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Prevalence of *Giardia duodenalis* among dogs seized by the Center for Control of Zoonoses (CCZ) of the city of Lages, Santa Catarina, Brazil

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ABSTRACT

The knowledge of the epidemiology of parasitic infections in stray and domestic animals, especially of its incidence and prevalence, is fundamental to adopting effective prophylactic measures. Stray dogs play an important role in environmental contamination favoring the transmission cycle of zoonotic agents. Among the parasitic infections that affect humans, *Giardia duodenalis* is the most common intestinal protozoa and was designated as a re-emerging infectious disease. This study aimed to determine the prevalence of *G. duodenalis* in dogs siezed by the Center for Control of Zoonoses (CCZ) of the city of Lages, Santa Catarina, Brazil using two diagnostic techniques. In 357 stool samples analysed, the prevalence of *G. duodenalis* cysts was 5.3% (19/357) and 4.8% (17/357) detected by floatation and sedimentation techniques, respectively. No correspondence between gender and age was found among the methods used for analyzing the infected dogs in this study. Our data suggested that two diagnostic techniques should be used in a complementary way to ensure that false negatives are not neglected.

Keywords: *Giardia duodenalis*; Dogs; Zoonoses; Brazil

1. INTRODUCTION

Urban growth and social change caused by human migration to the cities have favored the growth of the stray and domestic dog population in many developing countries [1]. From an epidemiological standpoint, stray dogs play an important role in environmental contamination, since they do not receive anti parasitic treatments and circulate in public areas favoring the spread of parasites [2].

Among the parasitic infections that affect humans and represent public health problems, *Giardia duodenalis* is one of the most common intestinal protozoa, having a broad geographical distribution throughout the world [3,4].

G. duodenalis (sin. *G. intestinalis* or *G. lamblia*) is a flagellated protozoan, whose life cycle consists of two stages: trophozoite and cyst, which contaminates food and water [5]. Cysts are usually responsible for transmitting the disease to the hosts, as they are resistant to changes in the environment, desiccation and gastric acidity from the stomach [6]. The infection is spread by the fecal-oral route to susceptible individuals and is influenced by the levels of environmental contamination and survival of the parasite [7]. The trophozoite inhabits the large intestine, but is not capable of invading the intestinal mucosa [8]. It affects the intestine, but less often affects the stomach [9], ileum and colon [10], and also the gallbladder [11].

The variety of vertebrate hosts is wide, including dogs, cats, rats, sheep, cattle, goats and horses, and also wild animals [12-14] The disease is responsible for malabsorption and diarrhea especially in children and immunosuppressed individuals [15,16].

Although the flagellate is common in dogs and cats, it is rarely associated with clinical disease in these animals [17]. However, giardiasis have been reported in animals living in kennels and catteries, acting as potential sources of infection for new animals to be placed in the envi-

ronment, which justifies the need to treat all animals regardless of whether they are experiencing symptoms or not [18,19].

The coproparasitological technique is the most common laboratory procedure for the diagnosis of gastrointestinal parasitic infections. Different techniques can be used for the diagnosis of *Giardia*, according to the sensitivity and resources of the routine laboratory tests. Generally, the diagnosis depends on the identification of cysts in stools with or without diarrhea, trophozoites in stools from a patient with diarrhea, contents of duodenal aspiration or biopsies of intestinal mucosa [20]. The zinc sulfate centrifugal flotation technique [21] was the technique chosen for performing routine diagnosis of parasitic structures, especially *Giardia* cysts and/or trophozoites [14]. There are also indirect diagnostic methods, such as ELISA (Enzyme-Linked Immunoabsorbent Assay) and PCR, an important epidemiological tool since different *Giardia* genotype is associated with the clinical severity of the infection [22].

This study aimed to determine the prevalence of *G. duodenalis* in dogs seized by the Center for Control of Zoonoses (CCZ) of the city of Lages (Santa Catarina), using two different diagnostic techniques.

2. MATERIAL AND METHODS

The city of Lages is located in the state of Santa Catarina, southern Brazil. The geographic coordinates are: South latitude 270˚48' West longitude 500˚20' and about 916 meters altitude; its climate is subtropical with an average temperature of 14.3˚C. It has a population of 156,727 inhabitants; the growth rate of the population is 1.38 and its population density is of 59.27 inhabitants/km^2 [23]. The CCZ is a public institution maintained by the city of Lages. It belongs to the Municipal Health Secretariat and is responsible for preventing and controlling zoonoses in the city and developing sanitary and epidemiological surveillance systems.

