Article

Supporting information

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Abstract
The sheep industry, an important economic activity in the Brazilian State of Parana, can be affected by gastrointestinal parasites. This study aimed to evaluate the occurrence of gastrointestinal parasites in sheep raised in the North of said state. Fecal samples were collected from 69 animals and processed through flotation and sedimentation. Parasites were found in 63 (91.3%) animals. The most frequent parasites were strongylid eggs (81.2%), followed by Eimeria sp (42%) and Giardia duodenalis (10.1%). To the best of our knowledge, this is the first report of Giardia duodenalis in sheep raised in the State of Parana. These findings indicate that sanitary management must be improved in order to control parasite infections and increase productivity.

Keywords: sheep; parasites; giardiasis; protozoa; helminths.

Introduction

Sheep farming is an activity widely exploited in tropical countries, where sheep are raised for their meat, milk and wool (Vieira, 2003). In developing countries, gastrointestinal parasitic diseases are one of the main

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factors in herd productivity reduction (Amarante et al., 2004; Sweeny et al., 2012).

Sheep are usually raised in small overcrowded areas, which causes intense pasture contamination. Even in extensive breeding, gastrointestinal parasites can become a major problem when they are associated with malnutrition, management errors, and anthelmintic therapy inefficiency (Amarante et al., 2004; Sweeny et al., 2012).

Reduced productivity and mortality are directly related to helminthiasis in sheep (Amarante et al., 2004). The protozoan Giardia duodenalis is relevant in this context. This flagellate has been reported as the cause of diarrhea and decreased weight gain in lambs (Aloisio et al., 2006). In addition, the fact that domestic ruminants and other animals are sources of infection for humans has garnered the scientific community interest in animal giardiasis (Olson, et al., 1995; Feng; Chiao, 2011; Coelho et al., 2017).

To propose adequate control, knowledge of the main gastrointestinal parasites is essential, since the indiscriminate and repetitive use of different chemical treatments results in the selection of resistant parasite populations (Cunha Filho et al. 1998; Melo et al., 2003; Almeida et al., 2010). The objective of this study is twofold: 1) to provide the first report of Giardia duodenalis, and 2) investigate gastrointestinal parasitism by both helminths and protozoa in sheep raised in the North of the Brazilian State of Paraná.

Material and methods

The study was conducted in the metropolitan area of Londrina, in the municipalities of Cambé (23º16’33”S, 51º16’42”W) and Ibirapu (23º16’09”S, 51º02’53”W), both located in the north of Paraná State. The animals were
raised in two different farms – Farm A (Cambé), and Farm B (Ibiporã) – and selected regardless of sex, age, and breed.

A questionnaire was used to collect the following data about the farms: source of water; nutritional, reproductive and sanitary management; facilities; type of raising; other domestic animals; individual aspects of each animal (i.e., age and sex). Data were collected by convenience sampling, with materials for coprological exams collected directly from the rectum (25 samples from Farm A; 44 from Farm B).

The fecal samples were chemically conserved in acetic formaldehyde solution and sent to the laboratory for further processing and analysis. They were examined for cestode, nematoid and intestinal protozoa through centrifugal flotation in zinc sulfate solution with density of 1.180 g/mL (Faust et al, 1938) and spontaneous sedimentation (Hoffmann et al, 1934). Samples were examined in an optical microscope, and the parasites were morphometrically identified. It was not possible to perform fecal culture and oocyst sporulation due to the use of chemical conservation.

Results and discussion

A total of 69 sheep were studied (25 from Farm A, 44 from Farm B): 69.6% (48) Santa Ines, 19.4 % (12) Texel, 13.0% (9) cross-breed (SI and ½ Texel). Ten animals (14.5%) were younger than 6 months old while 59 (85.5%) were adults, i.e., over 6 months old. All animals had good corporal score and normal feces.

