

Prevalence of *Giardia* in Household Dogs and Cats in the State of Rio de Janeiro using the IDEXX SNAP® *Giardia* Test

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ABSTRACT

A survey to estimate the prevalence of *Giardia* infections was conducted using a commercial *Giardia* antigen test kit (IDEXX SNAP® *Giardia* Test; IDEXX Laboratories, Inc., Westbrook, ME) in 1,837 dogs and 462 cats brought to participating clinics in the state of Rio de Janeiro. The overall estimated prevalence of *Giardia* infection was 9.7% in dogs and 15.6% in cats, and was lowest for animals six years of age or older (4.4%)

than in all other age groups ($P < 0.01$). Fecal centrifugation-flotation techniques frequently fail to identify infected animals because the passage of cysts in the feces is intermittent. The SNAP® *Giardia* test, with a sensitivity of 95% and a specificity of 99%, improves the ability of veterinarians and technicians to diagnose *Giardia* infections without having to perform repeated tests or have extensive training to be able to recognize the cysts.

INTRODUCTION

Giardia spp is a ubiquitous protozoan parasite responsible for diarrheal disease in

several domestic and wild animal species as well as in humans. The actual prevalence of *Giardia* is unknown, but it is felt to be much higher than its rate of detection.¹⁻³ Subclinical infections and difficulty in diagnosing *Giardia* spp in fecal examinations contribute to underestimations of its prevalence. Certain yeasts, plant seeds, and various debris items are sometimes mistakenly identified as *Giardia* cysts.¹ The cysts are shed infrequently, so it may be necessary to examine several fecal samples before they will be present. When they are present in a sample, the cysts can deteriorate rapidly in fecal floatation solutions.¹ Technicians in the clinic and sometimes even veterinarians are often not adequately trained to identify *Giardia* cysts.¹ In some cases, failure to isolate cysts from the feces of an animal with diarrhea of unknown cause will entice the veterinarian to make a presumptive diagnosis of giardiasis by evaluating the animal's clinical response to treatment with metronidazole.¹

Previous surveys of *Giardia* and other canine intestinal parasites have been conducted in areas of São Paulo, Paraná, and Rio de Janeiro states in Brazil using fecal centrifugation, flotation, and polymerase chain reaction techniques.^{2,4,5} The present study was initiated to estimate the prevalence of *Giardia* infections in a population of household dogs and cats brought to participating clinics for routine healthcare procedures in Rio de Janeiro, Brazil using the SNAP[®] *Giardia* Test, a rapid enzyme immunoassay for detection of *Giardia* antigen in canine and feline feces. Compared with immunofluorescence microscopy, the SNAP[®] *Giardia* Test (IDEXX Laboratories, Inc., Westbrook, ME) has a sensitivity of 95% (95% CI 87%–98%) and a specificity of 99% (95% CI 96%–100%). This test was previously used to study the prevalence of *Giardia* in dogs and cats in the United States presenting to clinics with clinical signs of gastrointestinal disease.⁶ In a study of laboratory dogs, all infected with *Giardia*, the SNAP[®] *Giardia* Test improved the ability of clinicians and inexperienced veterinary stu-

dents to identify infections over zinc sulfate flotation solutions.¹

MATERIALS AND METHODS

Dogs and cats selected for the survey were household pets brought to participating clinics (n=4) in the state of Rio de Janeiro for routine health procedures. The animals in this survey were sampled and examined from 2006 through 2007. Fecal samples were collected and refrigerated (2°C–8°C) until a sufficient number of samples was available for processing and testing.

Animal data recorded in the study included species (dog or cat) breed, name, age, gender, and region of residence. The presence or absence of clinical signs typical of *Giardia* infection, either from query of the owner or by observation in clinic, also was recorded for each animal sampled. In addition, the clinic was identified for all animals as well as the clinician collecting and examining the sample. Identification of the owner was recorded from some, but not all, animals sampled. Data were entered into a database to permit evaluation by some of these criteria.

Fecal samples were examined for the presence of *Giardia* antigen using the commercial SNAP[®] *Giardia* Test. The test identifies *Giardia lamblia*, otherwise known as *Giardia intestinalis* or *Giardia duodenalis*, the primary species in mammals. The presence of this antigen in fecal samples indicates the animal has ingested *Giardia* cysts, may be actively infected, and may be shedding cysts in feces. Clinicians using the test kit were familiar with the instructions for performing the evaluations. In addition to collection and examination of demographic data and results of the fecal sample examinations (recorded as positive or negative), clinicians evaluated the utility and ease of use of the commercial diagnostic test kit.

