS2 ribosomal DNA genetic markers and mitochondrial DNA markers can support epidemiological studies, in the development of improved diagnostic tests, new anthelmintics and vaccines (Carreno et al., 2009; Gasser et al., 2012). More recently, the use of next-generation sequencing (NGS) technologies has revolutionized the diagnosis of bacteria and viral infections, but is still rarely used for detection of nematode parasites (Avramenko et al., 2015). Considering the diversity of lungworm parasites infecting ruminants this kind of approach may be considered a promising tool. Recently, gastrointestinal parasites of domestic ruminants have been assessed by NGS techniques (Avramenko et al., 2015; Avramenko et al., 2017), hence the use of this technology may also be implemented for detection and species-identification of lungworms of ruminants.

4. Conclusion remarks and challenges for future research

The present review suggests the existence of adequate environmental conditions and hosts for the survival and development of lungworms infecting domestic ruminants especially in the Southeastern and Southern regions of Brazil. Lungworms, *D. viviparus* of cattle, and *D. filaria*, *M. capillaris*, and *P. rufescens* of small ruminants, have been reported over the past four decades in the country, but most of the information available was obtained after post-mortem examination. There is a need to better assess the distribution and prevalence of various lungworm species infecting

cattle, sheep and goat across Brazil through large-scale epidemiological studies. These studies would inform on optimal management and control practices applied by producers, aiming to reduce economic impact of clinical and subclinical lungworm infections. However, various challenges remain, including the need for more sensitive, field-friendly, and economically viable diagnostic tests.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2021.100645.

Ethical statement

The manuscript is a review article based on data previously published; therefore, the ethical statement of animal use is not applied in this case.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This article is based on the PhD thesis (Postgraduate Program in Animal Bioscience) of the first author, developed at the Federal Rural University of Pernambuco, with support from a fellowship from *Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco* (FACEPE).

References

Adem, J., 2016. Lungworm infection of small ruminant in Ethiopia. Adv. Life Sci. Technol. 43, 12–22.

Asmare, K., Sibhat, B., Haile, A., Sheferaw, D., Aragaw, K., Abera, M., Abebe, R., Wieland, B., 2018. Lungworm infection in small ruminants in Ethiopia: Systematic review and meta-analysis. Vet. Parasitol. Reg. Stud. Reports. 14, 63–70.

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S., 2015. Exploring the gastrointestinal "Nemabiome": Deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One. 10, 1–18.

Avramenko, R.W., Redman, E.M., Lewis, R., Bichuette, M.A., Palmeira, B.M., Yazwinski, T.A., Gilleard, J.S., 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. Int. J. Parasitol. 47, 893–902.

Barda, B., Zepherine, H., Rinaldi, L., Cringoli, G., Burioni, R., Clementi, M., Albonico M., 2013. Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. Parasit. Vectors. 220, 1–6.

Bassuino, D.M., Konradt, G., Bianchi, M.V., Snel, G.G.M., Sonne, L., Pavarini, S.P., Driemeier D., 2018. Causes of death in goats in Rio Grande do Sul state, Brazil: analysis of 322 cases (2000-2016). Braz. J. Vet. Res. Anim. Sci. 38, 2080–2087.

Bauer, B.U., Pomroy, W.E., Gueydon, J., Gannac, S., Scott, I., Pfister, K., 2010. Comparison of the FLOTAC technique with the McMaster method and the Baermann technique to determine counts of *Dictyocaulus eckerti* L1 and strongylid eggs in faeces of red deer (*Cervus elaphus*). Parasitol. Res. 107, 555–660.

Bekele, T., Shibbiru, T., 2017. Prevalence of ovine lungworm and associated risk factors in and around Debre Berhan town, Ethiopia. Int. J. Vet. Health Sci. Res. 5, 190–195.

Borges, F.A., Silveira, D.M., Graminha, E.B.N., Castagnolli, K.C., Soares, V.E., Nasciemento, A.A., Costa, A.J., 2001. Helminth parasites of cattle from Jaboticabal, São Paulo State, Brazil. Semina: Ci. Agrárias. 22, 49–53.

Cable, J., Barber, I., Boag, B., Ellison, A.R., Morgan, E.R., Murray, K., Pascoe, E.L., Sait, S.M., Wilson, A.J., Booth, M., 2017. Global change, parasite transmission and disease control: lessons from ecology. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 5, 1–17.

Cardoso, J.I.S., Oliveira, C.M.B., 1993. Fauna parasitária de caprinos na grande Porto Alegre. Rev. Bras. Parasitol. Vet. 2, 57–60.

Carreno, R.A., Diez-Baños, N., Hidalgo-Argüello, M.R, Nadler, S.A., 2009. Characterization of *Dictyocaulus* species (Nematoda: Trichostrongyloidea) from three species of wild ruminants in northwestern Spain. J. Parasitol. 95, 966–970.

Cassini, R., Párraga, M.A., Signorini, M., Regalbono, A.F., Sturaro, E., Rossi, L., Ramanzin, M., 2015. Lungworms in Alpine ibex (*Capra ibex*) in the eastern Alps, Italy: An ecological approach. Vet. Parasitol. 30, 132–138.

Cezaro, M.C., Tvarijonaviciute, A., Tecles, F., Céron, J.J., Eckersall, D.P., Ferreira, J.C., Schmidt, E.M., 2016. Changes in biochemical analyses in calves infected by nematode parasites in field conditions. Vet. Parasitol. 219, 1–6.

Cezaro, M.C., Neves, J.H., Cury, J.R.L.M., Dalanezi, F.M., Oliveira, R.M., Ferreira, J.C.P., Neto, V.A.K., Schmidt, E.M.S., 2018. Gastrointestinal and pulmonary

nematodes in calves naturally infected in the cities of Botucatu and Manduri, in the Brazilian state of São Paulo. Braz. J. Vet. Res. 38, 1286–1292.

Charlier, J., Ghebretinsae, A., Meyns, T., Czaplicki, G., Vercruysse, J., Claerebout, E., 2016. Antibodies against *Dictyocaulus viviparus* major sperm protein in bulk tank milk: Association with clinical appearance, herd management and milk production. Vet. Parasitol. 232, 36–42.

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. 5, 503–515.

Dank, M., Holzhauer, M., Veldhuis, A., Frankena, K., 2015. Association between *Dictyocaulus viviparus* status and milk production parameters in Dutch dairy herds. J. Dairy Sci. 11, 7741–7747.

Duarte, M.J.F., Gomes P, A.C., Sant'Anna, D.B., 1982. Prevalence and intensitv of helminth infection in calves at Cantagalo, RJ, Brazil. Pesq. Agropec. Bras. 17, 1521–1524.

Duarte, M.J.F., Miranda, I.M., 1984. Ocorrência de *Protostrongylus rufescens* (Nematoda: Protostrongylidae) em caprino do Estado do Rio de Janeiro. Pesq. Agropec. Bras. 19, 647–649.

Eysker, M., 1997. The sensitivity of the Baermann method for the diagnosis of primary *Dictyocaulus viviparus* infections in calves. Vet. Parasitol. 69, 89–93.

Fiedor, C., Strube, C., Forbes, A., Buschbaum, S., Klewer, A.M., Von Samson-Himmelstjerna, G., Schnieder, T., 2009. Evaluation of a milk ELISA for the

serodiagnosis of *Dictyocaulus viviparus* in dairy cows. Vet. Parasitol. 166, 255–261.

Forrester, S.G., Lankester, M.W., 1997. cd. J. Wildl. Dis. 33, 511–516.

Furlong, J., Novas, J.C.V., Cardoso Filho, J.B., 1985. Cattle parasitosis in the zona da mata region of Minas Gerais Brazil. Pesq. Agropec. Bras. 20, 1409–1413.

Gonçalves, P.C., Santos, O.P., Ueno, H., 1980. *Muellerius capillaris* em ovinos: ocorrência e identificação de adultos. Pesq. Agropec. Bras. 15, 137–141.

Gonçalves, R.C., Lisbôa, J.A.N., Souza, M.V., Almeida, C.T., Kuchembuck, M.R.G., Chiacchio, S.B., 2000. Aspectos clínicos e epidemiológicos da broncopneumonia dos bezerros em Botucatu, SP. Rev. Bras. Ci. Vet. 7, 144–147.

Gaglio, G., Cringoli, G., Rinaldi, L., Brianti, E., Giannetto, S., 2008. Use of the FLOTAC technique for the diagnosis of *Aelurostrongylus abstrusus* in the cat. Parasitol. Res. 103, 1055–1057.

Gasser, R.B., Jabbar, A., Mohandas, N., Höglund, J., Hall, R.S., Littlewood, D.T., Jex, A.R., 2012. Assessment of the genetic relationship between *Dictyocaulus* species from *Bos taurus* and *Cervus elaphus* using complete mitochondrial genomic datasets. Parasit. Vectors. 30, 1–11.

Habte, D., Simeneh, A., 2019. Prevalence, associated risk factors and species identification of lung worm infection in sheep in Dangla district, Western Amhara, North West Ethiopia. Int. J. Vet. Sci. Res. 5, 076–085.

Hagberg, M., Wattrang, E., Niskanen, R., Tråvén, M., Höglund, J., Lundén, A., 2005. Mononuclear cell subsets in bronchoalveolar lavage fluid during *Dictyocaulus*

viviparus infection of calves: a potential role for gamma/delta TCR-expressing cells in airway immune responses? Parasit. Immunol. 27, 151–61.

Henker, L.C., Schwertz, C.I., Lucca, N.J., Piva, M.M., Giacomin, P., Gris, A., Rhoden, L.A., Norbury, L.J., da Silva, A.S., da Rosa, R.A., Mendes, R.E., 2017. Dictyocaulosis in dairy cows in Brazil: an epidemiological, clinical-pathological and therapeutic approach. Acta Parasitol. 62, 129–132.

Hoberg, E.P., Brooks, D.R., 2015. Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 5, 1–7.

