

Occurrence of *Giardia intestinalis* in dairy goats and evaluation of risk factors for infection: research note

Ocorrência de *Giardia intestinalis* em caprinos destinados à produção leiteira e avaliação de fatores de risco para a infecção: nota de pesquisa

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Abstract

The aim of this study was to establish the occurrence of *Giardia intestinalis* in goat fecal samples from two dairy farms (A and B) located in Rio de Janeiro state, Brazil, assessing possible risk factors for infection. Fecal samples from all young animals (up to one year of age) (n=58) and from 10% of adult animals (n=18) were collected. Samples were collected directly from the rectum and were submitted to centrifuge-flotation in sugar saturate solution for microscopic search of *Giardia* cysts. From 76 analyzed samples, 17 (22.4%) were positive for *Giardia* cysts. All positive samples came from young animals of farm A. Also, dog fecal samples (n=9) were collected to evaluate their role in parasite transmission. Three dog samples (33.3%) were positive for *Giardia* cysts. Presence of helminth eggs was only observed in animals from farm A. *Eimeria* sp. oocysts were observed in fecal samples from both farms. The comparison of hygienic-sanitary and management conditions of both farms indicated that the high rate of infection in young animals from farm A was due to an association of risk factors: intensive raising of young animals in installations made of wooden slats floor, fecal accumulation underneath it, and the presence of domestic animals inside goat installations. The high *Giardia* positivity and the observation of risk factors for transmission call attention to the importance of effective prophylactic measures and patterns of management.

Keywords: dairy goat, *Giardia*, risk factors.

Resumo

Este estudo teve como objetivo estabelecer a ocorrência de *Giardia intestinalis* em amostras fecais de caprinos, provenientes de duas caprinoculturas (A e B) localizadas no estado do Rio de Janeiro, Brasil, avaliando possíveis fatores de risco para a infecção. Foram coletadas amostras fecais de todos os animais jovens (até um ano de idade) (n=58) e de 10% dos animais adultos (n=18). As amostras foram coletadas diretamente do reto e submetidas à técnica de centrifugo-flutuação em solução saturada de açúcar para pesquisa de cistos de *Giardia* por microscopia. Das 76 amostras analisadas, 17 (22,4%) foram positivas para cistos de *Giardia*, sendo todas provenientes de animais jovens da caprinocultura A. Também foram coletadas amostras de fezes caninas (n=9) da propriedade A, para avaliação do papel destes no ciclo de transmissão. Três (33,3%) amostras caninas foram positivas para cistos de *Giardia*. A presença de ovos de helmintos foi apenas observada em animais da propriedade A. Ambas as propriedades apresentaram positividade para oocistos de *Eimeria* sp. A comparação das condições higiênico-sanitárias e de manejo de ambas as caprinoculturas indicou que a alta taxa de infecção em animais jovens da caprinocultura A ocorreu por uma associação de fatores de risco: criação intensiva, instalações de piso de madeira ripada, acúmulo de fezes abaixo das instalações e a presença de animais domésticos no interior das instalações dos caprinos. A alta positividade para *Giardia* e a observação de fatores de risco para a transmissão chamam a atenção para a importância de medidas profiláticas e padrões de manejo eficazes.

Palavras-chave: caprino, produção leiteira, *Giardia*, fatores de risco.

Introduction

Gastrointestinal protozoa of the genus *Giardia* are responsible for considerable economic losses in livestock associated with morbidity and mortality (Aloisio et al., 2006; Geurden et al., 2010;

Sweeny et al., 2011). *Giardia intestinalis* infection in goats can be asymptomatic or associated with severe diarrhea and, depending on nutritional status, management, and concomitant presence of other pathogens, lead to animal death (Koudela and Vitovec 1998; Geurden et al., 2010).

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The transmission of this protozoa occurs most often through ingestion of cysts in contaminated water and food (Smith et al., 2007; Robertson, 2009). Several risk factors have been associated with *Giardia intestinalis* transmission in goats, such as age, housing of animals without separation of age groups, agglomeration, humidity, type and quality of food, hygienic-sanitary conditions, and construction of installations inadequate for the type of animal production (Bomfim et al., 2005; Geurden et al., 2010).

