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Original Article

Giardia duodenalis: prevalence and identification of the zoonotic assemblage B in cats from Lages, Santa Catarina, Brazil

Giardia duodenalis: prevalência e identificação da assemblage zoonótica B em gatos de Lages, Santa Catarina, Brasil

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Abstract

Giardia duodenalis is a zoonotic parasite that infects humans and several animal species, including cats. Although its importance to animal and public health is well recognized, the role of cats in transmitting the parasite to humans remains unclear. The aim of this study was to investigate *G. duodenalis* infection in cats from the municipality of Lages, Santa Catarina, Brazil. Between 2021 and 2023, 150 fecal samples were analyzed by zinc sulfate centrifugal flotation, spontaneous sedimentation, sucrose centrifugal flotation, polymerase chain reaction for amplification of the β-giardin and glutamate dehydrogenase genes, and *assemblage* characterization by restriction fragment length polymorphism. The association between infection and epidemiological variables was evaluated using the chi-square or Fisher's exact test, as appropriate ($p \leq 0.05$). Considering zinc sulfate centrifugal flotation as the reference method, sensitivity, specificity, and the *kappa* coefficient were determined. The prevalence of *G. duodenalis* was 18.7% (28/150), with *assemblage* B identified in two animals. All methods showed high specificity (>96%) and moderate sensitivity. The identification of *assemblage* B highlights the importance of molecular characterization for identifying genotypes with zoonotic potential and supporting control strategies based on the One Health concept.

Keywords: Giardiasis, zoonosis, epidemiology, cats.

Resumo

Giardia duodenalis é um parasito zoonótico que infecta humanos e diversos animais, incluindo gatos. Embora sua importância para a saúde animal e humana seja reconhecida, o papel dos gatos na transmissão para humanos permanece incerto. O objetivo deste estudo foi investigar a infecção por *G. duodenalis* em gatos do município de Lages, Santa Catarina, Brasil. Entre 2021 e 2023, foram coletadas 150 amostras fecais, analisadas por centrífugo-flutuação em sulfato de zinco, sedimentação espontânea, centrífugo-flutuação em sacarose, reação em cadeia da polimerase para amplificação dos genes β-giardina e glutamato desidrogenase e caracterização das *assemblages* por polimorfismo de comprimento dos fragmentos de restrição. A associação entre infecção e variáveis epidemiológicas foi avaliada pelos testes do qui-quadrado ou exato de Fisher, conforme apropriado

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($p \leq 0,05$). Considerando a centrífugo-flutuação em sulfato de zinco como método de referência, determinaram-se a sensibilidade, a especificidade e o coeficiente *kappa*. A prevalência de *G. duodenalis* foi de 18,7% (28/150), com identificação da *assemblage* B em dois animais. Todos os métodos apresentaram alta especificidade (>96%) e sensibilidade moderada. A identificação da *assemblage* B reforça a importância da caracterização molecular na identificação de genótipos com potencial zoonótico e para estratégias de controle baseadas no conceito de Saúde Única.

Palavras-chave: Giardíase, zoonose, epidemiologia, gatos.

Introduction

Giardia duodenalis, also known as *G. lamblia* or *G. intestinalis*, is a protozoan parasite of the small intestine responsible for extensive morbidity (Gardner & Hill, 2001), being one of the major intestinal parasites of animals, including humans, worldwide, with a higher prevalence in low-income countries (Rojas-López et al., 2022). It is the most prevalent pathogenic intestinal protozoan in humans (Adam, 2021) and one of the most common intestinal parasites in cats, in which it may cause infections ranging from asymptomatic infections to atypical clinical manifestations and persistent infections (Bowman & Lucio-Forster, 2010). Among the most common clinical signs are vomiting, watery diarrhea, and, in some cases, bloody diarrhea. The parasite was first described at the end of the seventeenth century by the Dutch microscopist Antonie van Leeuwenhoek, who reported, after examining his own feces under light microscopy, the presence of small organisms with flattened bodies and foot-like structures used to perform slight movements (Dobell, 1920).

