

## Prevalence of and Risk Factors for Feline *Tritrichomonas foetus* and *Giardia* Infection

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**Data were gathered for 117 cats from 89 catteries at an international cat show to examine prevalence and risk factors for feline *Tritrichomonas foetus* and *Giardia* infection. Prevalence of *T. foetus* was 31% among cats (36 out of 117) and catteries (28 out of 89) based on results of fecal smear examination (5 out of 36), fecal culture in modified Diamond's medium (9 out of 36), fecal culture in In Pouch TF medium (20 out of 36), or PCR amplification of the ribosomal RNA gene from feces with *T. foetus*-specific primers (34 out of 36). Catteries in which *T. foetus* was identified were more likely to have had a recent history of diarrhea, historical diagnosis of coccidia infection in adult cats, and a decreased number of square feet of facility per cat. Evidence did not exist for the ongoing transmission of *T. foetus* by water, food, or contact with other species.**

*Tritrichomonas foetus* was recently identified as a cause of large-bowel diarrhea in domestic cats (6, 7, 10, 15, 16). Based on morphology and sequence identity of rRNA, feline *T. foetus* is indistinguishable from bovine venereal *T. foetus* and porcine enteric *Tritrichomonas suis* (1, 2, 3, 8, 11, 13, 14, 18). The origin of *T. foetus* and the prevalence of infected cats are unknown. We thus performed an epidemiological study of feline *T. foetus* infection. The specific aims of the present study were to determine the prevalence of *T. foetus* infection within a geographically widespread group of suspected at-risk cats, to identify environmental risk factors for feline infection by *T. foetus*, and to determine the relative efficacy of direct fecal smear examination, fecal protozoal culture, and single-tube nested PCR for the diagnosis of *T. foetus* infection in naturally infected cats. Because of our clinical impression that *T. foetus* is often misidentified as *Giardia* and as a test of the ability of this study to disclose true risk factors for *T. foetus* infection if present, all cats were additionally tested for *Giardia* infection.

### MATERIALS AND METHODS

Survey distributions, fecal collection, and processing were performed at an international cat show in 2001. Catteries for which there was a completed survey and a freshly voided fecal sample from  $\geq 1$  cat present at the show were included. For each fecal sample, a single 0.9% saline smear was immediately prepared and viewed at  $\times 400$  magnification for motile trichomonads and *Giardia* trophozoites. A portion ( $\leq 0.1$  g) of feces was inoculated into In Pouch TF medium (Biomed Diagnostics; San Jose, Calif.) for the cultivation of *T. foetus* as described previously (4), and a portion (0.1 g) of the feces was suspended in 10 ml of sterile phosphate-buffered 0.9% saline and shipped overnight to the authors' laboratory for cultivation in modified Diamond's medium (Remel, Lenexa, Kans.) as described previously (4). Feces (2 g) were frozen, shipped same-day on dry ice to the authors' laboratory, and stored at  $-20^{\circ}\text{C}$ . Feces were examined for the presence of *Giardia*-specific antigen by enzyme-linked immunosorbent assay according to manufacturer instructions (ProSpecT *Giardia* microplate assay; Alexon-Trend, Ramsey, Minn.), and DNA was extracted from 200-mg samples of

feces and tested by means of PCR amplification of partial ITS1 and 5.8S ribosomal DNA by using *T. foetus*-specific primers as previously described (5).

Statistical analyses were performed with Analyze-It software (version 1.63; Analyze-It Software, Ltd., Leeds, England).

Fisher's exact test was used for the analysis of categorical data, and a chi-square test was used for variables with more than two responses. Odds ratios and 95% confidence intervals (using Woolf's approximation) were calculated where appropriate. All other variables were assessed using a Mann-Whitney U test. A calculated *P* value of  $\leq 0.05$  was considered statistically significant.

### RESULTS AND DISCUSSION

A voided fecal sample and completed survey were obtained for 117 cats from 89 catteries. This represented 12% of the total number of cats and 16% of the catteries present at the show. The sampled population of cats included 52 intact males, 41 intact females, 14 neutered males, and 4 spayed females. There were 66 adult cats ( $>6$  months of age) and 45 kittens ( $\leq 6$  months of age). The sexes and ages of the remaining six cats were not reported. Surveyed catteries contained a median number of 16 cats (range, 1 to 59), including a median of 10 adults (range, 8 to 12) and 6 kittens (range, 5 to 8).