The role of goats in zoonotic transmission of *Giardia intestinalis* has been extensively discussed in literature, especially the prevalence of infection in this host and the potential for environmental contamination (Smith et al., 2007; Robertson, 2009; Geurden et al., 2010; Feng and Xiao, 2011). However, fewer studies regarding *Giardia* prevalence in goats were performed worldwide (Díaz et al., 1996; Castro-Hermida et al., 2005a; Castro-Hermida et al., 2005b; Bomfim et al., 2005; Van der Giessen et al., 2006; Castro-Hermida et al., 2007a; Castro-Hermida et al., 2007b; Gueurden et al., 2008; Ruiz et al., 2008; Sprong et al., 2009; Berrilli et al., 2012), with only one study in Brazil (Bomfim et al., 2005).

Thus, the aim of this study was to establish the occurrence of *Giardia intestinalis* in two dairy goat farms located in Rio de Janeiro state, Brazil, verifying the association of possible risk factors for the infection.

Material and methods

The study was performed in two dairy goat farms located in Niterói municipality, Rio de Janeiro state, Brazil, which were named A and B. The farms were chosen based on convenience of location and availability to participate in the study. Up to three visits to each farm were performed from November 2009 to June 2010.

A questionnaire was used to characterize the two farms. The aim of this questionnaire was to obtain information about installations, management, and hygienic-sanitary conditions of the animals to assess risk factors. The information were obtained through owner/responsible interview and observations made during the visit.

Housing for the animals in both farms was built of masonry or wood. Hygienic conditions of the farms were classified according to the following criteria: good (good sanitation); satisfactory (satisfactory sanitation); average (few accumulation of feces, food remains, and humidity) and unsatisfactory (considerable accumulation of feces, food remains, and humidity).

Goat fecal samples were collected directly from the rectum of all animals up to one year of age, and of 10% of animals with more than one year of age.

Additionally, because dogs were observed inside goat installations in farm A, nine canine fresh fecal samples were collected from the soil in order to evaluate their potential for parasite transmission. To avoid cross contamination, only the superficial part of the samples that were not in contact with the soil were collected. All fecal samples were stored under refrigeration until processing.

Fecal samples were processed according to Huber et al (2007). Briefly, samples were homogenized with distilled water and filtered through disposable sieves with a gauze overlay.

The filtered material was placed inside two 15mL test tubes and centrifuged at 402.48xg for 10 minutes, after which the supernatant was discarded. One of the tubes was stored under refrigeration, and sugar saturated solution with specific density of 1.30g/ml was added to the sediment of the second tube. After homogenization the material was centrifuged at 402.48xg for 5 minutes. The tube was then topped up with sugar solution, covered with a slide coverslip and left at rest for 3 minutes. The coverslip was mounted on a glass slide and examined under an optic microscope with and without phase contrast.

Results

From a total of 76 fecal samples collected, 58 came from young animals (up to one year of age) and 18 came from adult female animals (> 1 year of age). Among the young animals, 43 were female and 15 were male. Microscopic analysis of fecal samples showed 29.31% (17/58) of young animals positive for *Giardia intestinalis* cysts. Adult goats were not diagnosed with *Giardia*.

A total of 42 fecal samples, 31 from young animals (23 female and 8 male) and 11 from adult animals, were collected at farm A. Microscopic analysis of fecal samples showed 100% animal infection for at least one parasite species. Poliparasitism occurred in 92.9% (39/42) of animals. Young goats were positive for *Giardia intestinalis* (54.8%) in addition to other parasite species (Table 1). However, adult animals were not parasitized by *Giardia* even though other parasite species were diagnosed (Table 1).

A total of 34 fecal samples, 27 from young animals (20 female and 7 male) and 7 from adult animals, were collected at farm B. Microscopic analysis of samples did not reveal *Giardia intestinalis* parasitism in those animals, albeit some of the samples were positive for the genus *Eimeria* (Table 1). Finally, of the nine canine fecal samples collected from the soil of farm A, 3 (33.3%) were positive for *Giardia intestinalis* cysts.

Both farms showed differences in management and hygienic-sanitary conditions, which are compared in Table 2.

Discussion

The present study revealed a high rate of infection in young animals (29.31%), which is even higher when only samples of young animals from farm A are considered (54.8%).

According to Robertson (2009), the prevalence of *Giardia* sp. in goats worldwide varies between 10 and 40%. This variation is not only due to differences inherent to each region, but also to differences between studies, especially regarding animal age and type of diagnostic techniques employed.