The genus *Giardia* comprises nine species, according to data retrieved from GenBank (Schoch et al., 2020), of which only one parasitizes multiple species and is known as *G. lamblia*, *G. intestinalis*, or *G. duodenalis*. This species comprises at least eight assemblage groups (A–H), which differ significantly from one another and appear to represent distinct species. Assemblages A and B have high zoonotic potential and are found in humans and several mammalian species (Heyworth, 2016). In turn, assemblages C and D are associated with dogs, E with livestock, F with cats, G with rats, and H with marine vertebrates (Heyworth, 2016; Ryan & Cacciò, 2013). In cats, assemblages F and A are among the most commonly reported, although B, C, D, and occasionally E have also been identified in this species (Barbosa et al., 2023).

Among domestic animals, cats have a close relationship with humans as companion animals (Yoshiuchi et al., 2010). However, the role of dogs and cats as sources of human infection with *G. duodenalis* remains under debate (Lucio et al., 2017). Despite the widespread ownership of pets and the close contact between humans and companion animals, the overall risk of zoonotic transmission to humans remains uncertain, and epidemiological studies involving animals and their owners, together with molecular subtyping tools, are needed to better understand the extent of transmission and reverse transmission of *Giardia* between companion animals and humans (Barbosa et al., 2023).

Host infection occurs through the ingestion of cysts present in contaminated water or, less commonly, contaminated food or through direct fecal–oral contact, and the cysts can survive for prolonged periods under different environmental conditions (Adam, 2001). In addition, companion animals may contribute to the environmental dispersal of infective stages (Barrera et al., 2026). The shedding of *Giardia* cysts may be variable and sporadic, and few studies have quantitatively evaluated cyst shedding in cats (Barbosa et al., 2023), with concentrations ranging from zero to more than 7,143 cysts per gram of feces (Cox et al., 2005). In this context, the investigation of *G. duodenalis* in companion animals, together with molecular characterization, contributes to understanding the epidemiology of the parasite and its potential relevance at the animal–human–environment interface from a One Health perspective (Barrera et al., 2026).

Therefore, the aim of the present study was to investigate *G. duodenalis* infection in cats from the municipality of Lages, Santa Catarina, Brazil, in order to determine its prevalence and the risk factors associated with infection.

Material and Methods

Between November 2021 and February 2023, 150 fecal samples were collected from cats living in the municipality of Lages, Santa Catarina, Brazil (Figure 1; IBGE, 2025). The animals were referred to the municipal Zoonosis Control Center to participate in the municipal population control program through ovariohysterectomy or orchiectomy. At the time of the procedure, the animals showed no clinical signs; cats presenting clinical abnormalities, including diarrhea or vomiting, were not submitted to surgical sterilization and, consequently, were not included in the study.