Statistical evaluations were performed by the chi-square test for comparisons of positive tests within combined results (dogs and cats) for selected evaluation criteria, including age category, gender, gastrointes-

tinal signs (diarrhea present or absent), and region. The estimated prevalence of *Giardia* in households with less than five animals versus those with five or more animals was summarized.

RESULTS

The SNAP® *Giardia* Test was reported by all clinicians to be easy to use and performed well for the large numbers of fecal samples examined in the clinics for this study.

Study Population

The population sampled included 2299 animals (1837 dogs and 462 cats) (Table 1). All animals resided in the state of Rio de Janeiro, and the majority of the animals were from Rio de Janeiro city, Niterói, Região dos Lagos, and Baixada Fluminense. Approximately 6% of the dogs and less than 1% of the cats resided in other regions within the state. The majority of the animals were older than 2 years. The distribution by gender was similar for both dogs and cats. Gastrointestinal signs were present in approximately 12% of the dogs and 27% of the cats sampled in the survey.

Test Findings

The overall estimated prevalence of *Giardia* infection was 9.7% in dogs and 15.6% in cats. The percentage of infected animals among those 6 years of age or older (4.4%) was significantly ($P < 0.01$) lower than in all other age groups (1–6 months, ≥ 6 months–2 years, or > 2 –6 years). The percentage infected among animals > 2 to 6 years of age (8.3%) was significantly ($P < 0.01$) less than in the two younger age groups, but was significantly ($P < 0.01$) higher than the percentage infected in the oldest age group. Detection of *Giardia* antigen was statistically similar between animals less than 6 months of age (23.6%) and those 6 months to 2 years of age (16.6%).

There was no significant difference in the detection of *Giardia* antigen between males and females of either species (Table 1). *Giardia* antigen was detected in 11.2% of male dogs and cats, similar to 10.7% in females (Table 1).

The presence or absence of gastrointestinal signs did not appear to be a significant predictor of finding *Giardia* antigen in this population of animals. Approximately 13% of the animals testing positive had diarrhea, whereas approximately 11% of the animals positive for *Giardia* were free of these signs (Table 1).

The only difference regionally was a significantly ($P < 0.01$) lower prevalence of *Giardia*-positive animals in the region of Baixada Fluminense than in Rio de Janeiro, Niterói, or Região dos Lagos (3.8% vs 10.4%, 13.3%, and 15.5%, respectively), (Table 1).

The majority of animals came from households having fewer than five animals (76%); however, animals from households with 5 or more animals had a higher percentage positive (20%) than those from households with fewer than 5 animals (5.8%). Also, households with the greater number of animals were more likely to have 1 or more animals positive for *Giardia* (41.5%) than were the households with fewer animals (6.3%), (Table 2).

DISCUSSION

In the present study, *Giardia* antigen was detected in the feces of nearly 10% of the household dogs and 16% of the cats brought to clinics in areas of Rio de Janeiro for routine health procedures. Age differences were observed in the prevalence of *Giardia* in these dogs and cats in that a higher percentage of young animals (< 6 months–2 years) were positive for *Giardia* antigen than were animals 2 years of age or older.⁵ These findings are consistent with those of Meireles and coworkers.² However, in the study of parasite species in dogs residing in São Paulo, *Giardia* spp infections were significantly higher among adults than in young dogs.⁵ In another South American study, Huber and coworkers found no association of *Giardia* infection with age in a study of dogs in Paraná state.⁴ It is likely that several factors, unrelated to age, are contributing to the development of infections, and that the significant differences detected in the present study were incidental to these other unidentified factors.^{2,4,5}

