Gastrointestinal Parasites in Sheep from the Brazilian Pampa Biome: Prevalence and Associated Factors

Parasitos Gastrintestinais em Ovinos do Bioma Pampa Brasileiro: Prevalência e Fatores Associados

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Abstract

Parasitic infections are common in sheep farming worldwide. A cross-sectional study was carried out to determine the prevalence and associated risk factors among gastrointestinal parasites and sheep herds from the Brazilian Pampa biome, Rio Grande do Sul state, Brazil. Twenty-one farms were visited, where rectal faecal samples were individually collected from 309 animals. The sheep industry on the studied farms was characterised by small farms with a low level of technification, an extensive grazing system and meat production. Of all samples analysed, strongyle nematodes had the highest prevalence (77.02%), followed by *Eimeria* spp. (70.55%), *Moniezia expansa* (20.39%) and *Strongyloides papillosus* (17.48%). Mixed infection, between helminths and protozoans, was detected in 68.61% of samples. All herds were positive for strongyle and *Eimeria* spp. A predominance of *Haemonchus* spp. and *Trichostrongylus* spp. nematodes was observed in the herds. Younger animals were significantly more affected by *Eimeria* and *M. expansa*. In the semi-intensive and intensive systems, a higher frequency of *Eimeria* and strongyle infections was observed. Parasite infection was significantly reduced at low animal densities. The Brazilian Pampa region presents a high prevalence of gastrointestinal parasites among sheep; age, breeding system and stocking density were factors associated with parasite infection.

Keywords: sheep industry, strongyle, Eimeria, Haemonchus, Trichostrongylus.

Resumo

As infecções parasitárias são comuns na criação de ovinos em todo o mundo. Um estudo transversal foi realizado para determinar a prevalência e fatores de risco associados entre parasitoses gastrintestinais e rebanhos ovinos do bioma Pampa brasileiro, estado do Rio Grande do Sul, Brasil. Vinte e uma fazendas foram visitadas, onde foram coletadas amostras de fezes individuais da ampola retal de 309 animais. A produção ovina era caracterizada por pequenas propriedades com baixo nível de tecnificação, os animais eram criados em sistema extensivo e para produção de carne. De todas as amostras analisadas, os estrongilídeos (Nematoda: Strongylida) foram mais prevalentes (77,02%), seguido por *Eimeria* spp. (70,55%), *Moniezia expansa* (20,39%) e *Strongyloides papillosus* (17,48%). Infecção mista, entre helmintos e protozoários, foi detectada em 68,61% das amostras. Todos os rebanhos foram positivos para estrongilídeos e *Eimeria* spp. Foi observada predominância dos nematódeos *Haemonchus* spp. e *Trichostrongylus* nos rebanhos. Animais mais jovens foram significativamente mais afetados por *Eimeria* e *M. expansa*. Nos sistemas semi-intensivo e intensivo, observou-se maior frequência de infecções por *Eimeria* e estrongilídeos. A infecção parasitária foi significativamente menor em fazendas com baixa densidade animal. A região do Pampa brasileiro apresenta alta prevalência de parasitos gastrintestinais em ovinos; idade, sistema reprodutivo e densidade animal foram fatores associados à infecção parasitária.

Palavras-chave: ovinocultura, estrongilídeos, Eimeria, Haemonchus, Trichostrongylus.



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Introduction

Brazil is considered a megadiverse country possessing six terrestrial biomes: Amazonia, Atlantic Forest, Caatinga, Cerrado, Pantanal and Pampa (Roesch et al., 2009). The Brazilian Pampa covers the southern half of the Rio Grande do Sul state and constitutes the Brazilian portion of the South American Pampas that extends through the territories of Uruguay and Argentina, occupying 68.8% of the state's area (Instituto Brasileiro de Geografia e Estatística, 2019a). The Pampa biome is formed by large areas dominated by herbaceous vegetation (natural and/or managed pastures). These natural grasslands serve as a source of forage for cattle and sheep farming; livestock production has always been one of the most relevant economic activities of the Rio Grande do Sul state (Instituto Brasileiro de Geografia e Estatística, 2019a; Roesch et al., 2009).