In a study at the Canary Island, Ruiz et al. (2008) found positivity of 42.2% in 315 young animals through the association of three diagnostic techniques (microscopy, immunofluorescence and PCR). However, if only the microscopy results are considered, the positivity goes down to 26%, emphasizing the need to combine techniques to achieve a more reliable diagnosis.

Thus, we believe that the use of only one diagnostic technique, coupled with the use of only one fecal sample from each animal, could have underestimated the real prevalence of this parasite in the studied region. Additionally, studies demonstrate that because of its easy transmission and high viability of cysts in the environment, when a longitudinal research of animals is made the prevalences could reach 100% (Koudela and Vitovec, 1998; Becher et al., 2001).

Table 1: Absolute and relative frequencies of parasites diagnosed by microscopy through centrifuge-flotation in sugar solution technique of goat fecal samples from farms A and B.

INTESTINAL PARASITES	FARM A				FARM B			
	YOUNG GOATS (UP TO 1 YEAR / N=31)		ADULT GOATS (> 1 YEAR / N=11)		YOUNG GOATS (UP TO 1 YEAR / N=27)		ADULT GOATS (> 1 YEAR / N=7)	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
<i>G. intestinalis</i>	17 (54.8%)	14 (45.2%)	0	11 (100%)	0	27 (100%)	0	7 (100%)
<i>Eimeria</i> sp.	30 (96.8%)	1 (3.2%)	11 (100%)	0	25 (92.6%)	2 (7.4%)	7 (100%)	0
<i>Strongylid</i>	22 (71%)	9 (29%)	11 (100%)	0	0	27 (100%)	0	7 (100%)
<i>Moniezia</i> sp.	2 (6.5%)	29 (93.5%)	0	11 (100%)	0	27 (100%)	0	7 (100%)

Table 2: Comparison of major characteristics of management and hygienic-sanitary conditions observed between the two studied farms.

FARM CHARACTERISTICS	FARM A		FARM B	
	YOUNG ANIMALS	ADULT ANIMALS	YOUNG ANIMALS	ADULT ANIMALS
Type of Installations	Suspended floor of wooden slats	Masonry floor not suspended	Suspended floor of wooden slats	Suspended floor of wooden slats
Hygiene conditions	unsatisfactory	unsatisfactory	unsatisfactory	unsatisfactory
Breed	Saanen	Saanen	Mixed breed/Saanen	Mixed breed/Saanen
Raising system	Intensive	Semi-intensive	Intensive	Intensive
Water origin	Nascent	Nascent	Artesian well	Artesian well
Presence of other animals in installations	Yes	No	No	No
Quarantine of animals	No	No	Yes	Yes
Feeding Management	Ration, hay and chopped grass on feeder.	Pasture, silage, ration and hay on feeder.	Ration, hay and chopped grass on feeder.	Ration, hay and chopped grass on feeder.
Presence of <i>Giardia intestinalis</i> cysts	Yes	No	No	No

The results obtained in this study were very similar to those described by Bomfim et al. (2005), which was conducted on the mountain region of Rio de Janeiro state, and found positivity in 30.6% of young animals. Data obtained in the present study are particularly relevant because they were obtained from a non-mountainy region of the same state, confirming the high prevalence of this parasite in goats and calling attention for the importance of those animals as environmental contaminants (Robertson, 2009).

The finding of *Giardia* infection in only young animals could be explained by the fact that their immune system is still under development. They also shed a higher amount of cysts in feces, which facilitates the diagnosis (Xiao, 1994; Koudela and Vitovec, 1998). On the other hand, adult animals have better-developed immunity and shed smaller amounts of cysts in feces, which may

not be detected on parasitological exams (Xiao, 1994; Castro-Hermida et al., 2005a).

Castro-Hermida et al. (2005a) noted a prevalence of up to 30% in adult goats, with the higher cyst shedding occurring in females during the first weeks after labor. However, this study used immunofluorescence as a diagnostic technique, which presents a higher sensitivity when compared to microscopy, and the samples were collected during the birth period. Thus, the methodological differences could have contributed for the discrepancies observed when comparing the results from this study with those described by Castro-Hermida et al. (2005a).