Table 1. Results of In-Office ELISA Testing of Dogs and Cats for *Giardia* spp Infections in Rio de Janeiro, Brazil (2006-2007)

| Category | Species | <i>Giardia</i> spp antigen | |
|--------------------|----------|--------------------------------|--------|
| | | No. Positive/Total in Category | (%) |
| Age (yr) | | | |
| < 0.5 | Dog | 65/273 | (23.8) |
| | Cat | 12/58 | (20.7) |
| | Combined | 77/331 ^a | (23.6) |
| 0.5–2 | Dog | 52/343 | (15.2) |
| | Cat | 24/108 | (22.2) |
| | Combined | 76/451 ^a | (16.6) |
| >2–6 | Dog | 32/592 | (5.4) |
| | Cat | 35/217 | (16.1) |
| | Combined | 67/809 ^b | (8.3) |
| >6 | Dog | 30/629 | (4.8) |
| | Cat | 1/79 | (1.3) |
| | Combined | 31/708 ^c | (4.4) |
| Gender | | | |
| Male | Dog | 84/822 | (10.2) |
| | Cat | 30/200 | (15.0) |
| | Combined | 114/1022 | (11.2) |
| Female | Dog | 95/1015 | (9.4) |
| | Cat | 42/262 | (16.0) |
| | Combined | 137/1277 ^{NS} | (10.7) |
| Diarrhea | | | |
| Present | Dog | 23/228 | (10.1) |
| | Cat | 22/124 | (17.7) |
| | Combined | 45/352 | (12.8) |
| Absent | Dog | 156/1609 | (9.7) |
| | Cat | 50/338 | (14.8) |
| | Combined | 206/1947 ^{NS} | (10.6) |
| Region | | | |
| Rio de Janeiro | Dog | 79/1016 | (7.8) |
| | Cat | 65/365 | (17.8) |
| | Combined | 144/1381 ^a | (10.4) |
| Niterói | Dog | 51/348 | (14.7) |
| | Cat | 4/66 | (6.1) |
| | Combined | 55/414 ^a | (13.3) |
| Região dos Lagos | Dog | 29/188 | (15.4) |
| | Cat | 2/12 | (16.7) |
| | Combined | 31/200 ^a | (15.5) |
| Baixada Fluminense | Dog | 6/167 | (3.6) |
| | Cat | 1/16 | (6.3) |
| | Combined | 7/183 ^b | (3.8) |
| Others | Dog | 14/118 | (11.7) |
| | Cat | 0/3 | (0) |
| | Combined | 14/121 ^{ab} | (11.6) |

NS = Not significant

Values within columns of categories having different superscript letters are significantly different ($p < 0.01$)

Others = Mountain resorts, southern state and São Gonçalo municipality

Table 2. Results of In-Office ELISA Testing of Dogs and Cats for *Giardia* spp Infections in Rio de Janeiro, Brazil by Number of Animals in the Household

| No. of Animals in Household | Animals | Households |
|-----------------------------|--------------------|--------------------|
| | No. Positive/Total | No. Positive/Total |
| <5 animals | 81/1397 (5.8%) | 67/1058 (6.3%) |
| >5 animals | 150/749 (20%) | 22/53 (41.5%) |
| Total | 231/2146 (10.8%) | 89/1111 (8.0%) |

Data to identify all owners of the animals tested were incomplete, but enough data were collected to determine that households with 5 or more animals had a much higher prevalence of infected animals. Likewise, animals that resided with 4 or more animals were more likely to be positive for *Giardia* antigen. These were not unexpected findings, since animals within a household would be likely to encounter the same environmental conditions to be exposed to sources of *Giardia* infection. It is possible that a single animal or fewer than 5 animals within a household might be better controlled and less likely to be exposed to sources of infection; however, this is merely speculation, with no evidence to support or refute this hypothesis collected in the present study.

Regional differences in the prevalence of *Giardia*-infected animals in the study might be related to factors such as water sources, and conditions and environmental sanitation practices. Baixada Fluminense, which demonstrated the lowest prevalence of *Giardia* infection among the animals tested, is the coastal lowland region in the state of Rio de Janeiro. Intensive farming, especially near Rio de Janeiro, is practiced. Although much of the region was drained in the 1930s, it is a region that is extremely poor, and animals are generally not kept in good sanitary conditions. However, the authors hypothesize that perhaps the animals have multiple parasite infections, which leads to extreme competition among species. This in turn minimizes *Giardia* infection rates.