However, in recent decades sheep farming in Rio Grande do Sul state has declined (Instituto Brasileiro de Geografia e Estatística, 2006, 2019b). Among the main obstacles for sheep farming to become commercially viable, parasitic problems stand out. They generally do not occur with the presence of a single parasitic genus, but in a mixed form. In southern Rio Grande do Sul state, gastrointestinal parasitosis was the most important cause of sheep morbidity and mortality, with estimated economic losses of R\$2,016,000/year (Oliveira et al., 2017), something around U\$36,400,000/year. Gastrointestinal parasites cause some of the most common infections in sheep industry. They can cause subclinical weight loss to lethal pathologies, with anaemia, diarrhoea and severe protein loss. Disease complexes involving parasitic infections should not be seen as a simple enumeration of parasite species pathogenic to animals, listing their location and host, but approached dynamically, considering the factors that predispose their establishment and clinical manifestations (Minho, 2014).

In Brazil, a range of gastrointestinal parasites (Nematoda, Cestoda and Protozoa) have already been identified in usually mixed-parasitic infections in sheep (Ferraz et al., 2019; Osório et al., 2021; Salgado et al., 2017). However, there are few studies that help to elucidate this distribution and potential risks associated with gastrointestinal parasites in sheep from the Brazilian Pampa biome. Regional epidemiological studies describing parasitosis dynamics, including the parasite, host and environment, are critical to understanding the factors that influence disease severity within a population in a defined environment (Fiel & Steffan, 1994). Therefore, the aim of this study was to report epidemiological data of gastrointestinal parasite infections in sheep from southern Rio Grande do Sul, Brazil, evaluating the prevalence of associated risk factors.

Material and methods

Study area

A cross-sectional study was carried out on 21 farms from eleven municipalities located in the Pampa biome, Rio Grande do Sul state, Brazil (Figure 1). Occupying the southernmost part of the country, this region presents great differences compared to other areas of Brazil. The climate in this state is predominantly humid subtropical (Alvares et al., 2013). The temperatures present wide seasonal variation, with hot summers and harsh winters, with the occurrence of frost and occasional snow precipitation. Average temperatures range between 15 and 18 °C, with minima from -10 °C to maxima of 40 °C. Concerning precipitation, the region presents a relatively balanced distribution of rainfall throughout the year, with an average precipitation range from 1,299 to 1,500 mm (Rio Grande do Sul, 2021).

Sample collection and coprological analysis

The minimum sample size (n = 264) was estimated using EpiInfoTM epidemiological calculators, based on the sheep population (n = 499,898) of the study area (Instituto Brasileiro de Geografia e Estatística, 2019b). A confidence level of 95%, expected frequency of 80% and statistical error of 5% were considered. From March 2018 to September 2019, 309 faecal samples were collected rectally and analysed for the detection of parasite eggs and/or oocysts. On each farm, at least 10% of the total sheep were included in the study. Each sample was first examined macroscopically for the possible detection of cestode fragments and proglottids. All individual samples were analysed using the modified Gordon and Whitlock technique (Ueno & Gonçalves, 1998). This technique is widely used in laboratory routines, allowing the identification and quantification

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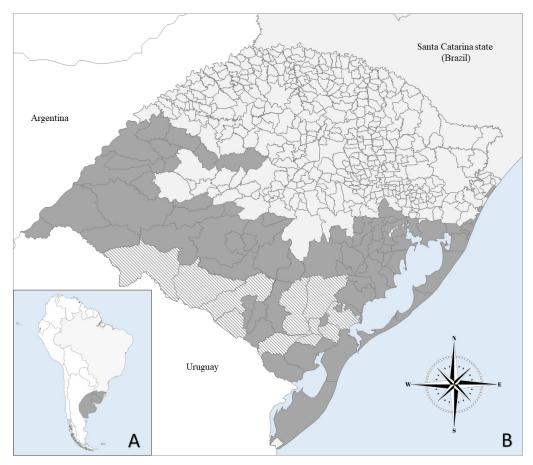


