



Short communication

Occurrence of *Sarcocystis gigantea* macrocysts and high frequency of *S. tenella* microcysts in sheep from southern BrazilCamila E. Minuzzi^{a,*}, Alfredo S. Cezar^b, Patricia Bräunig^a, Luiza P. Portella^a, Fernando de S. Rodrigues^a, Luis Antonio Sangioni^a, Fernanda S.F. Vogel^a^a Laboratório de Doenças Parasitárias (Ladopar), Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, prédio 44, sala 5139, 97105-900 Santa Maria, RS, Brazil^b Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil

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ABSTRACT

The aim of this study was to investigate the occurrence of sarcocysts in sheep slaughtered in the Rio Grande do Sul state, Brazil. Heart and esophagus samples from 130 sheep were subjected to macroscopic and microscopic examination, followed by molecular analysis. Ten sheep (7.7%) had *Sarcocystis gigantea* macrocysts in esophagus, as identified by gene sequencing. Microcysts were present in 96.1% of the sheep, with a higher frequency ($p < .05$) in the heart (91.5%) compared to the esophagus (81.5%) samples. The microcysts were identified as *Sarcocystis tenella* by gene sequencing. Our results revealed a high frequency of *Sarcocystis* spp. infection in sheep from southern Brazil. To the authors knowledge, this is the first molecular confirmation of *S. gigantea* presence in Brazil.

1. Introduction

Sheep can act as intermediate host (IH) for at least four *Sarcocystis* species: *Sarcocystis tenella* and *Sarcocystis arieticanis* which form microscopic muscle cysts (microcysts) and have canids as definitive hosts (DH); and *Sarcocystis gigantea* and *Sarcocystis medusiformis* which form macroscopic muscle cysts (macrocysts) and have felids as DH. Microcysts are formed mainly in the heart, esophagus, tongue, and diaphragm muscles; but they can also be found in several skeletal muscles and in the central nervous system. In turn, macrocysts generally appear in esophagus and diaphragm muscles of sheep (Oryan et al., 1996; Dubey & Lindsay, 2006; Gual et al., 2017).

Macrocyst-forming *Sarcocystis* have been commonly found in sheep from some countries of Asia and Europe (Oryan et al., 1996; Pipia et al., 2016) and they were also reported in sheep from Oceania (McKenna, 2009). However, reports on these macrocysts are not common in Africa (Dahmani et al., 2017) or the Americas (Gual et al., 2017).

Sarcocystosis usually presents a subclinical course in small ruminants. However, microcysts-forming *Sarcocystis* can cause anorexia, fever, decreased weight gain, neurological disease, and abortion in sheep (Dubey et al., 2015a). Macrocysts are generally non-pathogenic to sheep, but their presence can cause losses to the sheep industry due to organ and carcass condemnation (Martínez-Navalón et al., 2012).

Sheep wool and meat production are very traditional activities in southern Brazil, especially in the state of Rio Grande do Sul (RS) that holds around 3.5 million sheep or 20% of the total Brazilian sheep flock. However, little is known on the potential losses caused by *Sarcocystis* spp. on the sheep industry and no studies including molecular identification of *Sarcocystis* species have been performed in sheep from southern Brazil. Therefore, the aim of this study was to investigate the occurrence of macro- and microscopic *Sarcocystis* species in sheep slaughtered in the Rio Grande do Sul state.

2. Material and methods

The 130 sheep included in this study were slaughtered in two officially inspected abattoirs located in the municipalities of Santa Maria ($n = 101$) and Santiago ($n = 29$), both in the RS, southern Brazil. The animal slaughtering and evisceration procedures were performed in accordance to the Brazilian legal protocols of ethics and animal welfare under supervision of the Official Veterinary Inspection Service technicians. During the inspection of the sheep viscera, a piece of 20 cm of the esophagus (including cervical and thoracic segments) and a piece of 50 g of the heart ventricles apex were collected. Collections were performed once a week during the months of June to September 2016 to avoid concentration of many sheep from the same flock.

* Corresponding author.

E-mail address: camila.minuzzi03@gmail.com (C.E. Minuzzi).<https://doi.org/10.1016/j.vprsr.2018.12.002>

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Esophagus and heart tissue samples were macroscopically examined by the naked eye searching for macrocysts and lesions. When present, all the macrocysts were morphologically examined and one macrocyst was excised from each animal and stored at -80°C for further molecular analysis. For the fresh microscopic examination, 50 g of each tissue sample was individually cut, macerated, mixed with PBS (pH 7.3), filtered through a gauze to a petri dish, and examined under light microscopy at $400\times$ magnification. Each sample was classified as positive or negative for the presence of sarcocysts. The frequencies of microcyst-positive hearts and microcyst-positive esophagus were compared by the Chi-Square test using the SAS software. A confidence level of 95% was considered for statistical significance ($p < .05$).