Holmgren, S., Hagberg, Gustavsson, M., Lundén, A., Wattrang, E., 2014. Cytokine mRNA expression in bronchoalveolar lavage cells during *Dictyocaulus viviparus* infection in calves. Parasit. Immunol. 36, 78–86.

Holzhauer, M., van Schaik, G., Saatkamp, H.W., Ploeger, H.W., 2011. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. Vet. Rec. 169, 1–5.

Jorgensen, R.J., Ronne, H., Helsted, C., Iskander, A.R., 1982. Spread of infective *Dictyocaulus viviparus* larvae in pasture and to grazing cattle: experimental evidence of the role of *Pilobolus* fungi. Vet. Parasitol. 10, 331–339.

Kuchboev, A.E., Krücken, J., Karimova, R.R., Ruziev, B.H., Pazilov, A., 2017. Infection levels of protostrongylid nematodes in definitive caprine and intermediate gastropod hosts from Uzbekistan. J. Helminthol. 91, 236–243.

Kafle, P., Leclerc, L.M., Anderson, M., Davison, T., Lejeune, M., Kutz, S., 2017. Morphological keys to advance the understanding of protostrongylid biodiversity

in caribou (*Rangifer* spp.) at high latitudes. Int. J. Parasitol. Parasit. Wildl. 6, 331–339.

Kowal, J., Kornaś, S., Nosal, P., Basiaga, M., Wajdzik, M., Skalska, M., Wyrobisz, A., 2016. Lungworm (Nematoda: Protostrongylidae) infection in wild and domestic ruminants from Małopolska region of Poland. Ann. Parasitol. 62, 63–66.

Ianniello, D., Pepe, P., Alves, L.C., Ciuca, L., Maurelli, M.P., Amadesi, A., Bosco, A., Musella, V., Cringoli, G., Rinaldi, L., 2020. Why use the Mini-FLOTAC to detect Metastrongyloid larvae in dogs and cats? Acta Parasitol. 65, 546–549.

Landim, V.J.C., Costa, A.J., Costa, G.H.N., Rocha, U.F., Barbosa, O.F., Moraes, F.R., 2001. Parasitic nematodes in weaned calves from the Northeast region of São Paulo state, Brazil. Ars. Vet. 17, 42–50.

Lima, J.D., Muniz, P., Lima W.S., Gonçalves, L.C.B., Silva, A.C., Guimarães, A.M., 1995. Eficácia de doramectin contra nematódeos gastrintestinais e pulmonares de bovinos naturalmente infectados de Minas Gerais. Rev. Bras. Parasitol. Vet. 4, 49–52.

Lurier, T., Delignette-Muller, M.L., Rannou, B., Strube, C., Arcangioli, M.A., Bourgoin, G., 2018. Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach. Prev. Vet. Med. 154, 124–131.

Macedo, L.O., Miranda, L.F., Oliveira, J.C.P., Ramos, C.A., Verocai, G.G., Carvalho, G.A., Alves, L.C., Ramos, R.A.N., 2020. *Protostrongylus rufescens* in goats: Morphological and molecular characterization. Small Rumin. Res. 182, 11–14.

Machado, T.M.M., Lima, W.S., 1988. *Muellerius capillaris* (MUELLER, 1889) em caprinos no Estado de Minas Gerais. Arq. Bras. Med. Vet. e Zoot. 1, 69–70.

Mangiola, S., Young, N.D., Sternberg, P.W., Strube, C., Korhonen, P.K., Mitreva, M., Scheerlinck, J.P., Hofmann, A., Jex, A.R., Gasser, R.B., 2014. Analysis of the transcriptome of adult *Dictyocaulus filaria* and comparison with *Dictyocaulus viviparus*, with a focus on molecules involved in host-parasite interactions. Int. J. Parasitol. 44, 251–261.

Mansfield, L.S., Gamble, H.R., Baker, J.S., Lichtenfels, J.R., 1993. Lungworm infection in a sheep flock in Maryland. J. Am. Vet. Med. Assoc. 202, 601–606.

May, K., Brügemann, K., König, S., Strube, C., 2018. The effect of patent *Dictyocaulus viviparus* (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs. Parasit. Vectors. 11, 1–11.

McCarthy, C., Höglund, J., Christley, R., Juremalm, M., Kozlova, I., Smith, R., Van Dijk, J., 2019. A novel pooled milk test strategy for the herd level diagnosis of *Dictyocaulus viviparus*. Vet. Parasitol. 1, 1–7.

McCarthy, C., Van Dijk, J., 2020. Spatiotemporal trends in cattle lungworm disease (*Dictyocaulus viviparus*) in Great Britain from 1975 to 2014. Vet. Rec. 19, 1–7.

McNulty, S.N., Strübe, C., Rosa, B.A., Martin, J.C., Tyagi, R., Choi, Y.J., Wang, Q., Hallsworth, P.K., Zhang, X., Ozersky, P., Wilson, R.K., Sternberg, P.W., Gasser, R.B., Mitreva, M., 2016. *Dictyocaulus viviparus* genome, variome and transcriptome elucidate lungworm biology and support future intervention. Sci. Rep. 9, 1–17.

Millen., D.D., Pacheco, R.D.L., Meyer, P.M., Rodrigues, P.H.M., Arrigoni, M.B., 2011. Current outlook and future perspectives of beef production in Brazil. Anim. Front. 2, 46–52.

Molento, M.B., Depner, R.A., Mello, M.H., 2006. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing-season calves. Vet. Parasitol. 141, 373–376.

Morelli, S., Diakou, A., Di Cesare, A., Schnyder, M., Colombo, M., Strube, C., Dimzas, D., Latino, R., Traversa, D., 2020. Feline lungworms in Greece: copromicroscopic, molecular and serological study. Parasitol. Res. 119, 2877–2883.

Oliveira, G.P., 1988. Epidemiology of dairy cattle gastrointestinal nematodes in the São Carlos region, SP, Brazil. Pesq. Agropec. Bras. 23, 189–195.

Panuska, C., 2006. Lungworms of ruminants. Vet. Clin. North Am Food Anim. Pract. 22, 583–593.

Pereira, R.G., Silva, A.S., Mendes, R.E., Bottari, N.B., Schwertz, C.I., Lucca, N.J., Henker, L.C., Morsch, V.M., Schetinger, M.R.C., Machado, G., 2017. Butyrylcholinesterase activity in dairy cows naturally infected by *Dictyocaulus viviparous* and treated with eprinomectin. Comp. Clin. Pathol. 26, 155–158

Ploeger, H.W., 2002. *Dictyocaulus viviparus*: re-emerging or never been away? Trends Parasitol. 1, 329–332.

Pyziel, A.M., Dolka, I., Werszko, J., Laskowski, Z., Steiner-Bogdaszewska, Ż., Wiśniewski, J., Demiaszkiewicz, A.W., Anusz, K., 2018. Pathological lesions in

the lungs of red deer *Cervus elaphus* (L.) induced by a newly-described *Dictyocaulus cervi* (Nematoda: Trichostrongyloidea). Vet. Parasitol. 15, 22–26.

Rinaldi, L., Maurelli, M.P., Musella, V., Santaniello, A., Coles, G.C., Cringoli, G., 2010. FLOTAC: an improved method for diagnosis of lungworm infections in sheep. Vet. Parasitol. 169, 395–398.

Rode, B., Jørgensen, R.J., 1989 Baermannization of *Dictyocaulus* spp. from faeces of cattle, sheep and donkeys. Vet. Parasitol. 30, 205–211.

Rosa, F.B., Caprioli, R.A., Silva, T.M., Galiza, G.J.N., Barros, C.S.L., Irigoyen, L.F., Fighera, R.A., Kommers, G.D., 2013. Doenças de caprinos diagnosticadas na região Central no Rio Grande do Sul: 114 casos. Braz. J. Vet. Res. 33, 199–204.

Sales, D.P., Santos, A.C.G., Silva e Silva, A.N., Gonçalves, L.N.R., Júnior, G.S.A., Costa, V.A., 2017. Nota de pesquisa: Ocorrência de helmintose pulmonar em caprinos e ovinos da microrregião da baixada maranhense, estado do Maranhão – Brasil. Vet. Cient. FHO Uniararas. 5, 1–10.

Sekiya, M., Zintl, A., Doherty, M.L., 2013. Bulk milk ELISA and the diagnosis of parasite infections in dairy herds: a review. Ir. Vet. J. 66, 1–12.

Schade, J., Albernaz, R.M., Reis, A.C.F., Fonteque, J.H., 2020. Dictyocaulosis in cattle in Londrina, PR, Brazil. Vet. Zootec. 27, 1–6.

Schunn, A.M., Conraths, F.J., Staubach, C., Fröhlich, A., Forbes, A., Schnieder, T., Strube C., 2013. Lungworm Infections in German dairy cattle herds-seroprevalence and GIS-supported risk factor analysis. PLoS One. 8, 1–10.

Sidone, O.J.G., Haddad, E.A., Mena-Chalco, J.P., 2016. A ciência nas regiões brasileiras: evolução da produção e das redes de colaboração científica. TransInf. 28, 15–32.

Silva, M.C., Barros, R.R., Graça, D.L., 2005. Outbreak of dictyocaulosis in cattle in Santa Maria, RS, Brazil. Ci. Rural. 35, 629–632.

Silva, A.D., Silva, A.S., Baldissera, M.D., Schwertz, C.I., Bottari, N.B., Carmo, G.M., Machado, G., Lucca, N.J., Henker, L.C., Piva, M.M., Giacomin,P., Morsch, V. M., Schetinger, M., Rosa, R.A., Mendes, R.E., 2016. Oxidative stress in dairy cows naturally infected with the lungworm *Dictyocaulus viviparus* (Nematoda: Trichostrongyloidea). J. Helminthol, 91, 462–469.