Figure 1. (A) Location of the Pampa biome in South America; (B) Highlighted, Pampa biome in the state of Rio Grande do Sul, Brazil. In stripes, municipalities where sheep stool samples were collected for the diagnosis of gastrointestinal parasite infection in 2018 and 2019.

of parasite eggs and oocysts. Furthermore, this technique allows estimation of the number of eggs and oocysts per gram (E/OPG) of faeces shed by infected sheep. EPG positive samples were selected for coproculture, using farm pooled samples, in accordance with Roberts and O'Sullivan (Ueno & Gonçalves, 1998). Infectious larvae (L_3) were identified using the Ueno and Gonçalves (1998) identification key.

Intensity of coccidia and nematode infection was semi-quantitatively scored via a three-score scaling system, adapted from Idris et al. (2012) and Ueno and Gonçalves (1998), respectively. For *Eimeria* (Protozoa), the evaluated samples were graded as low (< 1,800 OPG), moderate (1,800 to 6,000 OPG) and high (> 6,000 OPG), and for Nematoda infections as low (< 1,000 EPG), moderate (1,000 to 2,000 EPG) and high (> 2,000 EPG). For Cestoda infections, sheep were differentiated as non-infected and infected.

Questionnaire interview and statistical analysis

A structured questionnaire was administered via face-to-face interviews with the farmers, to assess their knowledge of sheep health, husbandry and farm management practices. This information was used to search for risk factors associated with gastrointestinal parasite infections. Visual observations were also made to confirm the questionnaire responses and assess farming practices. Risk factors were estimated based on previous studies in livestock (Sorathiya et al., 2017; Squire et al., 2019).

Descriptive analyses were performed using EpiTools epidemiological calculators (Sergeant, 2018), and univariate and multivariate analyses with IBM^{*} SPSS^{*} Statistics, version 20.0. A univariate analysis was performed using Pearson's chi squared test (*x*²) or Fischer's exact test

(when less than five observations were observed in the test quadrant) to identify the individual and management characteristics associated with parasite presence (du Prel et al., 2010). *P*-values less than 0.05 were considered statistically significant. Variables that showed an association with $p \le 0.2$ by the x^2 test or Fischer's exact test were selected for multiple model construction. Finally, Generalised Estimating Equations (GEE), a multiple logistic regression model, was used to verify the association between the factors.

Results

In this study, most farms were of small size with a low level of technification (61.90%). Extensive grazing systems were used on 57.14% of farms, using native pastures, without separation by age or sex. In the semi-intensive farming system, used by 38.10% of farms, animals were housed in a simple structure and released to graze during the day and/or received some form of feed supplementation. The intensive system was used by 4.76% of farms, on which the animals were finished in confinement (housed without grazing).

The herds were mainly composed of mixed-breed (30.09%) or Corriedale breed (29.77%), and mostly raised for slaughter (71.43%). Importantly, in this study, only one farm raised animals exclusively for wool. Furthermore, on most farms other animals shared the same area as sheep, such as cattle (80.95%), horses (42.86%), goats (9.52%) and swine (4.76%). Diarrhoea was not observed in the analysed sheep although, on some farms, poor body condition, apathy and faecal staining around the hindquarters was noted.

Of all samples analysed, strongyle nematodes had the highest prevalence, followed by *Eimeria* spp., *Moniezia expansa*, and *Strongyloides papillosus*. Mixed infection, between helminths and protozoans, was detected in 68.61% of the samples. All farms (100%) had at least one animal shedding strongyle eggs and/or *Eimeria* oocysts (Table 1). Animals younger than 18 months had a higher prevalence, burden of infection and O/EPG mean for all parasites found (Table 2 and Table 3). Larval cultures indicated the presence of *Haemonchus* spp. (90.91%), *Trichostrongylus* spp. (63.64%), *Oesophagostomum* spp. (18.18%) and *Cooperia* spp. (9.1%).