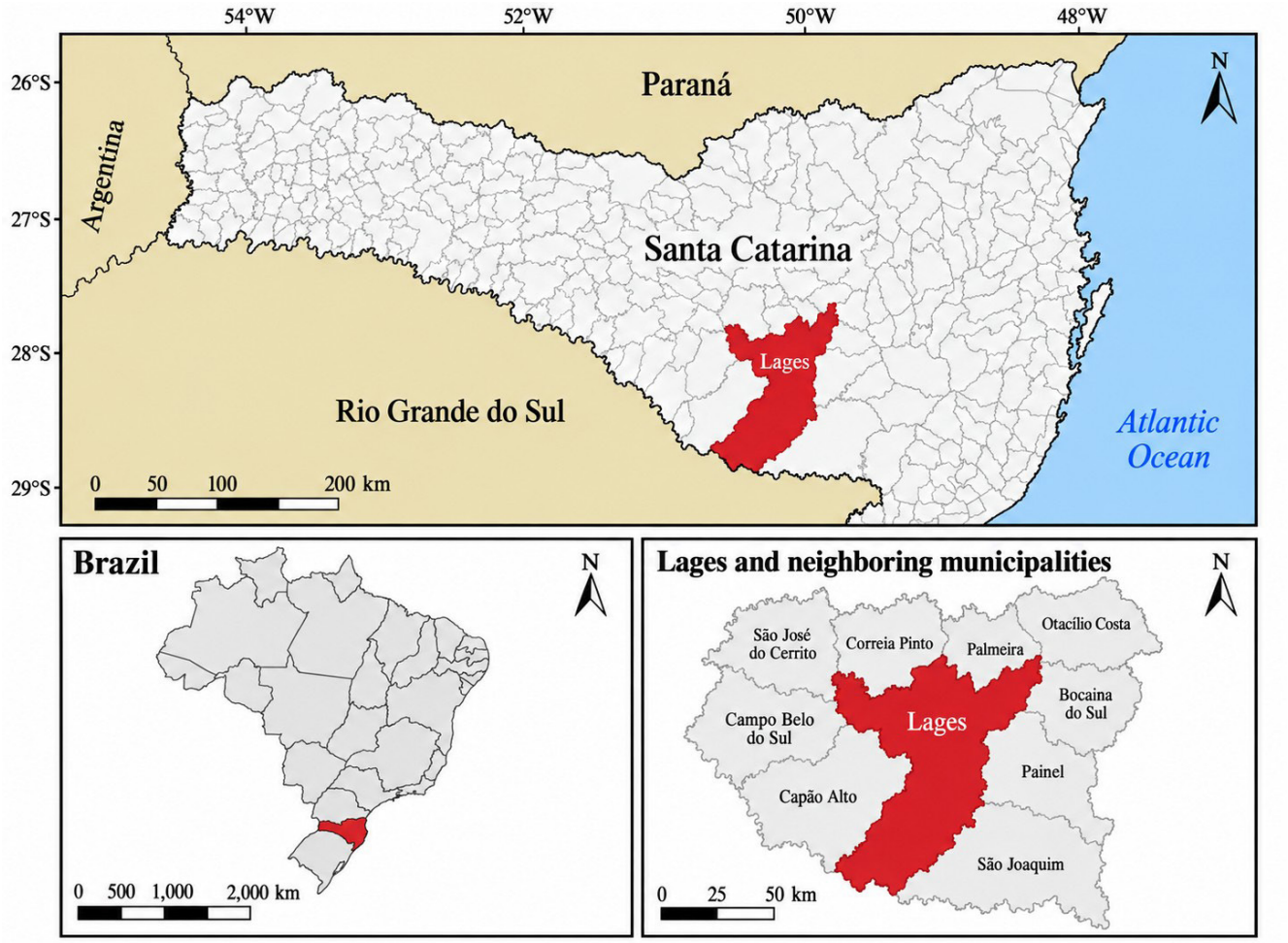

Figure 1. Geographic location of the municipality of Lages, Santa Catarina State, Brazil, and its neighboring municipalities. Map prepared by the authors using the 2025 Digital Municipal Mesh provided by the Brazilian Institute of Geography and Statistics (IBGE, 2025).

The sample size was calculated based on an estimated feline population for the municipality of Lages. According to ABINPET (2019), the population of companion cats in Brazil was 23.9 million, corresponding to approximately one cat for every nine inhabitants, considering the estimated Brazilian population (IBGE, 2021). This proportion was similar to that previously reported for Lages by Stalliviere et al. (2009), with approximately one cat for every 10.7 inhabitants. Based on the estimated population of Lages (IBGE, 2020), the feline population was estimated at 17,310 animals. Considering a 5% margin of error, a 95% confidence level, and an expected prevalence of 11%, based on the mean prevalence of *G. duodenalis* reported in Brazil (Carrasco et al., 2016; Gennari et al., 1999; 2016; Godoy et al., 2013), and according to the calculations proposed by OPS (1979), a total sample size of 150 cats was established.

The animals were classified according to sex (male and female), age (young ≤ 1 year and adult > 1 year), origin (owned and stray), and breed (purebred or mixed-breed). Owned cats were further classified according to anthelmintic treatment within the previous two months, outdoor access, contact with other animals (of the same or different species), and the presence of rodents in the environment. The collected data were recorded and tabulated for subsequent analysis of the association between these variables and the observed prevalence.

Samples were collected sequentially, regardless of the animals' characteristics (sex, age, breed, etc.). After collection, the samples were placed in plastic containers, properly identified, and stored under refrigeration (4–7 °C) until processing by the zinc sulfate centrifugal flotation technique (Faust et al., 1938), modified spontaneous sedimentation (Hoffman et al., 1934), modified sugar (sucrose) centrifugal flotation (Sheather, 1923) for the detection of cysts and/or trophozoites, in addition to Polymerase Chain Reaction (PCR) for parasite DNA detection.

For DNA extraction from fecal samples, the commercial QIAamp® Fast DNA Stool Mini Kit (QIAGEN®, Hilden, Germany) was used according to the manufacturer's instructions, with modifications based on the optimized protocol described by Hawash (2014). These modifications included lysis at 100 °C for 10 min, a condition used in that protocol to enhance the disruption of protozoan oocysts/cysts and DNA recovery, increasing the vortexing time with the InhibitEX buffer to 5 min, and reducing the elution volume to 100 µL. All heating procedures were performed in a Kasvi K80-02D dry bath, while centrifugation steps were carried out at room temperature using a Solab SL-703 centrifuge.