Silva, A.S., Baldissera, M.D., Bottari, N.B., Doleski, P.H., Carmo, G.M., Schwertz, C.I., Lucca, N.J., Henker, L.C., Piva, M.M., Giacomin, P., Stefani, L.M., Leal, D., Machado, G., Mendes, R.E., 2017. Ectonucleotidase and adenosine deaminase as inflammatory marker in dairy cows naturally infected by *Dictyocaulus viviparus.* Comp. Immunol. Microbiol. Infect. Dis. 51, 9–13.

Tolossa, Y.H., 2019. Lungworms infection of domestic ruminants with particular to Ethiopia: A review. Int. J. Adv. Res. Biol. Sci. 9, 89–103.

Valente, R., Robles, M., Diaz, J.I., 2020. Gastropods as intermediate hosts of *Angiostrongylus* spp. in the Americas: bioecological characteristics and geographical distribution. Mem. Inst. Oswaldo Cruz. 115, 1–9.

Verocai, G.G., Hoberg, E.P., Simard, M., Beckmen, K.B., Musiani, M., Wasser, S., Cuyler, C., Manseau, M., Chaudhry, U.N., Kashivakura, C.K., Gilleard, J.S., Kutz, S.J., 2020a. The biogeography of the caribou lungworm, *Varestrongylus*

elegueneniensis (Nematoda: Protostrongylidae) across northern North America. Int. J. Parasitol. Parasites Wildl. 8, 93–102.

Verocai, G.G., Chaudhry, U.N., Lejeune, M. 2020b. Diagnostic methods for detecting internal parasites of livestock. Vet. Clin. North Am. Food Anim. Pract. 36, 125–143.

Viña, M., Panadero, R., Díaz, P., Fernández, G., Pérez, A., Díez-Baños, P., Morrondo, P., López, C. M., 2013. Evaluation of the use of pooled fecal samples for the diagnosis of protostrongylid infections in sheep. Vet. Parasitol. 197, 231–234.

Wapennar, Barkema, H.W., Eysker, M., O'Handley, R.M., 2007. An outbreak of dictyocaulosis in lactating cows on a dairy farm. J. Am. Vet. Med. Assoc. 231, 1715–1718.

Yimer, A., Desie, A., 2016. Epidemiological studies on ovine lungworm species in Northern Ethiopia. J. Vet. Sci. Technol. 7, 1–4.

Zvinorova, P.I., Halimani, T.E., Muchadeyi, F.C., Matika, O., Riggio, V., Dzama, K., 2016. Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. Small Rumin. Res. 143, 75–83.

Figure captions

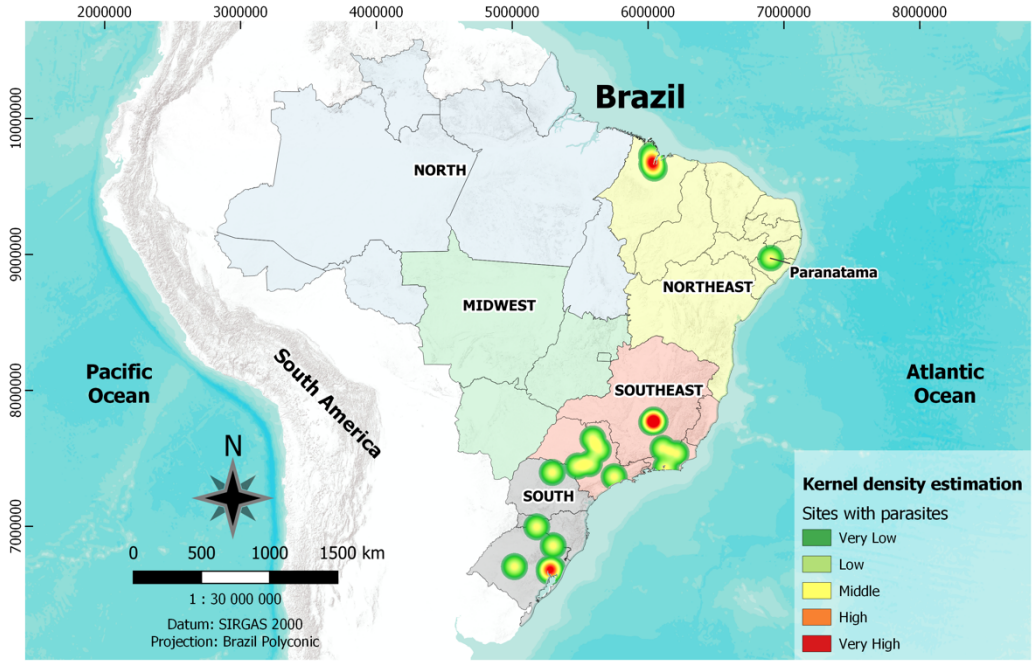
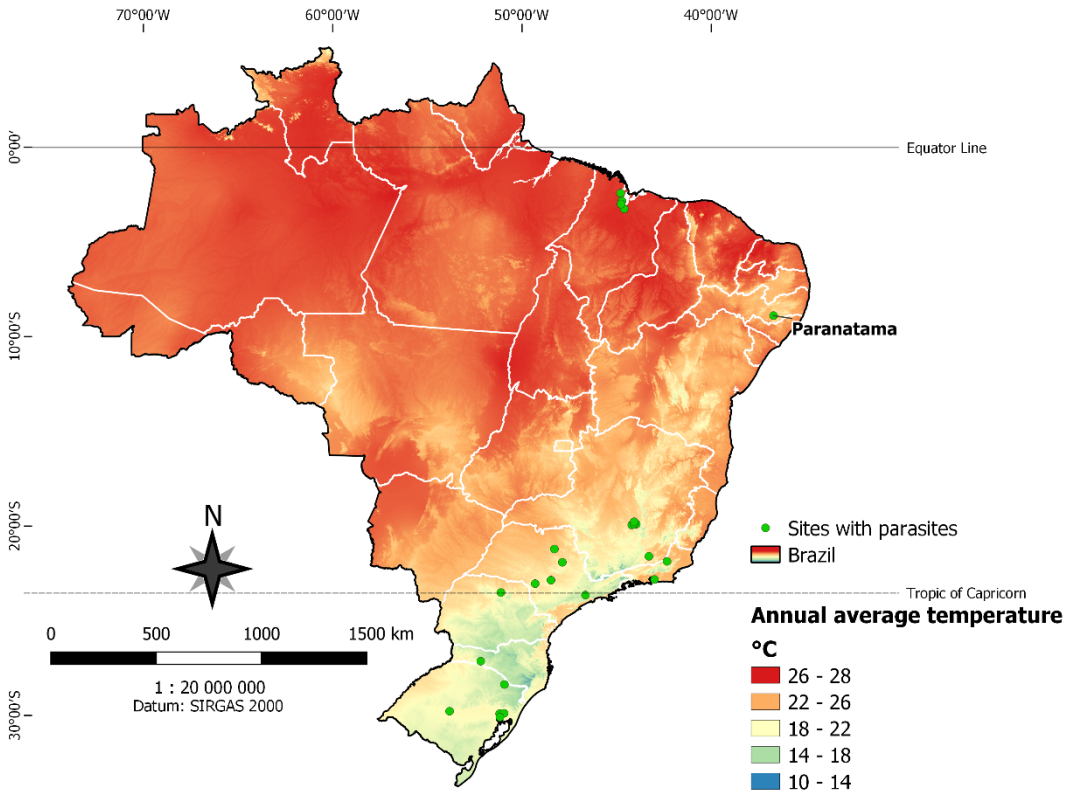


Figure 1: Geographic distribution of lungworm infection according to published records in cattle, goats and sheep the Brazil.



Supplementary file 1: Overall distribution of lungworms infecting ruminants in Brazil according to the mean temperature.

Table 1. Distribution of lungworms in cattle from Brazil.

Geographical area / State	Municipalities	Type of study	Aim of the study	Animals tested (n)	Positive Animals (%)	Diagnostic Method	Reference
Southeastern Brazil							
Rio de Janeiro	Cantagalo	ORP	Occurrence of lungworm	35	20.0	Necropsy	Duarte et al. (1982)
Minas Gerais	Coronel Pacheco	ORP	Occurrence of lungworm	48	95.8	Necropsy	Furlong et al., (1985)
São Paulo	São Carlos	ORP	Occurrence of lungworm	40	13.9	Necropsy	Oliveira (1988)
Minas Gerais	Belo Horizonte	ORP	Drug efficacy	20	100.0	Necropsy	Lima et al. (1995)
São Paulo	Botucatu	ORP	Occurrence of lungworm	133	10.5	Necropsy	Gonçalves et al. (2000)

São Paulo	Jaboticabal	ORP	Occurrence of lungworm	42	16,7	Necropsy	Borges et al. (2001)
São Paulo	São Paulo	ORP	Occurrence of lungworm	55	9.1	Necropsy	Landim et al. (2001)
São Paulo	Botucatu e Manduri	ORP	Occurrence of lungworm	86	22.1	Baermann	Cezaro et al. (2016)
São Paulo	Botucatu e Manduri	ORP	Occurrence of lungworm	140	37.9	Baermann	Cezaro et al. (2018)
Southern Brazil							
Rio Grande do Sul	Santa Maria	ORP	Occurrence of lungworm	15	13.3	Necropsy	Silva et al. (2005)
Rio Grande do Sul	Santa Maria	SC	Drug efficacy	70	100.0	Necropsy and Baermann	Molento et al. (2006)

Santa Catarina	Arabutã	ORP	Occurrence of lungworm	32	71.9	Necropsy and Baermann	Silva et al. (2016)
Santa Catarina	Arabutã	ORP	Occurrence of lungworm	22	68.9	Necropsy and Baermann	Silva et al. (2017)
Santa Catarina	Arabutã	ORP	Occurrence of lungworm	23	71.9	Necropsy and Baermann	Henker et al. (2017)
Santa Catarina	Arabutã	ORP	Occurrence of lungworm	22	68.9	Necropsy and Baermann	Pereira et al. (2017)
Paraná	Londrina	ORP	Occurrence of lungworm	9	55.6	Necropsy and Baermann	Schade et al. (2020)

ORP: Original Research Paper; SC: Short Communication.