Regarding the factors associated with gastrointestinal parasitosis, age and some management practices were statistically relevant (p < 0.05) (Table 3). Young animals were statistically more prone to *Eimeria* (p < 0.0001 OR = 8.73) and *M. expansa* (p < 0.0001 OR = 5.75) infections. In semi-intensive and intensive production systems, there was a higher prevalence of *Eimeria* (p < 0.0001 OR = 8.86) and strongyle infections (p < 0.0001 OR = 17.88), compared to the extensive system. In farms with low animal density, a lower frequency of strongyle (p = 0.0081 OR = 0.46), *Eimeria* (p < 0.0001 OR = 0.29), *S. papillosus* (p = 0.0041 OR = 0.2) and *M. expansa* (p = 0.02 OR=0.35) infections was observed (Table 3).

Discussion

Sheep farming is a traditional activity in the Brazilian Pampa, Rio Grande do Sul state. However, this activity has been affected by alternating periods of progress and crisis that changed strategies and dynamics in the sheep industry (Viana & Waquil, 2013). The farmers in this study reported some factors that made them reduce or practically abandon the activity, such as animal theft, predation by wild and domestic canids, expansion of agriculture, sheep susceptibility to infections

Table 1. Prevalence of gastrointestinal parasite infections in sheep from Rio Grande do Sul state, Brazil.

Parasite	Prevalence in animals (%)	CI (95%)	Mean (O/EPG)	Prevalence on farms (%)	
Strongyle	77.02	72.02-81.36	1349.84	100	
<i>Eimeria</i> spp.	70.55	65.24-75.36 3126.21		100	
Moniezia expansa	20.39	16.27-25.23	336.57	52.38	
Strongyloides papillosus	17.48	13.65-22.10	58.58	47.62	

O/EPG = Oocysts/eggs per gram of faeces; CI = Confidence interval

Table 2. Burden of infection, mean and range of O/EPG in sheep from Rio Grande do Sul state, Brazil.

Parasite	Age –	B	urden of infectio	Mean	Range of	
		Low	Moderate	High	(O/EPG)	O/EPG concentrations
<i>Eimeria</i> spp.	Young	61.40%	23.39%	15.20%	5492,40	100 - 120,000
	Adult	97.83%	1.45%	0.72%	3126.21	100 - 7,600
Strongyle-type	Young	53.22%	22.81%	23.98%	1411,11	100 - 19,700
	Adult	62.32%	16.67%	21.01%	1349.84	100 - 15,700

O/EPG = Oocysts/eggs per gram of faeces.

 Table 3.
 Prevalence of gastrointestinal parasite infection in sheep considering age, farm size, animal density and farming system from Rio Grande do Sul state, Brazil.

Variable	Categories	N .	Strongyle		Eimeria spp.		Moniezia expansa		Strongyloides papillosus	
			Pos ¹	%	Pos ¹	%	Pos ¹	%	Pos ¹	%
Age	Young (<18 months)	171	136	79.53%ª	152	88.89%ª	53	30.99%ª	33	19.30%ª
	Adult	138	102	73.91%ª	66	47.83% ^b	10	7.25% ^b	21	15.22%ª
Farm size	Small	143	116	81.12%ª	106	74.13%ª	36	25.17%ª	30	20.98%ª
	Medium	68	51	75.00%ª	52	76.47%ª	15	22.06%ª	16	23.53%ª
	Large	98	69	70.41%ª	60	61.22% ^b	12	12.24% ^b	8	8.16% ^b
Animal density	Low	63	37	58.73% ^a	36	57.14%ª	6	9.52%ª	5	7.94%ª
	Moderate	37	27	72.97% ^{ab}	28	75.68% ^b	9	24.32% ^b	11	29.73% ^b
	High	209	174	83.25% ^b	154	73.68% ^b	48	22.97% ^b	38	18.18% ^b
Farming system	Intensive	27	23	85.19%ª	22	81.48%ª	1	3.70%ª	2	7.41% ^a
	Semi-intensive	56	44	78.57% ^b	45	80.36%ª	8	14.29% ^{ab}	19	33.93% ^b
	Extensive	226	171	75.66% ^b	151	66.81% ^b	54	23.89% ^b	33	14.60% ^{ab}