According to the CCZ, the estimated dog population in Lages is of 25,000 animals, which represents an average of 0.16 dog/inhabitant. For the research, dog feces were collected in the CCZ between June 2011 and July 2012. The study was approved by the Ethics Committee for Animal Experiments from the Center of Agroveterinary Sciences at the University of Santa Catarina (UDESC) under the protocol 134/2011.

Samples and Parasitological Methods

Fecal samples were collected directly from the rectum of 357 dogs seized by the CCZ and sent to the Parasitology Laboratory of the Universidade do Planalto Catarinense (UNIPLAC) and processed within 24 hours. The parasitological tests were performed using two methods for each sample, verifying the presence of cysts and/or trophozoites of *G. duodenalis*.

The methods chosen for the analysis were: 1) The centrifugal floatation technique: Two grams of feces were homogenized in 10 ml of water and filter into a centrifuge tube and centrifuge at 400 g for 2 min. The supernatant is removed and a solution of zinc sulfate 33% (density = 1.180) is added and the tubes were shaken for 2 minutes. The tubes were centrifuged at 400 g for 2 min. The upper solution was removed with a Pasteur pipette and put on a slide. Two drops of Lugol are added [21]. 2) Spontaneous sedimentation technique: Two grams of feces were homogenized in 10 ml of water using a glass rod and filtered to a glass conical sedimenttation vessel. Water was added to the edge of the vessel. After approximately 1 hour, removed the sediment with Pasteur pipette and deposited on a slide. Two drops of Lugol were added [24,25]. The parasitic structures were visualized an optical microscope with the 40× objectives.

The chi-square test was used for statistical analysis, and the Test-t was used to compare the diagnosis methods and dog's gender, with a significance level of $p < 0.05$.

3. RESULTS

The city is divided into 65 neighborhoods [26] and for the study, stool samples were collected from stray dogs seized in 62 neighborhoods (95.38%). In 18 of those (29.03%), all located in the periphery (Araucária, Caravágio, Ferrovia, Guarujá, Habitação, Maria Luiza, Penha, Petrópolis, Ponte Grande, Promorar, Santa Catarina, Santa Clara, Santo Antonio, Tributo, Várzea, Vila Maria, Vila Mariza and Vila Nova), the animals were infected with *G. duodenalis* cysts, as shown in **Figure 1**.

The 357 stray dogs consisted of 195 (54.6%) females and 152 (45.4%) males aged from 1 month to 10 years. Of the 357 stool samples analyzed, none of them showed signs of diarrhea. Cysts of *G. duodenalis* were found with a prevalence of 5.3% (19/357) and 4.8% (17/357) for floatation and sedimentation methods, respectively.

The zinc sulfate centrifugal flotation is the technique choose for performing routine diagnosis of parasitic structures and it has good diagnostic sensitivity [14], but the association of two evaluation techniques for *G. duodenalis*, in this case sedimentation technique, regardless their equivalence, should improved the diagnosis. The total positivity for cysts of *G. duodenalis* was 36 samples, of which 22 (61%) were exclusively detected by one of the methods employed and 14 samples (39%) were positive by two methods (**Table 1**). This means that if the two methods were not complimentary, 61% of the samples would be negative for the parasite.