The prevalence of *T. foetus* infection was 31% among the cats (36 out of 117) and catteries (28 out of 89) included in the study. Diagnosis of *T. foetus* infection was made on the basis of results of direct smear examination of feces (5 out of 36), culture of feces in modified Diamond's medium (9 out of 36), culture of feces with In Pouch TF (20 out of 36), or demonstration of *T. foetus* ribosomal DNA in feces by PCR (34 out of 36). The prevalence of *Giardia* sp. infection was 31% (36 out of 117) among the individual cats and 35% (31 out of 89) among the catteries tested. Diagnosis of *Giardia* sp. infection was made on the basis of results of fecal enzyme-linked immunosorbent assay for *Giardia*-specific antigen (36 out of 36). *Giardia* sp. trophozoites were not seen by direct smear examination of feces from any cat. Prior reports on the prevalence of *Giardia* have ranged from 2.4 to 60% depending on the source of cats, means of testing, and geographic location (9, 12, 17). Coinfection with *T. foetus* and *Giardia* sp. was diagnosed in 12% (14 out of 117) of cats and 16% (14 out of 89) of catteries.

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TABLE 1. Responses of cattery owners to survey questions relating to risk factors for *T. foetus* or *Giardia* infection<sup>a</sup>

Identified risk factor(s) (no. of catteries responding)	All catteries			Catteries with identified infection						
	No. (%)	Median (CI)	No. (%)	Median (CI)	OR (95% CI)	P	No. (%)	Median (CI)	OR (95% CI)	P
<b>Clinical signs</b>										
Loose stools or diarrhea in any cats within the past 6 mos ( <i>n</i> = 88)	62 (70)		24 (86)		3.47 (1.07–11.32)	0.052	23 (77)			
Loose stools or diarrhea in adult cats within the past 6 mos ( <i>n</i> = 85)	48 (56)		18 (69)				21 (75)		3.33 (1.22–9.07)	0.027
Known diagnosis in adults cats ( <i>n</i> = 89)	0 (0)		0 (0)				0 (0)			
<i>T. foetus</i>	13 (15)		3 (11)				6 (19)			
<i>Giardia</i> sp.	7 (8)		5 (18)		6.41 (1.16–35.43)	0.059	4 (13)			
Coccidia	0 (0)		0 (0)				0 (0)			
<i>Cryptosporidium</i>										
<b>Facilities and management</b>										
Cats allowed free roam of owners' living space ( <i>n</i> = 89)	76 (85)		26 (93)				30 (97)		7.83 (0.97–63.35)	0.044
Total number of cats in cattery ( <i>n</i> = 89)		16 (14–20)		16 (12–22)				20 (14–28)		0.085
Square feet of facility per cat ( <i>n</i> = 62)		84.5 (69–111)		71.4 (50–100)		0.056		71.4 (50–100)		0.024
<b>Population</b>										
Species other than cats in cattery ( <i>n</i> = 88)	33 (38)		7 (25)				17 (55)		3.11 (1.25–7.76)	0.025
Physical contact between cats and other species ( <i>n</i> = 88)	24 (27)		6 (21)				13 (42)		3.01 (1.14–7.97)	0.045
Any outdoor contact (direct or by contact with indoor-outdoor species) ( <i>n</i> = 89)	30 (34)		9 (32)				16 (52)		3.35 (1.33–8.46)	0.018
<b>Water source</b>										
Municipal or well ( <i>n</i> = 87)	75 (86)		25 (89)				30 (97)		7.76 (0.94–64.22)	0.053
Bottled ( <i>n</i> = 87)	12 (14)		3 (11)				1 (3)			
Drink from toilet ( <i>n</i> = 87)	9 (10)		3 (11)				4 (13)			

<sup>a</sup> CI, confidence interval; OR, odds ratio. *P* values that were ≤0.10 are shown.

An association between *T. foetus* and *Giardia* sp. infection was not significant ( $P = 0.075$ ).