Table 2 Distribution of lungworms in goats and sheep from Brazil.

Geographical area / State	Municipalities	Type of study	Aim of the study	Animals tested (n)	Positive Animals (%)	Diagnostic Method	Population	Reference
Southeastern Brazil								
RJ	São Gonçalo	ORP	Occurrence of lungworm	1	100	Necropsy	Goat	Duarte and Miranda (1984)
MG	Contagem, Betim and Ribeirão das Neves	SC	Occurrence of lungworm	1219	4	Baermann	Goat	Machado and Lima (1988)
Southern Brazil								
RS	Vacaria	ORP	Occurrence of lungworm	120	40	Necropsy	Sheep	Gonçalves et al. (1980)
RS	Porto Alegre, Canoas and Gravataí	ORP	Occurrence of lungworm	23	4	Necropsy	Goat	Cardoso and Oliveira (1993)
RS	Santa Maria	ORP	Occurrence of lungworm	46	2.1	Necropsy	Goat	Rosa et al. (2013)

RS	Porto Alegre	ORP	Occurrence of lungworm	167	1.2	Necrpsy	Goat	Bassuino et al. (2018)
Northeastern Brazil								
MA	São João Batista, Cajapió, Anajatuba and Bequimão	ORP	Occurrence of lungworm	240	25	Baermann	Goat / Sheep	Sales et al. (2017)
PE	Paranatama	SC	Occurrence of lungworm	217	18.9	Baermann	Goat	Macedo et al. (2020)

ORP: Original Research Paper; SC: Short Communication.

5.3 Artigo 3

(Artigo publicado no periódico do Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 74, p. 205-209, 2022)

Prevalence of *Protostrongylus* sp. in ruminants in a semi-arid region of Northeastern Brazil

[Prevalência de *Protostrongylus* sp. em ruminantes na região semiárida do nordeste do Brasil]

RESUMO

Objetivou-se neste estudo determinar a prevalência de vermes pulmonares em ruminantes do semiárido, nordeste do Brasil. Amostras fecais (n=429), de bovinos (n=219), caprinos (n=122) e ovinos (n=88) foram coletadas e laboratorialmente analisadas pela técnica de Baermann. Larvas de *Protostrongylus* sp. foram detectadas em 8,19% (10/122) dos caprinos. Elas apresentaram o comprimento médio de 351µm (±29,06µm) e largura média de 19µm (±1,46µm). Todos os animais positivos eram mantidos em sistema de criação semi-intensivo e não apresentavam sinais clínicos sugestivos da infecção por nematódeos pulmonares. Bovinos e ovinos foram negativos. Este estudo fornece dados sobre a infecção por parasitos pulmonares em caprinos de uma importante área de criação de pequenos ruminantes no Nordeste do Brasil, onde informações sobre esses parasitos são quase inexistentes. Apesar da ausência de sinais clínicos nos animais deste estudo, medidas sanitárias são preconizadas para prevenir a infecção por esses nematódeos e reduzir o impacto econômico que eles podem causar na produção pecuária.

Palavras-chave: caprinos, vermes pulmonares, epidemiologia

Lungworms are an important group of nematodes that infect domestic and wild animals throughout the world (Panuska, 2006; Bezerra-Santos *et al.*, 2020). In general, these parasites colonize the lower respiratory tract, resulting in a wide

plethora of injuries and, occasionally, causing fatal pneumonia (Panayotova-Pencheva and Alexandrov, 2010). In livestock animals, aside from their pathogenic implications, infections by lungworms also cause significant economic losses, due to the reduction of milk production, as well as mortality of animals. For example, the estimated costs may achieve up to 167 € per cow, as demonstrated during an outbreak in The Netherlands (Holzhauer *et al.*, 2011).

Cattle are more commonly affected by *Dictyocaulus viviparus*, the causative agent of parasitic bronchitis in calves at first grazing season (Schunn *et al.*, 2013). On the other hand, small ruminants can be infected by different species such as *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens* (Panuska, 2006). Morphologically, the differentiation of larvae of lungworms is difficult, and it is based on the observation of the posterior extremity and data of measure (Kafle *et al.*, 2015). It is known that several epidemiological factors (e.g., climatic conditions, rainfall, humidity, farm management systems) may influence the occurrence of lungworms in ruminants (Samadi *et al.*, 2019). Additionally, the presence of intermediate hosts (snails and slugs) may play an important role in the epidemiology of these nematodes (Kuchboev *et al.*, 2017; Tolossa, 2019).

Despite the scant data on distribution of these parasites, they have been reported in different continents such as Americas (Wapenaar *et al.*, 2007; Henker *et al.*, 2017; Macedo *et al.*, 2020), Europe (Kowal *et al.*, 2016), Africa (Bekele and Shibbiru, 2017; Asmare *et al.*, 2018) and Asia (Lat-Lat *et al.*, 2007). Recently, a very comprehensive review of the literature on the occurrence lungworms in domestic ruminants in Brazil (Macedo *et al*., 2021) cited only two reports of *P. rufescens* infecting small ruminants. Firstly, in the state of Rio de Janeiro, and almost four decades forward in the state of Pernambuco (Duarte and Miranda, 1984; Macedo *et al.*, 2020). The recent retrieval of *Protostrongylus* in Northeastern Brazil has stimulated researchers to study several aspects such as the economic and sanitary impact on ruminant populations. Any information obtained is crucial to improve the quality of the creation of these animals in this region, where the rearing of ruminants represent an important economic activity for subsistence of many families. In fact, it is believed that dairy farming is the main economic activity in the Northeastern region (Oliveira *et al*., 2007), with an average production of 35 million liters of milk / year (Municipalities, 2016).

Therefore, the aim of this study was to determine the prevalence of lungworms in ruminants reared in a semi-arid region of Northeastern Brazil.

A transversal study was performed in farms (n=30) of ruminant rearing, located in the Garanhuns microregion (8º53'25'' South and 36º29'34'' West), which is comprised by 21 municipalities, located in state of Pernambuco, Northeastern Brazil. There is a predominance of a semi-arid climate with an annual average of temperature of 22°C (from 17°C to de 30°C), rainfall mean of 147 mm (from 25mm to 295 mm), and air relative humidity of 90%.

The Ethics Committee for Animal Experimentation (ECAE) of *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (approval number: 21/2019).

The minimum sample size (n=384) was estimated based on the cattle (n=307,347), goat (n=35,770), and sheep population (n=99,606) of the study area (IBGE, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004). The farms were randomly selected by convenience (Reis, 2003).

Animals from one month to 5 years old were included in this study, and all individuals of each farm were sampled. The historical of deworming was absent in these farms.

Animals enrolled in this study were from semi-intensive (n=343) and intensive (n=90) rearing systems. They were fed with *Cynodon dactyl* (Tifton grass) and *Brachiaria* spp. and supplemented with concentrated and mineral salt.

From March 2019 to December 2020, fresh fecal samples (n=429) were collected from the rectum of cattle (n=219), goats (n=122) and sheep (n=88) using plastic gloves. The material was stored in isothermal boxes at 4oC until laboratory processing.

Each sample was individually processed by the Baermann technique (Forrester and Lankester, 1997). Larvae found were morphologically analyzed and features of anterior and posterior ends were recorded (Boev, 1975). Measurements were obtained using the software TCapture 4.3.

Descriptive statistical analysis was performed to obtain relative and absolute frequencies. In addition, the Lilliefors test was used to verify the normality of the data. The relationship between the number of young (≤12 months old) and adult animals (>12 months old) parasitized, as well as the occurrence of

parasites in male and female, were evaluated through the Fisher's Exact test, with 5% significance level. The BioEstat software, version 5.3 was used to perform all the statistical analyses.

Lungworm larvae were detected in 8.19% (10/122) of goat samples, whereas all cattle and sheep scored negative. Larvae (n=25) of *Protostrongylus* sp. with an average length of 351 µm (±29.06 µm) and width mean of 19 µm (±1.46 µm) were detected. Morphologically, they presented the tip of the tail typical of subfamily Protostrongylinae, consisting of an elongated, thin, pointed, and slightly undulating process; the dorsal spine at the insertion of the tip of the tail (typical of the protostrongylid subfamilies Muelleriinae, Varestrongylinae, and Elaphostrongylinae) was absent. In addition, the presence of small granules was observed in the intestine.

Young goats (30.0%; 6/20) were statistically more parasitized than adults (3.9%; 4/102) (p=0.0013). Conversely, no significant difference was observed in the parasitism of females (7.3%; 8/110) and males (16.7%; 2/12) (p=0.2854). All positive animals were concentrated in three different farms in the municipality of Paranatama (Microrregion of Garanhuns). They were reared in a semi-intensive production system and did not present any clinical signs suggestive of the infection by lungworm nematodes. The overall positivity according to the age, sex and rearing system of animals is reported on Table 1.

Table 1. Prevalence of *Protostrongylus* sp. according to age, sex, and rearing system in goats from the Microregion Garanhuns, Northeastern Brazil.

	Goats % (n/N)
Age	
Young	30.0% (6/20)
Adult	3.9% (4/102)
Sex	
Female	7.3% (8/110)
Males	16.7% (2/12)
Rearing system	
Intensive	0.0% (0/58)
Semi-intensive	15.6% (10/64)

This study provides laboratorial evidence of the parasitism by *Protostrongylus* sp. in goats in Northeastern Brazil, as well as the absence of other lungworms parasites in cattle and sheep raised in the same region. In Brazil, data about this parasite are limited only to two reports (Duarte and Miranda, 1984; Macedo *et al.*, 2020). The overall positivity (8.19%; 10/122)

observed in this study is lower than that detected in previous research conducted in same area (i.e., 18.9%; 41/217) (Macedo *et al.*, 2020).