abDifferent lowercase letters on the same column and variable indicate a statistically significant difference (Chi-square p<0.05); Positive animals

and the devaluation of wool. Gradually, gaucho farmers are replacing traditional wool production with sheep meat as the main product in the supply chain (Viana & Waquil, 2013). This fact was observed in our study, in which almost all properties raised sheep for slaughter, in association with cattle breeding and crops (soybean, rice and corn).

Sheep susceptibility to parasitic diseases is also a productive obstacle (Sczesny-Moraes et al., 2010). Gastrointestinal parasitosis interfere with sheep growth and development. In addition, weight loss, diarrhoea and oedema due to hypoproteinaemia, reduced appetite, growth retardation and compromised bone development (Asín et al., 2021; Ruas & Berne, 2007) may also occur. In persistent infections, permanent morphological changes in the digestive system are generated, mainly due to tissue hypertrophy and hyperplasia, negatively interfering with digestive activity, which leads to a significant decrease in animal performance at slaughter (Steffan et al., 2012). It is worth mentioning that gastrointestinal parasitosis can be caused by several helminths or protozoa, sometimes in combination.

Sheep can be parasitised simultaneously by various nematode species (Ruas & Berne, 2007). The relative importance of different parasite species varies according to the infection intensity, prevalence, pathogenicity and ability to develop anthelmintic resistance. In a retrospective study conducted in Rio Grande do Sul southern region, Ferraz et al. (2019) also found a higher prevalence of strongyle eggs in sheep samples; however, they could not show which nematode

genera were present. For this purpose, coproculture was performed to identify infective strongyle larvae. *Haemonchus* spp. and *Trichostrongylus* spp. were the most common nematodes found in this study, agreeing with the findings of Santiago et al. (1976), Pinheiro et al. (1987) and Echevarria et al. (1996) in Rio Grande do Sul state. For decades, there appears to have been no significant change in the sheep helminth population in the region, even with possible changes in management, use of different anthelmintics or climate change. Even so, these parasites cause important parasitic diseases, being the main cause of sheep mortality (Oliveira et al., 2017). Haemonchosis is characterised by severe anaemia and can cause sudden death, without previous signs of gastrointestinal parasitism (diarrhoea, reduced body condition, oedema, etc.). In contrast, *Trichostrongylus* infection presents chronic diarrhoea and lower mortality, but with proven effects on weight loss and wool quality (Steffan et al., 2012). With this understanding, the clinical signs observed in the studied sheep, such as poor body condition, apathy and faecal staining around the hindquarters, could be related to helminthiasis.

Heavy parasitic infections can result in mortality; however, the reduction in herd productivity is the main consequence of verminosis (Amarante, 2014). In addition, indiscriminate and misguided use of anthelmintics is frequent, uneconomical and can lead to drug resistance (Vieira et al., 2014). Therefore, the development of ecological and integrated parasite management is needed to minimise the parasite population below the disease threshold level. For this, it is necessary to study all risk factors associated with parasite prevalence in a determinate geographic region (Sorathiya et al., 2017). The amount of parasite eggs and oocysts shed in the hosts' faeces is inversely proportional to the degree of resistance to parasitosis. Therefore, immune response efficiency plays a central role in the epidemiology of parasitic diseases. Several factors can influence the efficiency of immunity, such as age, management, nutrition, breed and individual susceptibility (Amarante, 2014). Considering the environmental, individual and management aspects that may interfere with immunity, risk factors related to the occurrence of gastrointestinal parasites in the studied region were hypothesised. After analysis, the results indicated that age and some management conditions presented a relevant association with a significant influence on sheep parasitosis.