Although clinical impressions have suggested that *T. foetus* is an infection of young cats (7), there were no differences in age or sex between uninfected cats and cats having *T. foetus* or *Giardia* sp. infection. The distribution of breeds and location of catteries infected with *T. foetus* or *Giardia* sp. were not different than the types of breeds and cattery locations present in the sample or overall show population. Risk factors significantly associated with either *T. foetus* or *Giardia* infection are shown in Table 1. Seventy percent (62 out of 88) of cattery owners reported having cats with diarrhea within the past 6 months. Affected catteries contained a median of two adult cats (range, 1 to 20) and a median of four kittens (range, 1 to 24) from two litters (range, 1 to 6) with diarrhea. There was a strong association between *T. foetus* infection and a history of diarrhea in the connected cattery. This finding supports the clinical significance of *T. foetus* infection as an associated cause of diarrhea in domestic cats. A history of diarrhea in kittens versus adults did not discriminate between catteries with and without *T. foetus*-infected cats. In contrast, catteries in which *Giardia*-infected cats were identified were significantly more likely to have had a history of diarrhea within the past 6 months involving adult cats. Notably, not a single cattery owner participating in the study was aware of *T. foetus* infection within their cattery. The survey results also provided no direct evidence that *T. foetus* organisms were historically misidentified as *Giardia* sp. In contrast, historical diagnosis of adult cats with coccidia infection was commonly reported by owners of catteries containing cats with *T. foetus*. This association is intriguing, insofar as coccidiosis is uncommon in adult cats. Because of their dissimilar appearance, it is unlikely that *T. foetus* was misidentified as coccidia in these cats. Another consideration for the failure to recognize the presence of *T. foetus* infection is whether veterinary care was sought for the majority of catteries where diarrhea was reported. A recent (<6 month) history of diarrhea was highly prevalent (70%) among the catteries. In particular, *T. foetus*-associated diarrhea waxes and wanes, is semiformed to a soft, unformed consistency rather than liquid, and is unassociated with signs of systemic illness (7). Although we did not score the fecal samples submitted by participants in the study, the consistency of the samples appeared to vary widely and may underscore a range of fecal consistencies considered normal or tolerable by cattery owners.

With regard to housing facilities and management practices, catteries with *Giardia*-infected cats housed larger numbers of cats than noninfected catteries. High housing density (low number of square feet of facility area per cat) was identified as a likely risk factor for both *T. foetus* and *Giardia* sp. infections and may account for the similar prevalences of the two infections in the cats reported here. Numerous risk factors were identified for the presence of *Giardia* and not for *T. foetus* infection. These risk factors are likely to reflect key differences in the life cycle between the two organisms. While both organisms are transmitted by the fecal-oral route, *Giardia* forms highly resistant, environmentally stable cysts, while *T. foetus* is incapable of prolonged survival outside the host. Thus, the potential for environmental contamination and exposure to cysts was identified as an important risk factor for *Giardia* infection but not for *T. foetus* infection. For example, the

presence of nonfeline species in the cattery and their physical contact with cattery cats were significant risk factors for *Giardia* sp. infection. Access to the outdoors was not a significant risk factor for either infection, although only two cattery owners actually allowed cats free range while outdoors. However, cattery owners allowing contact between cats and other species that were permitted indoor-outdoor access were at increased risk for having cats with *Giardia* sp. infection. Among cattery owners having cats diagnosed with *Giardia* sp., all but one reported use of municipal or well water. There was no apparent association between litter box management or type of litter used and the presence of either infection within the cattery.

With regard to *T. foetus*, we found no association between infection and any environmental variable aside from dense population housing. The proximity of the cattery to within 0.5 miles of agricultural species, type of diet fed to cattery cats (commercial or home cooked), and source of water were not associated with risk of infection. More than 40% of cattery owners also fed their cats table scraps, raw meat, catnip, or other supplements, none of which were significant risk factors.

Cattery owners traveled a median of 24 times per year for the purpose of showing their cats, and such travel was not statistically significantly associated with a risk of either infection. According to cattery owners, diseases of the ocular and respiratory systems, diarrhea, and skin disorders were relatively common in cats following the attendance of a cat show. When considered alone or collectively, these acquired illnesses were not statistically significantly associated with the presence of either infection. Cattery owners reported acquiring cats from 16 different countries. Acquisition of cats from outside the United States was not associated with an increased risk for either infection. Thus, the present study provides no direct evidence for recent or ongoing acquisition of *T. foetus* from other species (particularly cattle or swine), dietary sources (including ingestion of raw meat or toilet water), or exposure to international or domestic travel. However, these sources cannot be ruled out as the origin of *T. foetus* infection for the feline population at some time in the past. Assessment of the relatedness of feline *T. foetus* to bovine and porcine isolates may provide some insight into the potential origin of the feline organisms.