The dynamics of lungworm infection in ruminants is highly influenced by environmental conditions, which directly impact the development of larval stages and transmission opportunities (Habte and Simeneh, 2019). Therefore, the difference of parasitism between cattle/sheep and goats may be related to the period of the year, as well as the feeding behavior presented by these hosts. Cattle and sheep are known as grazers and are constantly challenged by parasites, developing a more effective immunity (Hoste *et al.*, 2010; Underwood *et al.*, 2015). On the other hand, goats are classified as intermediate selectors, and during the meal are less challenged by parasites, which difficult the development of resistance against nematodes (Hoste *et al.*, 2008).

Although, in grazing conditions both young and adult goats may be infected (Hoste *et al.*, 2008), in this study young animals were predominantly affected (p=0.0013). Immunological mechanisms against nematodes are differently expressed between young and adult animals (Hoste *et al.*, 2010). For this reason, clinical disease is more common at their first grazing season in young animals, whereas older goats developed a strong immunity over the time (Tolossa, 2019). Females and males were equally affected (p=0.2854). However, the association of positivity and sex requires additional analysis because in this study the number of males (n=12) evaluated was lower than females (n=110).

All positive animals were concentrated in three farms in a single municipality (i.e., Paranatama). This area is featured by a semi-arid climate with mean annual temperature of 22°C, mean rainfall of 147 mm and relative air humidity of 90%. It is known that the prevalence of lungworms is strongly influenced by different environmental aspects and climatic conditions (e.g., precipitation, humidity, and temperature) (Adem, 2016). From a climatic point of view, the region provides all suitable conditions for survival and development of *Protostrongylus* larvae (Fentahun *et al.*, 2016). Although, in this area the mollusk fauna has never been studied, it is known that the genus *Protostrongylus* uses different species of gastropods (e.g., *Vallonia* and *Helix*) as intermediate hosts (Kuchboev *et al.*, 2017; Tolossa, 2019). In Northeastern Brazil, the *Achatina fulica* may participate as an intermediate host for other nematodes (e.g., *Angiostrongylus* spp.) (Thiengo *et al.*, 2010); but the participation of this mollusk

species in the development of lungworms affecting ruminants has never been evaluated.

All infected goats were raised in a semi-intensive production system and did not exhibit any clinical signs suggestive of the infection by lungworms. A common practice adopted in these farms is the confinement of animals at night, and the free grazing during the day. This kind of system favors the contact with contaminated pastures and putative intermediate hosts (mollusks) (Tolossa, 2019). The management of each farm associated with the nutritional status, level of immunity acquired by animals and the period of infection, may explain the absence of clinical signs, the reduced number of infected animals, as well as the low larvae excretion (Habte and Simeneh, 2019). Additionally, it is important to highlight that the use of anthelmintic compounds may contribute to the absence of clinical signs. However, the historical of deworming was absent in the farms herein sampled, which difficult this kind of analysis.

This study provides important data on lungworm infection in goats in Northeastern Brazil. Although, no clinical signs had been observed in infected animals, these data sound as warning to veterinary practitioners, which may include these parasites in the list of putative causes of respiratory diseases. Finally, it is imperative the adoption of appropriate sanitary measures, as well as a good nutritional management of animals reared in this area to prevent infection by these nematodes and to reduce the economic impact they may cause. Further studies focusing especially on the determination of intermediate hosts involved in the life cycle of *Protostrongylus* will be useful to fill in an important gap on the natural history of this nematode.

Keywords: goats, lungworms, epidemiology.

ACKNOWLEDGEMENTS

Authors would like to thank Marcos Antônio Bezerra-Santos (University of Bari) for his suggestions on the manuscript. In addition, this article is based on the PhD thesis (Graduate Program in Animal Bioscience) of the first author, developed at the Federal Rural University of Pernambuco, with support from a

fellowship from Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

REFERENCES

ADEM, J. Lungworm infection of small ruminant in Ethiopia. *Adv. Life. Sci. Technol.*, v.43, p.12-22, 2016.

ASMARE, K.; SIBHAT, B.; HAILE, A. *et al*. Lungworm infection in small ruminants in Ethiopia: Systematic review and meta-analysis. *Vet. Parasitol. Reg. Stud. Reports.*, v.14, p.63-70, 2018.

BEKELE, T.; SHIBBIRU, T. Prevalence of ovine lungworm and associated risk factors in and around Debre Berhan town, Ethiopia. *Int. J. Vet. Health Sci. Res.*, v.5, p.190-195, 2017.

BEZERRA-SANTOS, M.A.; MENDOZA-ROLDAN, J.A.; ABRAMO, F. *et al*. Transmammary transmission of *Troglostrongylus brevior* feline lungworm: a lesson from our gardens. *Vet. Parasitol.*, v.285, p.1-7, 2020.

BOEV, S. Fundamentals of Nematology: Protostrongylids. Moscow: Nauka Publishers, 1975. 268p.

DUARTE, M.J.F.; MIRANDA, I.M. Ocorrência de *Protostrongylus rufescens* (Nematoda: Protostrongylidae) em caprino do Estado do Rio de Janeiro. *Pesq. Agropec. Bras.*, v.19, p.647-649, 1984.

FENTAHUN, S.; ABEBE, R.; MELKAMU, S.; ASRAT, M. Study on lungworm infection in small ruminants: Prevalence and risk factors in and around Gondar Town, Northwest, Ethiopia. *Int. J. Vet. Sci. Animal Husb.*, v.1, p.47-52, 2016.

FORRESTER, S.G.; LANKESTER, M.W. Extracting protostrongylid nematode larvae from ungulate feces. *J. Wildl. Dis.*, v.33, p.511-516, 1997.

IBGE - Brazilian Institute of Geography and Statistics. Municipalities – Municipal livestock production. 2016 Available from: <https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9107-

producao-da-pecuaria-municipal.html?=&t=o-que-e >. Accessed in: 20 may. 2021.

HABTE, D.; SIMENEH, A. Prevalence, associated risk factors and species identification of lung worm infection in sheep in Dangla district, Western Amhara, North West Ethiopia. Int. *J. Vet. Sci. Res.*, v.5, p.76-85, 2019.

HENKER, L.C.; SCHWERTZ, C.I.; LUCCA, N.J. *et al.* Dictyocaulosis in dairy cows in Brazil: an epidemiological, clinical-pathological and therapeutic approach. *Acta Parasitol.,* v.62, p.129-132, 2017.

HOLZHAUER, M.; VAN SCHAIK, G.; SAATKAMP, H.W.; PLOEGER, H.W. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. *Vet. Record.*, v.1, p.1-6, 2011.

HOSTE, H.; SOTIRAKI, S.; LANDAU, S.Y. *et al.* Goat-Nematode interactions: think differently. *Trends Parasitol.,* v.26, p.376-381, 2010.

HOSTE, H.; TORRES-ACOSTA, J.F.; AGUILAR-CABALLERO, A.J. Parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? *Parasite Immunol.,* v.30, p.79-88, 2008.

KAFLE, P.; LEJEUNE, M.; VEROCAI, G.G. *et al.* Morphological and morphometric differentiation of dorsal-spined first stage larvae of lungworms (Nematoda: Protostrongylidae) infecting muskoxen (*Ovibos moschatus*) in the central Canadian Arctic. *Int. J. Parasitol. Parasites Wildl.,* v.10, p.283-290, 2015.

KOWAL, J.; KORNAŚ, S.; NOSAL, P. *et al.* Lungworm (Nematoda: Protostrongylidae) infection in wild and domestic ruminants from Małopolska region of Poland. *Ann. Parasitol.*, v.62, p.63-66, 2016.

KUCHBOEV, A.E.; KRÜCKEN, J.; KARIMOVA, R.R. *et al.* Infection levels of protostrongylid nematodes in definitive caprine and intermediate gastropod hosts from Uzbekistan. *J. Helminthol.*, v.91, p.236-243, 2017.

LAT-LAT, H.; HASSAN, L.; SANI, R.A. *et al.* First report of bovine lungworm disease in South-East Asia. *Trop. Biomed.*, v.24, p.77-81, 2017.

MACEDO, L.O.; MIRANDA, L.F.; OLIVEIRA, J.C.P. *et al. Protostrongylus rufescens* in goats: Morphological and molecular characterization. *Small Rumin. Res.,* v.182, p.11-14, 2020.

PANAYOTOVA-PENCHEVA M.S.; ALEXANDROV, M.T. Some pathological features of lungs from domestic and wild ruminants with single and mixed protostrongylid infections. *Vet. Med. Int.,* v.1, p.1-9, 2010.

PANUSKA, C. Lungworms of ruminants. *Vet. Clin. North Am. Food Anim. Pract.*, v.22, p.583-953, 2006.

SAMADI, A.; FAIZI, N.; ABI, A.J. *et al.* 2019. Prevalence and pathological features of ovine lungworm infection in slaughtered animals in Nangarhar Province of Afghanistan. Comp. Clin. Pathol. 28, 1667–1673.

SCHUNN, A.M.; CONRATHS, F.J.; STAUBACH, C. *et al.* Lungworm Infections in German dairy cattle herds--seroprevalence and GIS-supported risk factor analysis. *PLoS One.,* v.5, p.1-10, 2013.

THIENGO, S.C.; MALDONADO, A.; MOTA, E.M. *et al.* The giant African snail *Achatina fulica* as natural intermediate host of *Angiostrongylus cantonensis* in Pernambuco, northeast Brazil. *Acta Trop.*, v.115, p.194-199, 2010.

TOLOSSA, Y.H. Lungworms infection of domestic ruminants with particular to Ethiopia: A review. *Int. J. Adv. Res. Biol. Sci.*, v.9, p.89-103, 2019.

UNDERWOOD, W.J.; BLAUWIEKEL, R.; DELANO, M.L. *et al.* Biology and diseases of ruminants (Sheep, Goats, and Cattle). *Lab. Anim. Sci.*, v.1, p.623-694, 2015.

WAPENAAR, W.; BARKEMA, H.W.; EYSKER, M.; O'HANDLEY, R.M. An outbreak of dictyocaulosis in lactating cows on a dairy farm. *J. Am. Vet. Med. Assoc.*, v. 231, p.1715-1718, 2007.