In addition to the differences inherent to the research methodology employed, several authors highlight that the differences in management and hygienic-sanitary conditions between the farms could affect the infection rate (Bomfim et al., 2005; Geurden et

al., 2010). Among those, the type of material used to build the installations (wooden slats or masonry) was reported as an important risk factor for parasite transmission (Bomfim et al., 2005). Installations built with wooden slats floors favor fecal accumulation underneath them and the proliferation of flies, which can act as mechanical vectors of cysts (Doiz et al., 2000; Graczyk et al., 2005). Similarly, masonry floors favor humidity and fecal accumulation, maintaining the environmental contamination and favoring reinfection (Maddox-Hyttel et al., 2006).

When comparing the housing of young and adult animals in farm A, there was a correlation between wooden slats floors and positivity for *Giardia*. Adult animals raised semi-intensively in masonry floors did not show positivity for this parasite. However, the type of installation could not be considered the only risk factor, since animals from farm B that were not positive for this parasite were also under intensive raising in wooden slats floor.

It is important to note that although hygienic-sanitary conditions in both farms were classified as unsatisfactory, the fecal material accumulated underneath the floor in farm B was removed and deposited in dunghill. Conversely, the fecal material in farm A remained underneath the floor, which contributes to the proliferation of flies and environmental contamination by cysts carried by rainwater. Moreover, several chickens were observed feeding from those fecal material, which can potentially favor the mechanical transmission of cysts (Majewska et al., 2009; Berrilli et al., 2012).

The presence of domestic animals in the same installations of goats was also reported as a risk factor for infection (Bomfim et al., 2005; Berrilli et al., 2012). In the present study, dogs and domestic birds were seen inside the young goat installations in farm A, which coincides with the only population found to be positive for *Giardia* cysts. This correlation becomes even more significant when one takes into consideration that *Giardia intestinalis* cysts were found in three canine fecal samples coming from the same farm. According to Traub et al. (2003), dogs could be reservoirs of this parasite or only act as mechanical transporters of cysts, contributing to environmental contamination and favoring goat infection, but molecular studies are still needed to confirm this hypothesis. Thus, we believe that the high rate of infection in young animals of farm A is due to an association of factors. More specifically, the intensive raising of young animals in installations made of wooden slats floor, associated with fecal accumulation underneath it, and the presence of domestic animals inside the goat installations, are both related to the high rate of infection.

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The finding of helminthes in animal feces only in farm A reinforces the importance of management and hygienic-sanitary conditions in goat infection. Several authors report frequent finding of helminthes in goats related to age, management, and sanitary conditions of livestock (Odoi et al., 2007; Khan et al., 2010; Guimarães et al., 2011; Ratanapob et al., 2012). We believe that the higher rates of adult infection in farm A were related to semi-intensive raising of animals, associated with inadequate hygienic-sanitary conditions of installations, in addition to the fact that this farm did not use quarantine in goats recently introduced to the livestock. Those characteristics were also highlighted in studies made by Khan and colleagues (2010) in Pakistan and Guimarães and colleagues (2011) in Brazil. Moreover, all adult animals studied were female at reproductive age, which could have contributed to a greater rate of helminth infection, since pregnant and lactant females tend to eliminate a higher number of eggs in feces due to stress and immune status (Khan et al., 2010).

The helminth infection in young goats only found in farm A can be justified by the greater susceptibility of those animals to infection due to an immune system immaturity inherent to age (Khan et al., 2010), besides having poor hygienic-sanitary conditions in their housing.

Several authors reported high *Eimeria* infection rates in goats from Brazil similar to those observed in the present study, varying from 69.79% to 100% (Freitas et al., 2005; Hassum & Menezes, 2005; Brito et al., 2009; Cavalcante et al., 2012; Coelho, Amarante & Bresciani, 2012). This protozoa infection is very common in young and adult goats (Freitas et al., 2005; Cavalcante et al., 2012) and is associated with favorable environmental conditions, management practices and presence of infected adult animals in close contact with young animals (especially during lactation period) (Gauly et al., 2004; Hassum & Menezes, 2005). All those features were observed in the studied population, contributing to the elevated percentage of infected animals reported.

The high infection rates of the studied population, together with the zoonotic potential of *Giardia intestinalis*, and the observation of possible risk factors for infection call attention for the need of more studies regarding epidemiology of *Giardia* in goats in Brazil. These studies could contribute to a better knowledge of the role of those animals in zoonotic transmission and to establish effective prophylactic measures to reduce environmental contamination and transmission risks.

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