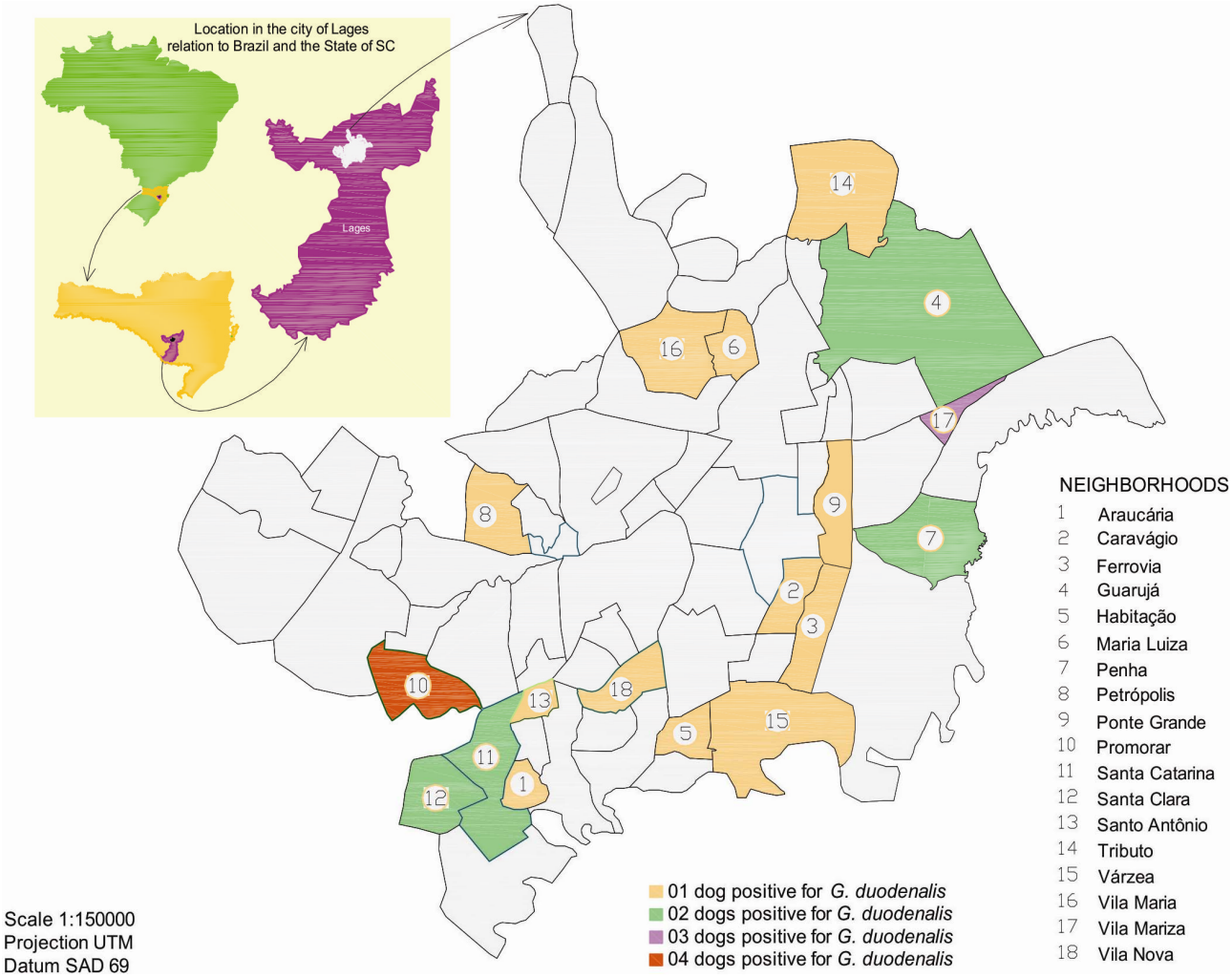

Figure 1. Distribution of the dogs seized by the CCZ in the neighborhoods of the city of Lages, in Santa Catarina.

Table 1. Compatibility of the results from the two diagnosis methods for *G. duodenalis*.

Method	Total Detection[1]	Exclusive Detection	Common Detection
Flotation	19	12	7
Sedimentation	17	10	7
Total	36	22	14

[1]There is no dependency between gender and the methods used for the diagnosis of *G. duodenalis* ($\chi^2 = 0.7846^{ns}$).

Table 2. Infected dogs with *G. duodenalis*, according to sex and diagnosis methods.

Method	Females	Males	Prob < t[1]
Flotation	3.0 ± 1.4	4.3 ± 3.5	0.18
Sedimentation	2.0 ± 1.1	4.6 ± 3.4	0.19
Prob < t[2]	0.46	0.42	

[1]Comparison at line and [2]column, using test t.

Regarding age, it was verified through the t-test that the age of the females infected with *G. duodenalis* was similar to the age of the males. The age of the infected females detected by the flotation method was the same as the one detected by the sedimentation method, *i.e.*, there is no difference between gender nor methods for the variable age (**Table 2**).

Through the chi-square test, it was found that there is no dependancy between the methods and dogs's gender, *i.e.*, the presence of *G. duodenalis* maintains the same pattern between the two assessment methods, so the methods are equivalent in terms of frequency for the diagnosis of the protozoan.

Considering age, the prevalence of *G. duodenalis* occurred mainly in dogs aged up to six years (**Table 3**), and regardless of the method used for the diagnosis, about 80% of the animals were within the first three age groups. There was one case of positive sample, for which the age of the dog was not estimated by the vets of the CCZ.

Table 3. Absolute and relative frequency (%) of *G. duodenalis* according to the age of male and female dogs.