During microscopic examination, ten microcysts were collected from each tissue sample and stored in microtubes at -80°C for further DNA extraction. Genomic DNA was extracted from each of the excised macrocysts (total of ten) and from each pool of ten microcysts collected from each sample of esophagus and heart using a commercial kit (Wizard Genomic DNA Purification Kit, Promega, USA). DNA samples were subjected to nested-PCR for amplification of a fragment belonging to the 18S rDNA gene of the genus *Sarcocystis* according to Silva et al. (2009). The external primer pair (first round PCR) Tg18s48F (5'-CCA TGCATGTCTAAGTATAAGC-3') and Tg18s359R (5'-GTTACCGTCACT GCCAC-3'); and the internal primer pair (second round PCR) Tg18s58F (5'-CTAAGTATAAGCTTTTATACGGC-3') and Tg18s348R (5'-TGCCACG GTAGTCCAATAC3') were used. Final (second round) PCR products (310 bp) were analyzed after electrophoresis using 2% agarose gel.

DNA product amplified from each macrocyst found and from ten of the pools of microcysts (five from the esophagus and five from the heart samples) were subjected to gene sequencing performed by ACTGene sequencing service (Porto Alegre, Brazil). The sequences obtained were analyzed using the Genbank database.

3. Results and discussion

As shown in the Fig. 1, sarcocysts were detected in 96.1% of the sheep. All of these positive sheep had microcysts (Fig. 2) detected in at least one tissue sample. Ten of these sheep (7.7%), which were all slaughtered in Santa Maria municipality, had macrocysts (Fig. 3) present in the esophagus samples. All the muscle macrocysts were characterized as white oval-shaped nodules of 3 to 5 mm diameter that were

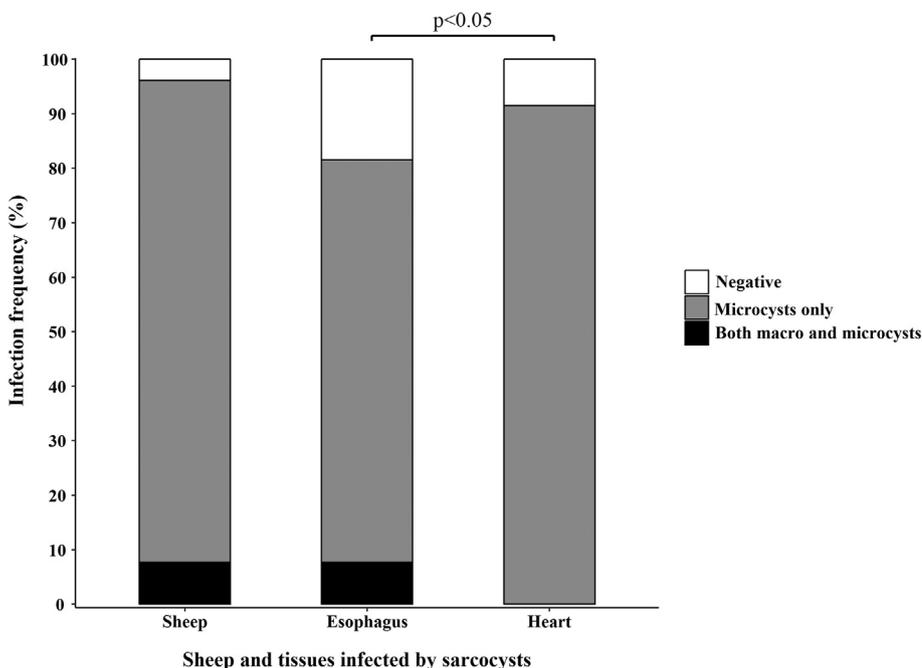


Fig. 2. Microcyst morphologically compatible with *Sarcocystis* spp. observed using light microscopy after isolation from sheep myocardium. 400 x magnification.



Fig. 3. Two white oval-shaped macroscopic sarcocysts present in sheep esophageal muscle layer.

encrusted in the external wall of the esophagus. The number of macrocysts found in each esophagus piece ranged from four to fifteen. Eight of the sheep infected with macrocysts had also microcysts in both esophagus and heart, while two sheep had macro and microcysts in the esophagus, and no cysts in the heart.

The frequency of microcysts was higher ($p < .02$) in the heart (91.5%) in comparison to the esophagus (81.5%) samples. Microcysts were found in both heart and esophagus in 76.9% of the animals. Therefore, 14.6% of the sheep had microcysts detected in the heart, but

Fig. 1. Percentage of sheep infected by sarcocysts (macrocyts/microcyts), and comparative frequency of positive samples detected using different tissues (esophagus and heart) of these sheep. Note: macrocyts were only detected in esophagus samples, but a higher frequency ($p < .02$) of microcyst-positive hearts was observed in comparison to the esophagus samples by the Chi-square test at a 95% confidence level.

not in the esophagus; while six sheep (4.6%) had microcysts exclusively detected in the esophagus. This high infection rate was not surprising since Portella et al. (2016) found microcysts in 76.2% of the myocardium samples taken from sheep slaughtered in southern Brazil. Moreover, microcysts were detected in 95.8% of the sheep studied in Bahia state, northeastern Brazil (Bittencourt et al., 2016). Although very few data on sheep sarcocystosis are available from South America, these studies together indicate a high prevalence of microcysts-forming *Sarcocystis* spp. in Brazilian sheep. Moreover, our results showed that the number of tissues examined can influence the frequency of detection of sheep infected by microcysts in prevalence studies.