Parasitic diseases can affect any animal in the herd; however, they are more severe in young animals, especially lambs. In this study, sheep younger than 18 months tended to be more susceptible to strongyle infection compared to adults; although, these differences were not significant (*p* > 0.05), similar to the studies of Dagnachew et al. (2011) and Sorathiya et al. (2017). These authors related the lack of statistical findings to the lower number of young animals compared to adults. However, this was not the case in our study. Genetic susceptibility may have influenced the parasite load found, with no statistical difference between age categories. Normally, strongyles are unevenly distributed in the herd; most sheep have low infections, while a small portion of the population suffers from severe parasitic infection due to individual genetic susceptibility (Maia et al., 2013). This differed to what was found in our study, where the strongyle EPG mean, prevalence and high burden of infection was similar between adult and young animals. This fact may be related to the presence of adult sheep naturally susceptible to verminosis in the studied herds.

Regarding age categories and other parasitic infections, a statistical difference (p < 0.05) was found between young sheep and infections by Eimeria and M. expansa. Coccidiosis is one of the most important parasitic diseases of small ruminants worldwide. It is caused by protozoan parasites of the genus Eimeria, causing decreases in productivity, low growth performance, diarrhoea and mortality. In this study, young sheep were more heavily parasitised by *Eimeria* spp. compared to adults. Eimeria sporulated oocysts are highly dispersed throughout the environment and are ingested from an early age by lambs with food and water. In healthy animals, kept under adequate management conditions, a low intake of oocysts induces the development of a protective immune response, which limits, but does not extinguish, the infection (Amarante, 2014). This makes it common to observe minimal amounts of oocysts shed by adult animals, which usually do not show clinical signs, but can serve as a source of infection for susceptible animals (Martins et al., 2022). This is compatible with the results of the OPG mean, parasite distribution and burden of infection between young and adult sheep. In turn, adult tapeworms (*M. expansa*) are common intestinal parasites of sheep and are often diagnosed by the presence of segments in the faeces. Infections are generally harmless, although occasionally clinical signs may be observed, including growth reduction, diarrhoea, intestinal torsion, respiratory signs and even convulsions (Abbott et al., 2012; Kelly et al., 2021; Liu et al., 2019). Young sheep were more commonly infected and the parasite appeared in different herds (52.38%). This suggests that reservoirs of the parasite (infected soil mites) are present on most farms and may develop into health problems if there are favourable conditions for the parasite and the infection goes unnoticed.

The production system can be a risk factor for gastrointestinal infections (Lopes et al., 2013; Martins et al., 2020; Salgado et al., 2017). Pasture-based sheep production is more challenging to young animals due to their high susceptibility to parasite infections (Greer et al., 2009), and justifies the use of the confinement system (intensive), because in some Brazilian regions, the edaphoclimatic conditions favour the maintenance of a high helminth population in the pastures (Zanette & Neumann, 2012). The confinement of newly weaned lambs with food rich in energy and protein, in a suitable, dry, protected, salubrious environment is the best alternative for finishing and producing quality lamb meat (Veríssimo et al., 2002). In addition, rearing lambs in a feedlot can contribute to parasite control (Salgado et al., 2017). However, in this survey, animals raised on intensive systems were kept in small and poorly maintained barns. In this breeding system, a higher frequency of *Eimeria* and strongyle infections was observed (Table 3). Furthermore, the farms where animals were finished in confinement had a low level of technification, no history of anthelmintic treatment prior to confinement, and the sheep lived in precarious hygienic and sanitary conditions. This may have contributed to the higher occurrence of strongyles in the intensive system. Furthermore, management directly influences the characteristics of *Eimeria* infections. Previous studies demonstrated that intensive and semi-intensive farming systems could facilitate the dissemination of *Eimeria* species (Lopes et al., 2013; Martins et al., 2020; Tomczuk et al., 2015). In intensive production systems, a high population density facilitates the transmission of the protozoan, with a large number of oocysts available in the environment (Lima, 2004).