Most animals (91.3%; 63) tested positive for parasites. The prevalence rates were 100% (24/24) and 86.7% (39/45) for Farm A and Farm B, respectively. This finding indicates a high occurrence of gastrointestinal parasites in sheep (91.3%) in the region, one which is higher than reported by Souza et al. (2012) for sheep in the Northern State of Rio Grande do Norte (76.56%).
The sheep industry in Paraná State is concentrated in small areas, with high levels of pasture contamination (Nieto et al., 2003). Grasses of stoloniferous habit are traditionally used in Brazilian pastures for feeding sheep, which form a dense plant mass, preventing the direct incidence of sunlight and forming a microclimate that is favorable for the survival of helminth larvae and the viability of eggs and oocysts. This leads to a high infection rate, as observed in both properties in this study.

Polyparasitism was found in 30 (47.6%) of the positive samples, and the most frequent association involved strongylid eggs and *Eimeria* sp. oocysts (Figure 1).

Figure 1: Polyparasitism in fecal samples of sheep raised in two farms located in the municipalities of Cambé (A) and Ibiporã (B) in the metropolitan area of Londrina, State of Parana, Brazil.

Table 1 provides the positive results distributed by farm and animal age.
Table 1: Parasites identified in fecal samples of sheep raised in two farms located in the municipalities of Cambé (A) and Ibiporã (B) in the metropolitan area of Londrina, State of Parana, Brazil.

| Parasitism       | Farm A                |               | Farm B                |               |
|------------------|-----------------------|---------------|-----------------------|---------------|
|                  | Young (<6 months)     | Adult (>6 months) | Young (<6 months)     | Adult (>6 months) |
| N=6              | N=19                  | N=4           | N=40                  |               |
| Strongylid       | 6 (100%)              | 18 (94.7%)    | 3 (75%)               | 29 (72.5%)    |
| Trichuris sp.    | 0                     | 0             | 2 (50%)               | 4 (10%)       |
| Moniezia sp.     | 1 (16.7%)             | 0             | 0                     | 1 (2.5%)      |
| Eimeria sp.      | 6 (100%)              | 12 (63.2%)    | 2 (50%)               | 10 (25%)      |
| Giardia duodenalis | 5 (83.3%)         | 0             | 2 (50%)               | 0             |

Strongylid eggs were the most frequent parasite as they were found in 56 (81.2%) samples, followed by Eimeria sp. oocysts (29; 42%). These findings are similar to those reported by Brito et al. (2009) in sheep in the Northern State of Maranhão: 63.54% for helminth eggs and 58.85% for Eimeria sp. oocysts. However, Souza et al. (2012) reported higher positivity for Eimeria sp. oocysts (56.25%; 36/49), followed by strongylid eggs (29.69%; 19/49) in sheep in the State of Rio Grande do Norte. These differences may have been influenced by the diagnosis techniques, number of samples, geographic location, breed, time of the year, and environmental conditions (CARNEIRO et al., 2006; BASSETTO et al., 2009; McMANUS et al., 2009).

Most animals were female (62; 89.9%). The majority of the females were in reproductive age, which may have contributed to the increased positive results, as pregnant and lactating females tend to have higher eggs per gram (epg) and oocyst elimination rate (see Amarante et al., 1992; Gauly et al., 2004).
This is the first report to date of natural infection by *Giardia duodenalis* in sheep in the State of Parana, with a rate of 10.1% positive animals (7/69). This parasite was only found in young animals, which is probably related to their developing immune system and elimination of large numbers of cysts in the feces (Xiao; Herd, 1994; Koudela; Vitovec, 1998).

The analysis of a single sample may have underestimated the real prevalence of this protozoan, since studies have proved that cyst elimination is intermittent (Sweeny et al., 2011). Souza et al. (2012) reported 18.75% (12/49) positive cases of *Giardia duodenalis* cysts in sheep in the State of Rio Grande do Norte. However, reports of this protozoan in sheep raised in Brazil are still scarce, which calls for further studies. Molecular studies could evaluate the real importance of these hosts as environmental contaminants and their potential risk for zoonotic transmission of this parasite.