Microcyst samples were identified as *S. tenella* by DNA sequence comparison, since the sequences found in the present study (~351 to 354 bp) showed 98–99% identity when aligned to *Sarcocystis tenella* isolates 18S rDNA gene sequences from BLAST/NCBI databases, such as: MF039329.1, KP263759.1, KP263758.1, KP263757.1, KC209737.1, KC209734.1, L24383.1. In addition, alignment to other *Sarcocystis* species resulted in up to: 97% identity to *Sarcocystis capracanis* 18S rDNA gene (sequence L76472.1), which was already described infecting goats in Brazil (Bittencourt et al., 2016); and 95% identity to *Sarcocystis heydorni* 18S rDNA gene (KX057997.1) or *Sarcocystis cruzi* 18S rDNA gene (AF017120.1), which can both infect cattle (Dubey et al., 2015a,b).

All the macrocysts found were identified as *S. gigantea* given their higher identity (98–100%) with *Sarcocystis gigantea* isolates 18S rDNA sequences (KY594259.1, KC209733.1, L24384.1, MG002175.1) available in BLAST/NCBI databases. These sequences also showed up to 96–97% identity with *Sarcocystis moulei* 18S rDNA partial sequences (KC508513.1 and L76473.1), which can infect goats (Dubey et al., 2015a).

The macrocyst-forming species *S. gigantea* and *S. medusififormis* have been commonly described in sheep from Asian and European countries (Oryan et al., 1996; Martínez-Navalón et al., 2012; Pipia et al., 2016). Furthermore, *Sarcocystis* spp. macrocysts were already reported in sheep from Africa (Dahmani et al., 2017) and Oceania (McKenna, 2009). Conversely, reports on macroscopic sarcocysts presence are very uncommon in small ruminants from the Americas.

In North America, Dubey et al. (1988) described the presence of *S. gigantea* in sheep from Northwest United States and Texas. At the moment, the unique molecular confirmation of the presence of *S. gigantea* in a country from the Americas had occurred in Argentina, where Gual et al. (2017) reported a case in which one sheep was found dead with several macrocysts present in the esophageal and pharyngeal muscles. To the authors knowledge, our results are the first molecular confirmation of the presence of *S. gigantea* in Brazil. Macrocysts found in the present study were morphologically similar to that recently described in three adult sheep slaughtered in a farm located in Uruguaiiana municipality, also from RS (Damboriarena et al., 2016). These data indicate that the frequency of macrocysts infections, as well as the potential losses to the sheep industry caused by organ and carcass condemnation, can be underestimated in southern Brazil.

Mixed infection by macro and microcysts and the higher prevalence of microcysts compared to macrocysts that were found in our study are in accordance with the pattern of sheep sarcocystosis reported from countries of Asia, Europe, and Africa (Dehaghi et al., 2013; Pipia et al., 2016; Dahmani et al., 2017). In southern Brazil, this scenario can be related to the regular exposure of sheep to the feces of canids (*S. tenella* DH). It occurs because canids generally have free-access to pastures, feeders, and water sources meant for sheep. In addition, dogs are commonly used in the livestock management and they can acquire the parasite by consuming viscera and carcass of sheep dead or slaughtered into the farms. On the other hand, cats (*S. gigantea* DH) have no close relationship with sheep, especially that raised in extensive systems, and the access of cats to sheep carcasses is also limited. Possibly, this condition can explain the low prevalence of *S. gigantea* in the studied sheep.

Furthermore, although other Apicomplexa protozoan (*Toxoplasma gondii*) that has cats as DH can be commonly found infecting sheep from southern Brazil (Ferreira et al., 2016), its more complex life-cycle (Dubey and Lindsay 2006), including the vertical transmission should be considered to explain the high prevalence of *T. gondii* and the low prevalence of *S. gigantea* in these sheep flocks.

In conclusion, our results showed a high frequency of microcysts-forming *S. tenella* infection in sheep from southern Brazil and provided the first molecular confirmation of the presence of macrocysts-forming *S. gigantea* in Brazilian sheep. These data revealed the importance of to establish control measures as: to limit the access of dogs and cats to the sheep pasture and facilities, and to avoid the access of dogs and cats to non-cooked viscera and carcasses of dead or slaughtered sheep at the farms.

Declaration of absence of conflicts of interest

I declare that there are no conflicts of interest between the authors of the article entitled: “Molecular identification of *Sarcocystis gigantea* macrocysts and *S. tenella* microcysts from sheep slaughtered in southern Brazil”, subject for appreciation in the journal Veterinary Parasitology: Regional Studies and Reports.

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