The extracted DNA was analyzed by nested PCR targeting the β-giardin gene (Lalle et al., 2005) and semi-nested PCR targeting the glutamate dehydrogenase (GDH) gene (Read et al., 2004). The β-giardin amplicons were analyzed by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) using the restriction enzyme Hae III, whereas the GDH amplicons obtained by semi-nested PCR were digested with Nla IV and Rsa I. After incubation in a thermal cycler, all products were subjected to electrophoresis on 2% agarose gel and stained with SYBR Safe®. All reactions were performed using a Veriti™ 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA). Table 1 shows the primer sequences used in the PCR assays.

Table 1. Primer sequences used for PCR detection of *Giardia duodenalis*.

Marker	Step	Sequence (5'- 3')	Product (bp)	Reference
β – giardina	First reaction	AAGCCCGACGACCTCACCCGCAGTGC	753	Lalle et al. (2005)
		GAGGCCGCCCTGATCTTCGAGACGAC		
	Second reaction	GAACGAACGAGATCGAGGTCCG	511	
		CTCGACGAGCTTCGTGTT		
GDH	First reaction	TCA ACG TYA AYC GYG GYT TCC GT	730	Read et al. (2004)
		GTT RTC CTT GCA CAT CTC C		
	Second reaction	CAG TAC AAC TCY GCT CTC GG	432	

Samples in which parasitic structures were detected by any of the three coproparasitological techniques and/or *G. duodenalis* DNA was detected by PCR were considered positive.

The chi-square (χ^2) test with Yates' continuity correction was used to assess the association between the observed prevalence and the analyzed variables when all expected frequencies were equal to or greater than five. When at least one expected frequency was lower than five, Fisher's exact test was used. A significance level of $p \leq 0.05$ and a 95% confidence interval were adopted. Odds, odds ratios, and their respective 95% confidence intervals were calculated.

Considering zinc sulfate centrifugal flotation as the reference method, sensitivity, specificity, accuracy, and the kappa coefficient (κ) were calculated to evaluate the performance and agreement of the diagnostic methods for the detection of *G. duodenalis*.

Statistical analyses were performed using R software (R Development Core Team, 2024).

This study was approved by the Animal Use Ethics Committee (CEUA) of CAV/UDESC under protocol Nº. 1773210621 on June 25, 2021.

Results

Of the 150 fecal samples analyzed, 28 (18.7%) were positive for *G. duodenalis* by at least one of the diagnostic methods used. The distribution of positive results according to sex, age, origin, and breed is shown in Table 2. No statistically significant association was observed between *G. duodenalis* positivity and any of these variables ($p > 0.05$).

Considering only the owned cats (Table 3), for which information was obtained from the owners, additional variables could be compared with *G. duodenalis* infection.

Table 2. Prevalence of *Giardia duodenalis* in fecal samples from cats living in the municipality of Lages, Santa Catarina, Brazil.

Variable	Category	n	%[1]	P	%[2]	%[3]	*p*-value	*Odds*	*Odds ratio*	95% CI
Sex	Male	45	30	10	22.2	35.7	0.615[a]	0.29	1.38	0.58 – 3.29
	Female	105	70	18	17.1	64.3		0.21		
Age	Adult	88	58.7	16	18.2	57.1	1[a]	0.22	1.09	0.50 – 2.39
	Young	62	41.3	12	19.4	42.9		0.24		
Origin	Owned	146	97.3	27	18.5	96.4	0.5665[b]	0.23	1.47	0.15 – 14.67
	Stray	4	2.7	1	25	3.6		0.33		
Breed	Purebred	7	4.7	2	28.6	7.1	0.6152[b]	0.4	1.8	0.33 – 9.79
	Mixed-breed	143	95.3	26	18.2	92.9		0.22		
Total		**150**	**-**	**28**	**18.7**	**100**	**-**	**-**	-	-

n = number of animals. P = number of positive animals. %[1] = percentage of animals in each category in relation to the total number of animals. %[2] = percentage of positive animals within each category. %[3] = percentage of positive animals within each category in relation to the total number of positive animals. *p* = statistical significance value. [a] chi-square (χ^2) test with Yates' continuity correction; [b] Fisher's exact test. Fisher's exact test was used when at least one expected frequency was lower than five. Odds = odds of being positive for *Giardia duodenalis* in each group. Odds ratio = ratio of the odds of being positive for *Giardia duodenalis* between the two groups, considering the group with the higher prevalence relative to the group with the lower prevalence within each category.