In an earlier study, 12.3% of 72 household dogs and 45% of 94 shelter dogs from the West Zone of Rio de Janeiro were

positive for *Giardia* cysts in the feces using centrifugation-flotation technique with saturated sugar solution.⁴ The authors stated that the actual prevalence of *Giardia* in these populations was most likely underestimated because the passage of *Giardia* cysts is intermittent, and only one

fecal sample was examined for each dog in the study. The researchers felt that repeated fecal examinations for dogs that tested negative for the first sample might have increased the infection rate in the population. Sensitivity of the Faust centrifugation-flotation technique using sugar solution or zinc sulfate has been reported to range from 50% to 70% for a single sample, but might be reach as high as 94% accuracy when repeated tests are performed.^{1,2}

Reliability of the flotation techniques using sugar solutions for diagnosing *Giardia* infections may be diminished because the high specific gravity of the solution tends to distort the cysts.⁶ Although recovery of *Giardia* cysts is reportedly improved somewhat by using zinc sulfate as opposed to a saturated sugar solution as the flotation medium, detection of the cysts in zinc sulfate solution is still rather difficult for inexperienced persons.⁶ In one study of animals with mixed parasite infections, the sugar solution performed better than the zinc sulfate solution for recovery of heavy parasite eggs in the feces. However, diagnosis of *Giardia* infection by both flotation techniques was inferior to the SNAP[®] *Giardia* Test.¹

Giardia cysts were identified in the feces from 5% of 119 household dogs and nearly 18% of 152 shelter dogs in São Paulo that were examined by a simple flotation, centrifugation-flotation and sedimentation techniques.⁵ In these studies, detection of *Giardia* cysts and *Cystoisospora* spp was significantly ($p < 0.05$) better with the Faust centrifugation-flotation technique than with the other two methods. However, it was concluded that none of the methods tested were able to identify all parasites in the dogs evaluated.

The Faust centrifugation technique, an assay using Sheather solution (modified by Benbrook) and polymerase chain reaction (PCR) techniques were used for detection of *Giardia* infections in 100 household dogs and 100 shelter dogs residing in metropolitan areas of Curitiba, Paraná state in southern Brazil.² The Faust centrifugation test detected significantly ($P < 0.05$) more samples containing *Giardia* cysts than the Benbrook technique did for both household and shelter dogs. Overall, the Faust technique detected *Giardia* in samples from 33 dogs (4% of shelter dogs and 9% of household dogs). PCR only detected 23 of the 33 samples (69%) that were positive by the Faust technique. Although the authors acknowledged the limited efficacy of the Faust technique, using it as the standard for comparison of the other two techniques, Benbrook's technique had a sensitivity of 66% and PCR had a sensitivity of 69%.

Even when recovery of *Giardia* cysts is successful by fecal flotation techniques, correct identification of the cysts can be very difficult, particularly without the proper training or microscopes capable of measuring the small cysts. Many pseudoparasites, including yeasts and plant debris, are frequently misidentified as *Giardia*.¹ In a wet lab conducted to evaluate the ability to identify cysts of *Giardia* and eggs of other parasites in the feces, 27 veterinarians and laboratory technicians who were each given a pooled fecal sample that they knew contained eggs of various intestinal parasites, *Cystoisospora* oocysts, and *Giardia* cysts.¹ Only 6 of the participants were able to recover and identify *Giardia* cysts by the various centrifugation techniques available in the lab. Conversely, all 27 participants obtained a positive result using the SNAP[®] *Giardia* antigen test.

Although some cases of giardiasis are asymptomatic, infections also produce weight loss, diarrhea, and poor growth rates in young animals.²⁻⁴ *Giardia* cysts in the environment, especially under crowded conditions, are somewhat difficult to remove,

and infected animals serve as a source of reinfection of other animals.⁷ Contamination of the environment can be minimized by prompt removal of feces from cages, kennels, and yards.

CONCLUSIONS

Given the relatively high prevalence of *Giardia* in this population of animals in Rio de Janeiro as well as that of dogs and cats in other surveys in other areas of South America and North America, and the potentially deleterious effects of severe clinical infections, it is important to be able to accurately and easily diagnose the presence of this parasite. Because the presence of cysts in the feces is sporadic, centrifugation-flotation techniques frequently fail to identify infected animals without examination of multiple samples. The SNAP[®] *Giardia* Test, with a sensitivity of 95% and a specificity of 99%, improves the ability of veterinarians and technicians to diagnose *Giardia* infections without requiring repeated tests or have extensive training to be able to recognize the cysts.

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