5.4 Artigo 4

(Artigo publicado na Revista Brasileira de Parasitologia Veterinária, v. 31, p. 1-5, 2022)

Larvoscopic study on *Dictyocaulus* sp. in the faeces of beef cattle in northeastern Brazil

Abstract

The lungworm *Dictyocaulus viviparus* has an important role in the bovine health and productivity worldwide, since infections can lead to substantial economic losses. Despite its importance, few studies investigating the epidemiological aspects of infections by this parasite has been conducted. The aim of this study to report the occurrence of lungworm infection in beef cattle herds reared in an important area of livestock production in the Northeastern region of Brazil. From September 2020 to August 2021, monthly fecal samples (*n* = 493) were collected from 46 beef cattle. Out of all animals assessed, lungworm larvae were detected in 8.7% (4/46). Animals did not present any clinical sign suggestive of the infection by lungworms parasites. Twenty larvae were retrieved, with the minimal number (*n* = 1) detected in October and December, and the maximum number (*n* = 13) in November. They presented a mean length of 363 µm (± 28.65 µm), mean width of 19 µm (±1.03 µm) and were morphologically similar to *Dictyocaulus* sp.. This study reports the occurrence of this parasite in this livestock production area. Finally, local veterinarians should be aware of the inclusion of this parasite in the differential diagnosis of other respiratory infections in beef cattle.

Keywords: Nematode; Lungworm; Epidemiology; Livestock production.

Resumo

O verme pulmonar *Dictyocaulus viviparus* tem um papel importante na saúde e produtividade bovina em todo o mundo, uma vez que infecções podem levar a perdas econômicas substanciais. Além de sua importância, poucos estudos investigando aspectos epidemiológicos das infecções por esse parasito têm sido realizados. Portanto, esta pesquisa teve como objetivo relatar a ocorrência de infecção por nematódeos pulmonares em rebanhos bovinos de corte criados em

uma importante área de produção pecuária na região Nordeste do Brasil. De setembro de 2020 a agosto de 2021, foram coletadas mensalmente amostras fecais mensais (n = 493) de 46 bovinos de corte. De todos os animais avaliados, larvas de vermes pulmonares foram detectadas em 8,7% (4/46). Os animais não apresentaram nenhum sinal clínico sugestivo de infecção por vermes pulmonares. Vinte larvas foram recuperadas, com o número mínimo (n = 1) detectado em outubro e dezembro, e o número máximo (n = 13) em novembro. Apresentavam comprimento médio de 363 µm (± 28,65 µm), largura média de 19 µm (±1,03 µm) e eram morfologicamente semelhantes a *Dictyocaulus* sp.. Este estudo relata a ocorrência deste parasito nesta área de produção pecuária. Por fim, os veterinários locais devem estar atentos à inclusão deste parasito no diagnóstico diferencial de outras infecções respiratórias em bovinos de corte.

Palavras-chave: Nematódeo; Verme pulmonar; Epidemiologia; Produção pecuária.

Infections by lungworms parasites play an important role in the ruminant's health worldwide (Kuchboev et al., 2012; Pyziel et al., 2018; Verocai et al., 2020; Macedo et al., 2021). In cattle this infection is featured by a parasitic bronchitis (PB) caused especially by the lungworm species *Dictyocaulus viviparus* (Nematoda: Dictyocaulidae), which has been considered a nematode economically important for these animals in different stages of their life (Schunn et al., 2013; Lurier et al., 2018; Forbes, 2018). This nematode has a direct life cycle and bovines are infected in pastures contaminated with third-stage larvae (L3) (Jorgensen, 1980). After infection, this parasite colonizes the lower respiratory tract, females lay eggs, which quickly hatch after release, then are swallowed and the first stage larvae are eliminated on feces (Panuska, 2006). The infection can lead to subclinical or severe clinical disease with clinical signs like coughing, dyspnea and eventually death (Ploeger et al., 2012; Holzhauer et al., 2011; May et al., 2018).

For a long time, the PB by *D. viviparus* was considered of clinical relevance mainly in first-year of age of calves. However, in recent decades outbreaks have been reported in adult herds, with considerable economic losses, due to the reduction of production and mortality (Van Dijk, 2004; Wapenaar et al., 2007;

Holzhauer et al., 2011; Vanhecke et al., 2020). For example, an outbreak reported in The Netherlands demonstrated that the loss in the production may achieve 4 kg/cow/day of milk (Holzhauer et al., 2011). On the other hand, in Belgium, animals with subclinical infection the loss mean was about 0.5 kg/cow/day of milk (Charlier et al., 2016). Unfortunately, this economical loss may be underestimated, especially when the mortality occurs, as already reported in beef cattle herd from Malaysia (Lat-Lat et al., 2007).

In Brazil, there are reports of infection by *D. viviparus* only in the Southern and Southeastern regions (Landim et al., 2001; Molento et al., 2006; Henker et al., 2017; Cezaro et al., 2018; Schade et al., 2020; Macedo et al., 2021). In most studies the occurrence was reported at postmortem examinations (Duarte et al., 1982; Gonçalves et al., 2000; Silva et al., 2005). Although no published data are available in the Northeastern region there are personal communications from veterinarians reporting clinical cases of respiratory disease suggestive of lungworm infection in cattle (Macedo et al., 2021).

The classic method to diagnosis lungworm infection in cattle is the Baermann technique, which is based on the retrieval and morphological identification of larvae in fresh fecal samples (Forrester and Lankester, 1997). However, along the time, greater attention has been given to gastrointestinal parasites and this method has been little applied, especially in bovines without clinical signs of PB (May et al., 2018). Additionally, the different levels of sensitivity of the Baermann method according to the age of the animals, has hampered the real knowledge about the occurrence of *D. viviparus* in bovines worldwide (Charlier et al., 2016; Lurier et al., 2018).

Therefore, the aim of the study was to report the occurrence of lungworm infection in beef cattle herds reared in an important area of livestock production in the Northeastern region of Brazil.

The study was performed on a beef cattle farm (Nellore breed), located in the municipality of Quipapá (8º83’64’’ South and 36º09’30’’ West), Zona da Mata region, state of Pernambuco, Northeastern Brazil. This area is characterized by a hot and humid tropical climate (*As*), with average annual temperature around of 23.4°C to 25.8, an annual rainfall mean of 400 mm to 900 mm, and air relative humidity of 90% (Medeiros et al., 2021).

The Ethics Committee for Animal Experimentation (ECAE) of *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (approval number: 21/2019).

In July 2020, two animals belonging to a herd of 48 animals (aging from 5 to 8 months-old, 6 male and 40 female) were attended to the Clinic of Bovines of Garanhuns (Federal Rural University of Pernambuco). These two animals died, and at post-mortem examination numerous filiform helminths (unidentified morphologically) were observed in the trachea and bronchi. The animals did not present clinical signs suggestive of the infection by lungworm parasites and, thus, the detection of nematodes was a finding.

After this event, the herd (n = 46) was monitored monthly and fecal sample from each animal was collected for a period of 12 months (from September 2020 to August 2021). Feces were collected directly from the rectum using plastic gloves. They were kept in isothermal boxes (8 °C) and processed in until 6 hours after collection. The herd was fed with *Panicum maximum* pasture, supplemented with corn silage and concentrate feed. The meteorological data (temperature, relative humidity and rainfall,) were obtained from of the National Institute of Meteorology (INMET).

Fresh fecal samples were collected and individually processed by the Baermann technique (Forrester and Lankester, 1997). Larvae found were morphologically analyzed and features of anterior and posterior ends were recorded (Soulsby, 1968). Measurements were obtained using the software TCapture 4.3.

Descriptive statistics were used to calculate relative, and absolute frequencies. Exact binominal 95% confidence intervals (CIs) of Wilson score interval, were established for proportions. The chi-square test statistic with Yates correction was used to compare proportions with a probability p value < 0.05 regarded as statistically significant, using the Epitools Epidemiological and Chi-Square Calculators.

A total of 493 samples from 46 animals were analyzed during the whole study, with a mean of 41 ± 4 animals assessed per month. Out of all samples analyzed, lungworm larvae were detected in 1.4% (7/493; 95% CI = 0.69-2.90). Among the animals 8.7% (4/46; 95% CI = 0.34-20.32) comprising two males and two females scored positive (p = 2.3103; χ^2 = 0.1285). During the entire whole

study period, these animals did not present any clinical sign suggestive of the infection by lungworms parasites.

A total of 20 larvae were retrieved, with the minimal number (*n* = 1) detected in October and December, and the maximum number (*n* = 13) in November. These presented a mean length of 363 µm (± 28.65 µm) and mean width of 19 µm (±1.03 µm). In addition, morphologically, they were similar to *Dictyocaulus* sp., with intestinal cells containing numerous granules, typical of the family Dictyocaulidae (Figure 1).

The overall distribution of larva during the study period according to the climatic data is reported on Figure 2.

This study assessed the occurrence of *Dictyocaulus* sp., infection in beef cattle herds reared in an important area of livestock production in the northeastern region of Brazil. Until now, occurrence of this parasite had only been reported in the southeastern and southern regions of this country (Landim et al., 2001; Molento et al., 2006; Henker et al., 2017; Cezaro et al., 2018). A previous study reported an outbreak with morbidity and lethality rates of 7.1% and 13.3%, respectively, in young animals from the state of Rio Grande do Sul (Silva et al., 2005).

Similarly, to what we report here, a study in Costa Rica the prevalence of infection (1.8%;9/549) remained low throughout the study period (Jiménez et al., 2007). It is important to note that a common practice adopted in the farm herein studied was the administration of anthelmintic drug (i.e., Ivermectin) every three months more precisely in November, February, and May. Undoubtedly, this was a determining factor to difficult the retrieval of lungworms larvae in animals of this study.