Table 3. Prevalence of *Giardia duodenalis* in fecal samples from owned cats living in the municipality of Lages, Santa Catarina, Brazil.

Variable	Category	n	%[1]	P	%[2]	%[3]	*p*-value	*Odds*	*Odds ratio*	95% CI
Anthelmintic treatment within the previous two months?	Yes	112	76.7	26	23.2	96.3	0.01575[a]	0.30	9.98	1.30 - 76.52
	No	34	23.3	1	2.9	3.7		0.03		
Outdoor access?	Yes	86	58.9	18	20.9	66.7	0.4893[a]	0.26	1.5	0.62 - 3.61
	No	60	41.1	9	15	33.3		0.18		
Lives with other cats?	Yes	138	94.5	26	18.8	96.3	1[b]	0.2	1.63	0.19 – 13.79
	No	8	5.5	1	12.5	3.7		0.14		
Lives with animals of other species?	Yes	102	69.9	17	16.7	63.0	0.5266[a]	0.20	0.68	0.28 – 1.63
	No	44	30.1	10	22.7	37.0		0.29		
Presence of rodents in the environment?	Yes	23	15.8	4	17.4	14.8	1[b]	0.21	0.96	0.30 – 3.09
	No	123	84.2	23	18.7	85.2		0.22		
Total		**146**	**-**	**27**	**18.5**	**100**	**-**	-	-	-

n = number of animals. P = number of positive animals. %[1] = percentage of animals in each category in relation to the total number of animals; %[2] = percentage of positive animals within each category; %[3] = percentage of positive animals within each category in relation to the total number of positive animals. *p* = statistical significance value. [a] chi-square (χ^2) test with Yates' continuity correction; [b] Fisher's exact test. Fisher's exact test was used when at least one expected frequency was lower than five. Odds = odds of being positive for *Giardia duodenalis* in each group. Odds ratio = ratio of the odds of being positive in the "Yes" category relative to the "No" category for each variable.

A statistically significant association was observed between *G. duodenalis* infection and previous anthelmintic treatment ($p = 0.01575$). Among cats that had received anthelmintic treatment within the previous two months, 23.2% were positive by at least one of the diagnostic methods, representing 96.3% of all positive owned cats.

The results regarding outdoor access, contact with other cats, contact with animals of other species, and the presence of rodents in the environment are shown in Table 3. None of these variables showed a statistically significant association with *G. duodenalis* positivity ($p > 0.05$).

Table 4 shows that, among the diagnostic methods evaluated, zinc sulfate centrifugal flotation showed the highest diagnostic performance for detecting *G. duodenalis* infection, since 75% of all positive animals were detected by this technique.

Also in Table 4, molecular analyses revealed that, of the 150 animals evaluated, four were positive by PCR. Of these, 50% (2/4) were identified as assemblage B. In the remaining two PCR-positive samples, it was not possible to determine the assemblages.

Table 4. Number of positive samples according to the diagnostic method used.

Diagnostic method	P	%[1]	%[2]	Assemblage
Zinc sulfate centrifugal flotation	21	14	75	-
Sucrose centrifugal flotation	4	2.7	14.3	-
Spontaneous sedimentation	9	6	32.1	-
PCR	4	2.7	14.3	B (2/4); undetermined (2/4)
Positive by at least one diagnostic method	**28**	**18.7**	**100**	-

P = number of positive samples. %[1] = percentage of positive animals by diagnostic method in relation to the total number of animals (*n* = 150); %[2] = percentage of positive animals by diagnostic method in relation to the total number of animals positive by at least one diagnostic method.

Table 5 presents the performance of the diagnostic tests for detecting *G. duodenalis* parasitism, expressed as sensitivity, specificity, accuracy, and agreement (kappa coefficient), using zinc sulfate centrifugal flotation as the reference method.

Table 5. Evaluation of the sensitivity, specificity, accuracy, and kappa coefficient (κ) of the diagnostic methods using zinc sulfate centrifugal flotation as the reference method.