Table 2 summarizes the differences in management and hygienic and sanitary conditions between the two farms. Farm sanitization must be performed systematically, with feces and food debris properly stored. Cleaning irregularities in Farm B may have favored the transmission of parasites that depend on infectious structures in the environment, such as *Trichuris* sp eggs, which were found in this farm. Thus, practices such as failing to quarantine the newly acquired animals and allowing the contact of the sheep with other ruminants, as observed in this property, may cause the transmission of parasites and increase the infection rate (Costa et al., 2011; Gouveia et al., 2013).
Table 2: Hygienic, sanitary, and management conditions of two sheep farms located in the municipalities of Cambé (A) and Ibiporã (B) in the metropolitan area of Londrina, State of Parana, Brazil.

| Characteristics                  | Farm A                                                                 | Farm B                                                                 |
|----------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|
| Creation system                  | Semi-intensive                                                         | Semi-intensive                                                         |
| Production purpose               | Meat production                                                        | Meat production                                                        |
| Source of food                   | Star grass and oats; concentrate feed only for ewes with newborns and finishing lambs; mineral supplementation in covered trough ad libitum | Brachiaria sp. and silage provided in the trough; provision of winter feed for the entire herd; mineral supplementation inside the sheep pen ad libitum |
| Source of water                  | Artesian well                                                          | Weir                                                                   |
| Antiparasitic treatment          | Every 3 months                                                         | Monthly                                                                |
| Type of facilities               | Sheep pen with concrete floor                                          | Sheep pen with mud for most animals. Sheep pen with ripped floor for the finishing lambs. |
| Cleaning of facilities           | Daily, without chemicals, only with mechanical scraping                | Monthly, without chemicals, only with mechanical scraping               |
| Replacement animals              | Yes                                                                    | Yes                                                                    |
| Quarantine of new animals        | Yes                                                                    | No                                                                     |
| Sheep farming only               | Yes                                                                    | No, also bovine farming.                                               |
| Contact with others domestic animals | No                                                               | Yes, contact between ovines, bovines, shepherd dogs and domestic birds. |

The presence of domestic fowl and dogs in the farm B may also have contributed to the transmission of parasites, since these animals may be mechanical transmitters of parasitic structures (Traub et al., 2003; Majewska et al., 2009). Besides, dogs are hosts of zoonotic *Giardia duodenalis* genotypes, acting as a vessel of this protozoan. This fact contributes to environmental contamination, being a risk factor for
parasite transmission (Bomfim et al., 2005; Sudré; Couto; Bonfim, 2012; Coelho et al., 2017).

The actual frequency of protozoa such as *Giardia* and *Cryptosporidium* is probably under diagnosed in the databases due to the parasitological techniques used in the routine control of small ruminants. Co-diagnostic methods allow greater sensitivity to research these agents in these animals. In addition, all animals in this study were asymptomatic, which may further contribute to the zoonotic risk of transmission.

The high infection rate and the report of *Giardia duodenalis* infection is indicative that improvement is necessary in the sanitary management of the animals under study. This may eventually result in reduced transmission rate, environmental contamination, and risks of human infection.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**Ethics committee**

This study was approved by the Animal Ethics Committee of Universidade Federal Fluminense (CEUA-UFF), Approval No. 239, as of 9 August 2012.

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**Ocorrência de Giardia sp associada à infecção por outros parasitos gastrointestinal em ovinos da região norte do estado do Paraná, Brasil**

**Resumo**
A ovinocultura, uma atividade econômica muito importante no Estado do Paraná, pode ser afetada por parasitas gastrointestinais. O objetivo do presente estudo foi avaliar a ocorrência de parasitas gastrointestinais em ovinos criados na região norte do Paraná. Amostras fecais foram coletadas de 69 animais e processadas por técnicas de flutuação e sedimentação.
Encontraram-se parasitas em 63 (91,3%) animais. Os parasitas mais freqüentes foram os ovos de estrongilídeos (81,2%), seguidos por Eimeria sp (42%) e Giardia duodenalis (10,1%). Até onde se pôde apurar, este é o primeiro relato de Giardia duodenalis em ovinos criados no Paraná. Esses resultados indicam que o manejo sanitário deve ser aprimorado para controlar infecções por esses parasitas e aumentar a produtividade.

Palavras-chave: ovinos; parasitas; giardiase; protozoários; helmintos.

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