Age	Flotation				Sedimentation			
Groups	Female (%)		Male (%)		Female (%)		Male (%)	
≤2	2	(33.3)	5	(38.5)	3	(60.0)	4	(30.0)
2.1 a 4	3	(50.0)	2	(15.4)	1	(20.0)	3	(30.0)
4.1 a 6	1	(16.7)	3	(23.1)	0	(0.0)	2	(20.0)
6.1 a 8	0	(0.0)	2	(15.4)	0	(0.0)	2	(0.0)
8.1 a 10	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
10.1 a 12	0	(0.0)	1	(7.7)	0	(0.0)	0	(0.0)
NI	0	(0.0)	0	(0.0)	1	(20.0)	1	(10.0)
Total	6		13		5		12	

4. DISCUSSION

There are many studies on prevalence of *G. duodenalis* in stray dogs populations worldwilde and particulary in Brazil [27]. Prevalence is variable and depends on a number of factors including age, living conditions, region studied and health status of the animal [28] and in diagnostic methodology employed as shown in this study.

In present work neighborhoods Vila Mariza and Promorar were the ones with the highest number of infected dogs with *G. duodenalis* (**Figure 1**) However, there is no explanation for these findings, since peripheral neighborhoods have the same physiographic and socio-economic characteristics. The neighborhood of Tributo was responsible for 10.08% (36/357) of the dogs seized during the study, mainly because of the close location to CCZ.

In this work it was found that positive results for *Giardia* cysts in dogs stool samples were different from other studies in Brazil. A prevalence of 80% in Rio de Janeiro (RJ), 70% in Curitiba (PR), 34% in Florianópolis (SC) and 32% in Belo Horizonte (MG) was found by [29]. A prevalence of 41% in Uberlândia (MG) was found by [30], a close result to 44.3% in dogs from the municipality of Santa Maria (RS) [31], but this high prevalence in the study was attributed to the large number of young dogs (89.1%). Also in the state of Rio Grande do Sul, in the city of Porto Alegre, *Giardia* cysts was found in 198 (38%) of the 526 samples analyzed using centrifugal floatation technique method [32], and in Canoas (RS), using the same technique, 34.04% of dog's stool samples positive for the protozoan [33]. However, the data for the dogs in Lages were closer to data obtained in Guarulhos (SP) of 13.25% (22/166) using different techniques from the ones used in this study [34].

The data obtained for these 357 samples were in agreement with other the authors [35,36] that reported that the prevalence of *Giardia*, especially in dogs, shows variable rates, depending on geographical location, the method used for the diagnosis and the characteristics of the population studied. In practice, the negative results in stool samples for *Giardia* are common, due to either inadequate sensitivity of the diagnostic tests or by intermittent removal of the parasite [37].

Comparative studies between dogs and children in their households in the city of Lages in 2005, found that 20% (20/100) of the children and 18% (19/105) of the dogs were *Giardia*-positive, according centrifugal floatation technique [38,39]. These more significant data than those found recently for the same region can be explained by the low temperatures and high levels of rainfall registered in the city in the year of 2011, which may have contributed to low environmental resistance of the parasite and by the improvement of living conditions experienced by the population, especially due to the implementation of sewage systems in various districts of the city that started in 2010.

Although no significant differences was found between males and females infected with the protozoa, as was observed in the studies in Porto Alegre [32] and in northern Greece [40], a higher number of infected adult males than females was observed [41]. According to the authors, neutered dogs tend to show a reduced prevalence of infection compared with sexually active animals. But, according to [42], the large number of infections occurred in females (7%) compared to males (3.4%).

No dependency relationship was found between age and the methods applied for the diagnosis using the Chi-square test. As reported by [43] no significant difference for cysts of *Giardia* among puppies in kennels (39%) and puppies in houses (34%) [43]. But, [44] and [45] mentioned that apparently healthy dogs have a prevalence of 10% to 20% for *Giardia* compared to animals aged less than 12 months, and this frequency ranged between 36% - 50% in his work in northern Greece [40], found positive results of 14,8% for dogs infected with the protozoan aged up to six months and 3,3% for dogs aged between 6 months and 10 years.

In Germany, *Giardia* infections occur more often in puppies housed in kennels due to the crowded conditions compared to adult and companion dogs [46]. This higher susceptibility of animals aged less than 12 months to the protozoan can be attributed to a certain degree of resistance that is acquired with age. Stray animals may be

more exposed to contaminated water, food and feces, increasing the risk of infection for the group, although it has not been proven in this study.

No dependency relationship was found in this study between the methods of flotation and sedimentation in relation to gender and age of the dogs infected with G. *duodenalis* seized by the CCZ. But, although the zinc sulfate centrifugal flotation technique (Faust *et al*. technique) is the technique chosen for the diagnosis of *Giardia* cysts and/or trophozoites, the two assessment methods should be used as complimentary methods.

Although the frequency of *G. duodenalis* have shown different distributions in the various geographic regions, its increase or decrease is strongly correlated with socioeconomic, environmental, ecological and prevention facptors that require a global commitment from not only the different health professionals, but also from politicians to allocate resources in order to conduct interactive control programs.

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