Diagnostic method	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Accuracy (%)	Kappa coefficient (κ)
Sucrose centrifugal flotation	55.3 (39.4 – 71.1)	100 (100 - 100)	89.82	0.66
Spontaneous sedimentation	56.8 (40.8 – 72.7)	97 (94.1 – 99.9)	88.24	0.61
PCR	51.2 (35.9 – 66.5)	97.7 (95.2 - 100)	86.71	0.57

Interpretation of kappa values: 0.01–0.20 = slight agreement; 0.21–0.40 = fair agreement; 0.41–0.60 = moderate agreement; 0.61–0.80 = substantial agreement; 0.81–1.00 = almost perfect agreement.

All three tests evaluated showed high specificity but moderate sensitivity. Spontaneous sedimentation showed the highest sensitivity (56.8%), followed by sucrose centrifugal flotation (55.3%) and PCR (51.2%), all with wide confidence intervals due to the limited number of positive samples. Sucrose centrifugal flotation achieved 100% specificity, with no false-positive results, whereas PCR showed a specificity of 97.7% and spontaneous sedimentation 97.0%. Regarding accuracy, the highest value was observed for sucrose centrifugal flotation (89.8%), followed by spontaneous sedimentation (88.2%) and PCR (86.7%). Analysis of the kappa coefficient, which measures agreement beyond chance, also indicated that sucrose centrifugal flotation showed the highest agreement (0.66), followed by spontaneous sedimentation (0.61) and PCR (0.57).

Discussion

The prevalence of *G. duodenalis* observed was 18.7% (28/150) (Table 2), which was higher than the 2% reported by Carrasco et al. (2016) in cats from Seropédica, Rio de Janeiro, and the 5.2% reported by Gennari et al. (2016) in cats from São Paulo. The latter authors also found no statistical association between *G. duodenalis* infection and the animals' age, breed, or sex ($p > 0.05$). In contrast, the prevalence found was similar to the 16.04% reported by Gennari et al. (1999), also in cats from the city of São Paulo.

Similar results regarding the variables evaluated in Table 2 were also reported by Sun et al. (2023), in a meta-analysis involving studies of dogs and cats from different regions worldwide, in which no significant differences

in *G. duodenalis* infection were observed according to the sex or age of cats ($p > 0.05$). Likewise, Veyna-Salazar et al. (2023), evaluating cats from Santiago de Querétaro, Mexico, found no association between *G. duodenalis* infection and the sex or origin of the animals (owned or stray) ($p > 0.05$).

Among owned cats, outdoor access, contact with other animals, and the presence of rodents were also not significantly associated with *G. duodenalis* positivity ($p > 0.05$) (Table 3). However, the small number of animals in some categories may have limited the precision of the estimates.

A statistically significant association was observed between *G. duodenalis* positivity and anthelmintic treatment within the previous two months ($p = 0.01575$). However, this finding should be interpreted with caution. The cats evaluated were not patients recruited from veterinary clinics or hospitals, but animals participating in the municipal population control program in the city of Lages, and they showed no clinical signs at the time of the procedure. Among positive owned cats, characteristics such as outdoor access and contact with other cats were frequent, although these variables were not statistically significantly associated with *G. duodenalis* positivity. In addition, treatment of giardiosis requires the use of drugs with activity against *G. duodenalis*, such as fenbendazole or metronidazole (ESCCAP, 2025). As no information was obtained regarding the active ingredients administered to the animals in the present study, the treatment regimen used, or whether a coproparasitological examination had been performed before anthelmintic administration, it is not possible to determine whether the previous anthelmintic treatment had activity against the parasite. Thus, the observed association should not be interpreted as evidence of a causal relationship between anthelmintic treatment and infection and may be related to confounding factors or other characteristics not evaluated in this population. This result differs from that reported by Sun et al. (2023), who found no significant association between *G. duodenalis* prevalence and the use of anthelmintic drugs ($p > 0.05$).

In the present study, PCR-RFLP identified assemblage B in two animals (Table 4), demonstrating the occurrence of this assemblage in cats from Lages, Santa Catarina, Brazil.