Our results indicated that males and females ($p = 3.206$; $\chi^2 = 0.733$) were equally affected, although of six males present in the herd, two were infected. Previous studies in small ruminants have also shown that males are commonly more affected suggesting that the different types of nutrition among these animals can influence lungworm infection (Borji et al., 2012). However, it is difficult to detect any effects of sex on the prevalence of lungworm infection, due to the large difference between males (n = 6) and females (n = 40) assessed in this study.

The infections by *Dictyocaulus* sp., in cattle are often suspected due to the clinical signs such as coughing and increased respiratory rate (Ploeger et al., 2012; May et al., 2018). Nevertheless, over the entire study period, none of the animals of the present study showed any clinical sign suggestive of the infection by lungworm infection. It is believed that cattle develop protective immunity and therefore do not develop clinical diseases. (May et al., 2018).

Although the prevalence herein detected was relatively low (i.e., 8.7%), the presence of lungworm was detected in five consecutive months (October/2020 to February/2021). Previous studies in the Southeastern region of the country demonstrate the presence of *D. viviparus* with prevalence of 50%, 35% and 28.5%, in autumn, winter and summer, respectively (Cezaro et al., 2018). The larval count ranged from 1 to 7 larvae per 40 g of feces for each animal, which is in accordance with previous studies (Eysker et al., 1994; May et al., 2018). However, we cannot discard the possibility of false negative results, especially because the Baermann techniques presents high sensitivity (100%) only for the detection of primary infection in young animals (Eysker, 1997).

In Brazil, the number of reports using the Baermann technique to detect this lungworm species is scant, as most records is performed during the post-mortem examination, although there is relatively simple in vivo diagnostic test that can be implemented (Verocai et al., 2020). For example, a recent retrospective epidemiological study in the last four decades showed that only 20.8% of animals were diagnosed using the Baermann technique in live animals (Macedo et al., 2021). In general, lungworm infection in ruminants may be of minor importance compared to gastrointestinal parasites and is therefore largely underdiagnosed, which leave gaps in the knowledge on the distribution and epidemiology of these parasites. Even if the absence of molecular analysis had been considered an important limitation of this study, the morphological identification of larvae in animals from a herd with a previous historic of infection by lungworm indicates the parasites in this area. Undoubtedly, molecular data will be addressed in the future to determine the parasite at level species.

Lastly, the data presented here demonstrated the rate occurrence of *Dictyocualus* sp. in fecal samples collected on beef cattle from Northeastern

Brazil. Although no clinical sings were observed among these animals, veterinarians and farmers need to be aware of the importance of this parasites in the differential diagnosis of other respiratory infections, such as viral and bacterial infections. Knowledge about this kind of infection in the region will be useful for guiding decisions anthelmintic treatment and other control strategies to prevent clinical disease, mortality and subsequently minimize potential economic losses.

References

Borji H, Azizzadeh M, Ebrahimi M, Asadpour, M. Study on small ruminant lungworms and associated risk factors in northeastern Iran. *Asian Pac J Trop Med* 2012; 5(11): 853-856. http://dx.doi.org/10.1016/S1995-7645(12)60159-X

Cezaro MC, Neves JH, Cury JRLM, Dalanezi FM, Oliveira RM, Ferreira JCP, et al. Gastrointestinal and pulmonary nematodes in calves naturally infected in the cities of Botucatu and Manduri, in the Brazilian state of São Paulo. Braz. *J Vet Res* 2018; 38(7): 1286-1292. http://dx.doi.org/10.1590/1678-5150-PVB-5225

Charlier J, Rinaldi L, Musella V, Ploeger HW, Chartier C, Vineer HR, et al. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. *Prev Vet Med* 2020; 182(1): 105103. http://dx.doi.org/10.1016/j.prevetmed.2020.105103.

Charlier J, Ghebretinsae A, Meyns T, Czaplicki G, Vercruysse J, Claerebout E. Antibodies against *Dictyocaulus viviparus* major sperm protein in bulk tank milk: Association with clinical appearance, herd management and milk production. *Vet Parasitol* 2016; 232(1): 36-42. http://dx.doi.org/10.1016/j.vetpar.2016.11.008

Duarte MJF, Gomes PAC, Sant'Anna DB. Prevalence and intensity of helminth infection in calves at Cantagalo, RJ, Brazil. *Braz J Agric Res* 1982; 17(1): 1521-1524.

Eysker M, Claessens EW, Lam TJGM, Moons MJ, Pijpers A. The prevalence of patent lungworm infections in herds of dairy cows in the Netherlands. *Vet Parasitol* 1994; 53(3-4): 263-267. http://dx.doi.org/10.1016/0304-4017(94)90189-9

Eysker M. The sensitivity of the Baermann method for the diagnosis of primary *Dictyocaulus viviparus* infections in calves. *Vet Parasitol* 1997; 69(1-2): 89-93. http://dx.doi.org/10.1016/s0304-4017(96)01099-0

Forbes A. Lungworm in cattle: epidemiology, pathology and immunobiology. *Livest Sci* 2018; 23(2): 59-66. http://dx.doi.org/10.12968/live.2018.23.2.59

Forrester SG, Lankester MW. Extracting protostrongylid nematode larvae from ungulate feces. *J Wildl Dis* 1997; 33(3): 511-516. http://dx.doi.org/10.7589/0090-3558-33.3.511

Gonçalves RC, Lisbôa JAN, Souza MV, Almeida CT, Kuchembuck MRG, Chiacchio SB. Aspectos clínicos e epidemiológicos da broncopneumonia dos bezerros em Botucatu, SP. *Braz J Vet Sci* 2000; 7(3): 144-147. http://dx.doi.org/10.4322/rbcv.2015.200

Henker LC, Schwertz CI, Lucca NJ, Piva MM, Giacomin P, Gris A, et al. Dictyocaulosis in dairy cows in Brazil: an epidemiological, clinical-pathological and therapeutic approach. *Acta Parasitol* 2017; 62(1): 129-132. http://dx.doi.org/10.1515/ap-2017-0015

Holzhauer M, van Schaik G, Saatkamp HW, Ploeger HW. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. *Vet Rec* 2011; 169(19): 494-494. http://dx.doi.org/10.1136/vr.d4736

Jiménez AE, Montenegro VM, Hernández J, Dolz G, Maranda L, Galindo J, et al. Dynamics of infections with gastrointestinal parasites and *Dictyocaulus viviparus* in dairy and beef cattle from Costa Rica. *Vet Parasitol* 2007; 148(3-4): 262-271. http://dx.doi.org/10.1016/j.vetpar.2007.06.015

Jorgensen RJ. Epidemiology of bovine dictyocaulosis in Denmark. *Vet Parasitol* 1980; 7(1): 153-167.

Kuchboev AE, Karimova RR, Ruziev BK, Azimov DA. Ecological aspects of nematodes of the family Protostrongylidae, parasites of Bovidae. *Sci Parasitol* 2012. 13(1): 17-25.

Landim VJC, Costa AJ, Costa GHN, Rocha UF, Barbosa OF, Moraes FR. Parasitic nematodes in weaned calves from the Northeast region of São Paulo state, Brazil. *Ars Vet* 2001; 17(1): 42-50.

Lat-Lat H, Hassan L, Sani RA, Sheikh-Omar AR, Hishamfariz M, Ng V. First report of bovine lungworm disease in South-East Asia. *Trop Biomed* 2007; 24(1): 77-81.

Lurier T, Delignette-Muller ML, Rannou B, Strube C, Arcangioli MA, Bourgoin G. Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach. *Prev Vet Med* 2018; 154(1): 124-131. http://dx.doi.org/10.1016/j.prevetmed.2018.03.017

Macedo LO, Lima TARF, Verocai GG, Alves LC, Carvalho GA, Ramos RAN. Lungworms in ruminants from Brazil: A retrospective epidemiological study over four decades. *Vet Parasitol Reg Stud Reports* 2021; 26(1): 100645. http://dx.doi.org/10.1016/j.vprsr.2021.100645

May K, Brügemann K, König S, Strube C. The effect of patent *Dictyocaulus viviparus* (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs. *Parasit Vectors* 2018; 11(1): 1-11. http://dx.doi.org/10.1186/s13071-017-2602-x

Medeiros RM, Holanda RM, França MV, Saboya LMF, Rolim Neto FC, Araújo WR, et al. Climate classification of Köppen in the area of Mata Pernambucana – Brazil. Rev. *Cient Multidisc* 2021; 2(1): 1-22. http://dx.doi.org/10.51359/2238-6211.2018.229388

Molento MB, Depner RA, Mello MH. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing-season calves. *Vet Parasitol* 2006; 141(3-4): 373-376. http://dx.doi.org/10.1016/j.vetpar.2006.01.061

Panuska C. 2006. Lungworms of ruminants. *Vet Clin North Am Food Anim Pract* 2006; 22(3): 583-593. http://dx.doi.org/10.1016/j.cvfa.2006.06.002

Pyziel AM, Dolka I, Werszko J, Laskowski Z, Steiner-Bogdaszewska Ż, Wiśniewski J, et al. Pathological lesions in the lungs of red deer Cervus elaphus (L.) induced by a newly-described *Dictyocaulus cervi* (Nematoda:

Trichostrongyloidea). *Vet Parasitol* 2018; 261(1): 22-26. http://dx.doi.org/10.1016/j.vetpar.2018.08.003

Ploeger HW, Verbeek PC, Dekkers CW, Strube C, Van Engelen E, Uiterwijk M, et al. The value of a bulk-tank milk ELISA and individual serological and faecal examination for diagnosing (sub)clinical *Dictyocaulus viviparus* infection in dairy cows. *Vet Parasitol* 2012; 184(2-4): 168-79. http://dx.doi.org/10.1016/j.vetpar.2011.08.032

Ploeger HW. *Dictyocaulus viviparus*: re-emerging or never been away? *Trends Parasitol* 2002; 18(8): 329-332. http://dx.doi.org/10.1016/s1471-4922(02)02317-6

Pyziel AM, Dolka I, Werszko J, Laskowski Z, Steiner-Bogdaszewska Ż, Wiśniewski J, et al. Pathological lesions in the lungs of red deer *Cervus elaphus* (L.) induced by a newly-described *Dictyocaulus cervi* (Nematoda: Trichostrongyloidea). *Vet Parasitol* 2018; 261(1): 22-26. http://dx.doi.org/10.1016/j.vetpar.2018.08.003

Schade J, Albernaz RM, Reis ACF, Fonteque JH. Dictyocaulosis in cattle in Londrina, PR, Brazil. *Vet Zootec* 2020. 27 (1): 1-6. http://dx.doi.org/10.35172/rvz.2020.v27.469

Schunn AM, Conraths FJ, Staubach C, Fröhlich A, Forbes A, Schnieder T, et al. Lungworm Infections in German dairy cattle herds-seroprevalence and GIS-supported risk factor analysis. *PLoS One* 2013 8(9): e74429. http://dx.doi.org/10.1371/journal.pone.0074429

Silva MC, Barros RR, Graça DL. Outbreak of dictyocaulosis in cattle in Santa Maria, RS, Brazil. *Rur Scie* 2005. 35(1): 629-632. http://dx.doi.org/10.1590/S0103-84782005000300022

Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals (Veterinary Helminthology & Entomology). 6ª ed., Baltimore, The Williams and Wilkins Company, 824p. 1968.