In the semi-intensive system, sheep received shelter at night and/or food supplementation in winter. This is a common practice in Rio Grande do Sul, due to the low temperatures and decreasing photoperiod, severely limiting forage production, which increases the risk of infection by grazing in areas with the highest density of infective larvae and oocysts. In this production system, there was a higher prevalence of strongyle, *Eimeria* and *S. papillosus* infections compared to extensive farming. The higher stock density and the proximity to the parasites' infective stages may explain these findings. Even with strategic nutritional supplementation, the low height of the pasture facilitates the ingestion of a large number of parasite infective stages, and consequently, infections and clinical cases of parasitosis (Steffan et al., 2012).

Stocking density revealed that smaller areas, with a higher number of grazing animals, had a higher prevalence of gastrointestinal parasites, as sheep are more prone to reinfection, similar to that observed by Vieira et al. (2014). In fact, low animal density was a protective factor (OR < 1) for strongyle, *Eimeria, S. papillosus* and *M. expansa* occurrence. Parasite infection can significantly reduce when sheep are reared at low densities (Vieira et al., 2014). As the grazing system becomes more extensive with a lower stocking density, animals can select the grazing area (away from faeces contaminated sites), decreasing the risk of disease (Steffan et al., 2012). Duarte et al. (2012) observed that properties with a small pasture area, low stocking rate and consequently, low population density, presented less risk of contamination by parasites, similar to our findings.

Despite having limitations, coprological examinations can be used to help decide if anthelminitic treatment is necessary, can be safely delayed or omitted. On some farms, coprological monitoring may allow anthelminitics to be used more efficiently, managing the risk of disease outbreaks or lost productivity (Abbott et al., 2012). In the Brazilian Pampa region, most sheep farmers are of low socioeconomic status, with no technical training, and are unable to invest in facilities and new technologies. Expenditure on anthelminitic treatment and parasite-related mortality are threatening small ruminant production in some regions and endangering other areas of greater animal production (Oliveira et al., 2017). Research on the epidemiology of sheep gastrointestinal parasitosis, investment in education and training programmes for infection prevention, with farmers and veterinarians, is crucial to establish adequate forms of strategic control, improving productivity and economic development of the sheep industry in Rio Grande do Sul.

Conclusion

The Brazilian Pampa region presents a high prevalence and wide distribution of gastrointestinal parasites in sheep. Therefore, during the control and treatment of parasitic diseases in these hosts, age, housing system and animal density should be considered as potential risk factors for disease occurrence. Additional attention should be given to young animals, reared at high stocking densities, and in semi-intensive and intensive production systems.

Ethics statement

All applicable guidelines for the care and use of animals were followed by the authors (Kilkenny et al., 2010). Informed consent was obtained from all participants interviewed in the study. The procedures performed were approved by the Ethics Commission on Animal Experimentation of the Universidade Federal de Pelotas (UFPel) under protocol number 15450/2018.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Authors' contributions

All authors made substantial contributions to this study. N.S.M., C.C.S., S.P.M., and A.S.M. collected and processed the samples. Data analysis and interpretation was performed by N.S.M. and J.L.R. The first draft of the manuscript was written by N.S.M. and all authors commented on previous versions of the manuscript. The final version of the manuscript was read, critically reviewed, and approved by all authors.

Availability of complementary results

Coprological analysis were carried out at Laboratório de Parasitologia (XIII), Departamento de Microbiologia e Parasitologia, Instituto Biologia, Universidade Federal de Pelotas.

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