The identification of assemblage B is a relevant finding because, together with assemblage A, it is recognized for its high zoonotic potential (Sun et al., 2023). Although assemblage F is generally predominant in cats (Lecová et al., 2020; Xu et al., 2016; Wu et al., 2022), studies conducted in Italy, the Czech Republic, Poland, Japan, China, Australia, and Iran have also detected assemblages A and/or B in cats, often associated with mixed infections, suggesting that these animals may act as occasional hosts and potential sources of human infection (Guadano Procesi et al., 2022; Iijima et al., 2018; Jaros et al., 2011; Karimi et al., 2023; Lecová et al., 2020; Piekarska et al., 2016; Zhang et al., 2025; Yang et al., 2015).

Assemblage B has been reported in cats from different regions of the world, although its frequency varies considerably according to the geographical, population, and methodological context. Studies conducted in Turkey (Önder et al., 2021; Sursal et al., 2020) demonstrated the exclusive occurrence of assemblage B in cats, suggesting that this genotype may be well adapted to urban environments with high population density and close interspecific contact. A similar result was reported in Campo Grande, Mato Grosso do Sul, Brazil, where Prado et al. (2023) identified only assemblage B in cats, representing the first report of this genotype in that state. These findings are consistent with those of the present study and reinforce the epidemiological relevance of assemblage B in Brazilian feline populations.

Therefore, the results obtained indicate that the presence of zoonotic assemblages, such as assemblage B, highlights the need for continuous molecular surveillance. The identification of assemblages allows a more accurate assessment of zoonotic risk and contributes to control strategies based on the One Health concept.

When comparing the diagnostic methods used, considering zinc sulfate centrifugal flotation as the reference method (Table 5), all tests showed high specificity (> 96%) but moderate sensitivity. This finding indicates that these methods have a greater ability to correctly identify non-infected animals, thereby reducing the occurrence of false-positive results. On the other hand, moderate sensitivity may result in failure to detect a relevant proportion of infected animals, limiting the use of these tests as stand-alone screening tools. Sucrose centrifugal flotation showed the best overall agreement, whereas spontaneous sedimentation had slightly higher sensitivity and PCR showed the lowest sensitivity under the conditions evaluated. The lower performance of PCR may be associated with false-negative results in fecal samples containing low concentrations of cysts due to the small amount of DNA obtained after extraction and the limited amount of amplified product after amplification and purification (Sudré et al., 2014), making it difficult to detect mild infections in asymptomatic carriers with low parasite burdens, particularly in animals (Uchôa et al., 2018).

Therefore, when selecting a diagnostic test, the need for accuracy and the availability of resources should be considered, seeking a balance between sensitivity and specificity to ensure accurate and reliable detection of the parasites under investigation.

Conclusion

The occurrence of *Giardia duodenalis* in cats from the municipality of Lages, Santa Catarina, Brazil, was confirmed, with the identification of assemblage B, which is recognized for its zoonotic potential. Considering the close contact between owned cats and their owners, this finding reinforces the epidemiological relevance of investigating the parasite at the animal–human health interface. The observed association between positivity and previous anthelmintic treatment reinforces the importance of coproparasitological examinations for diagnosing infection and guiding the use of specific treatment when indicated. Among the diagnostic methods evaluated, zinc sulfate centrifugal flotation showed the best performance for detecting asymptomatic cats. The identification of assemblage B reinforces the importance of continuous molecular surveillance, contributing to the assessment of zoonotic potential and the development of control strategies based on the One Health approach.

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Data availability

All data supporting the conclusions of this study are available in the text.

Ethics declaration

This study was submitted to the Animal Ethics Committee (CEUA) of CAV/UDESC and was approved under protocol No. 1773210621 on June 25, 2021.

Conflict of interest

The authors declare that there are no conflicts of interest of any nature.

Author contributions

Mayckon Antonio Cardoso Padilha: conceptualization, funding acquisition, data curation, formal analysis, methodology, investigation, writing – original draft, writing – review & editing. Felipe Rieth de Lima: methodology, investigation. João Victor Teixeira Albanaes: methodology, investigation. Ana Clécia dos Santos Silva: methodology, investigation, resources. Rafaela Maria Boson Jurkevicz: methodology, investigation, resources. Ana Paula Remor Sebolt: methodology, investigation. Geanice Ledo: investigation, resources. Jenniffer Gerber: investigation, resources. Mariana Ester Antunes: investigation, resources. Luiz Daniel de Barros: supervision, resources. Andreas Lazaros Chryssafidis: supervision, resources. Anderson Barbosa de Moura: conceptualization; funding acquisition; methodology; project administration; supervision; writing – review & editing.

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