Van Dijk J. The epidemiology and control of dictyocaulosis in cattle. *Cattle Pract* 2004; 12(1): 133-145.

Vanhecke M, Charlier J, Strube C, Claerebout E. Association between *Dictyocaulus viviparus* bulk tank milk antibody levels and farmer-reported lungworm outbreaks. *Vet Parasitol* 2020; 288(1): 109280. http://dx.doi.org/10.1016/j.vetpar.2020.109280

Verocai GG, Hoberg EP, Simard M, Beckmen KB, Musiani M, Wasser S, et al. The biogeography of the caribou lungworm, *Varestrongylus eleguneniensis* (Nematoda: Protostrongylidae) across northern North America. *Int J Parasitol Parasites Wildl* 2020; 11(1): 93-102. http://dx.doi.org/10.1016/j.ijppaw.2020.01.001

Wapennar W, Barkema HW, Eysker M, O'Handley RM. An outbreak of dictyocaulosis in lactating cows on a dairy farm. *J Am Vet Med Assoc* 2007; 231(11): 1715-1718. http://dx.doi.org/10.2460/javma.231.11.1715

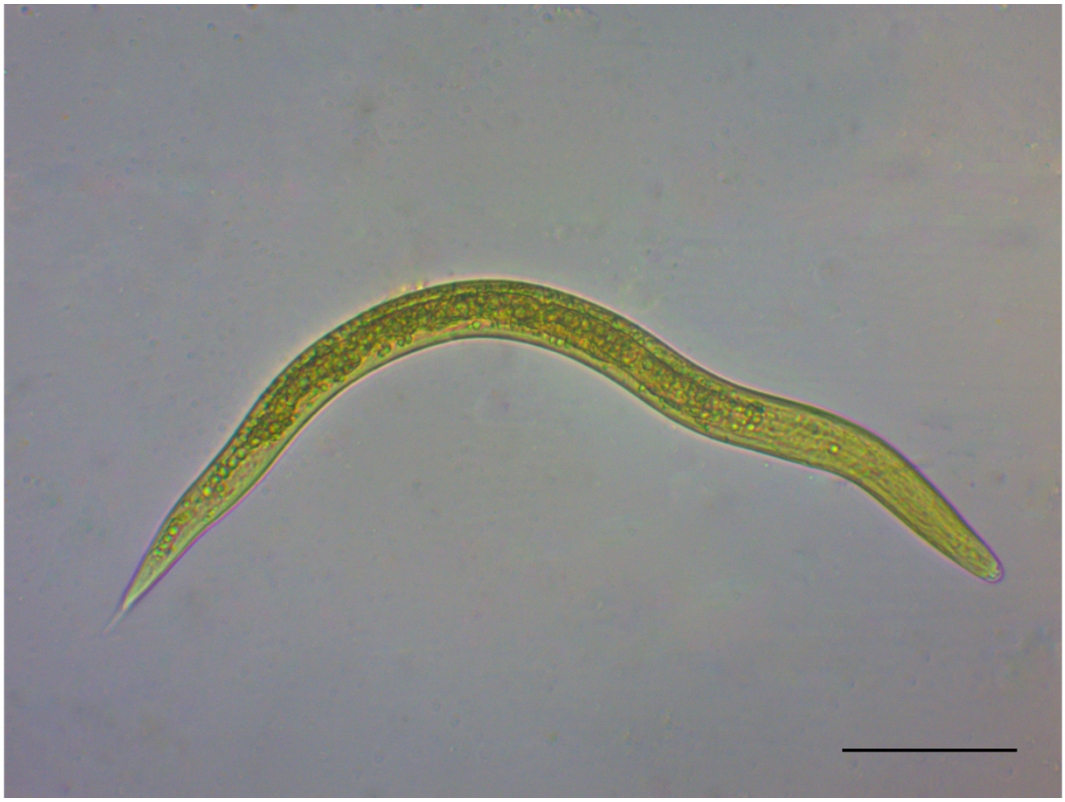


Figure 1. First-stage larva (L1) of *Dictyocaulus* sp., detected in feces from cattle northeastern Brazil (scale-bar = 50 µm).

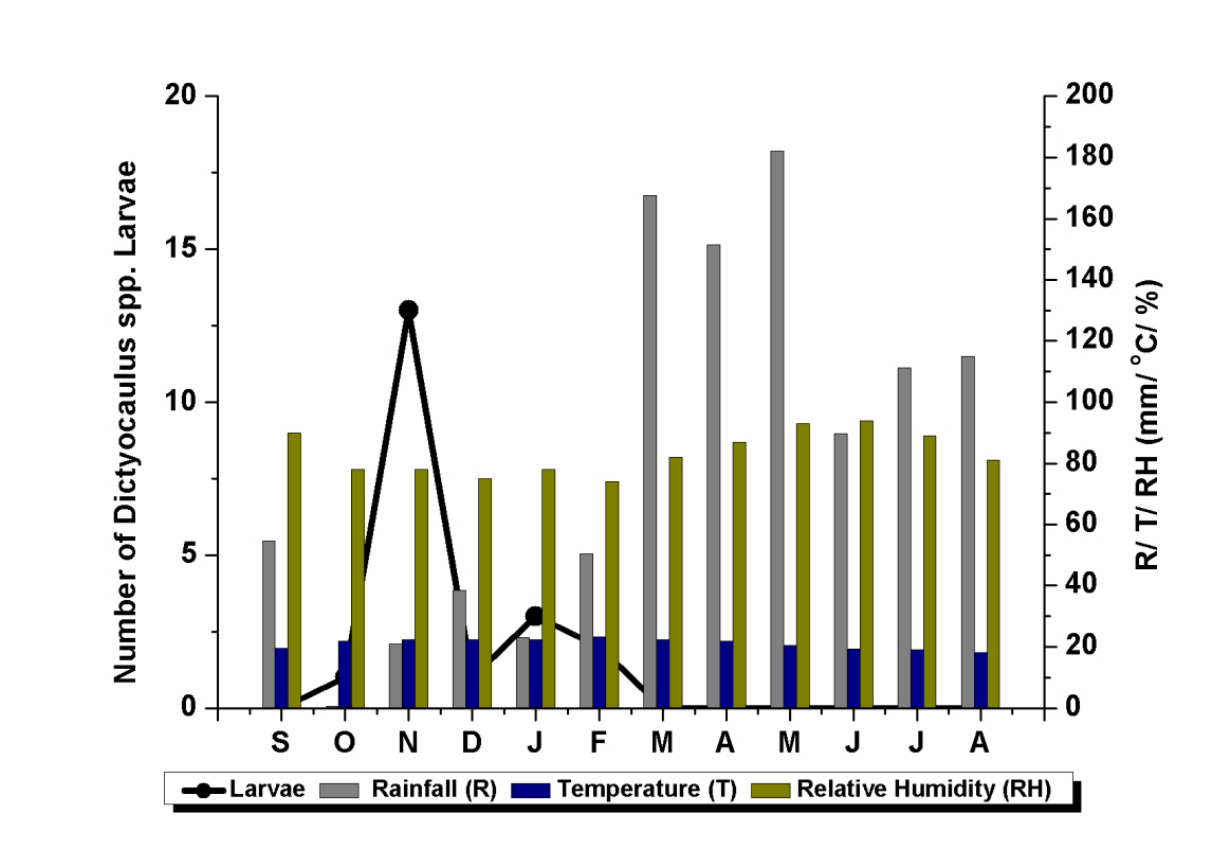


Figure 2. Overall distribution of larvae during the study period according to the climatic data.

6. CONCLUSÕES GERAIS

Os dados aqui apresentados demonstraram que a infecção por nematódeos broncopulmonares tem sido relatada nas últimas quatro décadas no Brasil. Com a maior parte de relatos nas regiões Sul e Sudeste do país, cujas temperaturas amenas provavelmente contribuem para ocorrência. Assim como, foi demostrado a infecção por *D. viviparus*, em bovinos e *P. rufescens* em caprinos, são presentes nestes rebanhos na região nordeste do Brasil. Fornecendo relevantes dados epidemiológicos e morfológicos sobre estes parasitos. No entanto, mais estudos são necessários para elucidar dados moleculares e aspectos biológicos, como os hospedeiros intermediários envolvidos em seu ciclo de vida.

Considerando a ocorrência destes parasitos, médicos veterinários locais devem estar atentos à inclusão deste parasito no diagnóstico diferencial de outras infecções respiratórias, assim como medidas sanitárias são preconizadas para prevenir a infecção por esses nematódeos e reduzir o impacto econômico que eles podem causar na produção pecuária.