In conclusion, the present study demonstrates a high prevalence of *T. foetus* infection in purebred domestic show cats and a strong association between infection and the presence of diarrhea within the connected cattery. Based on a comparison of diagnostic methods, we concluded a relative efficacy for the detection of *T. foetus* organisms to be as follows: direct fecal smear examination < fecal culture < single-tube nested PCR. These results suggest that *T. foetus* is likely to be greatly underdiagnosed if fecal smears are the only means used for diagnosis. There is currently no effective antimicrobial treatment for *T. foetus* infection. Thus, it may be that the clearest and perhaps most preventable risk factor for *T. foetus* infection was a high density of cats housed within a facility.

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## REFERENCES

1. De Carli, A., and J. Guerrero. 1976. Antigenic comparison between *Tritrichomonas suis* and *T. foetus*. II. Gel immunodiffusion. Rev. Latinoam. Microbiol. **18**:167–171.
2. Doran, D. J. 1957. Studies on trichomonads. I. The metabolism of *Tritrichomonas foetus* and trichomonads from the nasal cavity and cecum of swine. J. Protozool. **4**:182–190.
3. Felleisen, R. S. J. 1997. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitology **115**:111–119.
4. Gookin, J. L., D. M. Foster, M. F. Poore, M. E. Stebbins, and M. G. Levy. 2003. Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats. J. Am. Vet. Med. Assoc. **222**:1–4.
5. Gookin, J. L., A. J. Birkenheuer, E. B. Breitschwerdt, and M. G. Levy. 2002. Single-tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. J. Clin. Microbiol. **40**:4126–4130.
6. Gookin, J. L., M. G. Levy, J. M. Law, M. G. Papich, M. F. Poore, and E. B. Breitschwerdt. 2001. Experimental infection of cats with *Tritrichomonas foetus*. Am. J. Vet. Res. **62**:1690–1697.
7. Gookin, J. L., E. B. Breitschwerdt, M. G. Levy, R. B. Gager, and J. G. Benrud. 1999. Diarrhea associated with trichomonosis in cats. J. Am. Vet. Med. Assoc. **215**:1450–1454.
8. Hampl, V., A. Pavlicek, and J. Flegr. 2001. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. Int. J. Syst. Evol. Microbiol. **51**:731–735.
9. Hill, S. L., J. M. Cheney, G. F. Taton-Allen, J. S. Reif, C. Bruns, and M. R. Lappin. 2000. Prevalence of enteric zoonotic organisms in cats. J. Am. Vet. Med. Assoc. **216**:687–692.
10. Levy, M. G., J. L. Gookin, M. F. Poore, A. J. Birkenheuer, M. J. Dykstra, and R. W. Litaker. 2003. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline trichomonal diarrhea. J. Parasitol. **89**:99–104.
11. Mattos, A., A. M. Sole-Cava, G. DeCarli, and M. Benchimol. 1997. Fine structure and isozymic characterization of trichomonadid protozoa. Parasitol. Res. **83**:290–295.
12. McGlade, T. R., I. D. Robertson, A. D. Elliot, and R. C. A. Thompson. 2003. High prevalence of *Giardia* detected in cats by PCR. Vet. Parasitol. **110**:197–205.
13. Pakandl, M., and L. Grubhoffer. 1994. Some properties of the sialic-acid binding systems in *Tritrichomonas suis* and *Tritrichomonas foetus*. Comp. Biochem. Physiol. **108**:B529–B536.
14. Robertson, M. 1960. The antigens of *Tritrichomonas foetus* isolated from cows and pigs. J. Hyg. 9 Camb. **58**:207–212.
15. Romatowski, J. 1996. An uncommon protozoan parasite (*Pentatrichomonas hominis*) associated with colitis in three cats. Feline Pract. **24**:10–14.
16. Romatowski, J. 2000. *Pentatrichomonas hominis* infection in four kittens. J. Am. Vet. Med. Assoc. **216**:1270–1272.
17. Spain, C. V., J. M. Scarlett, S. E. Wade, and P. McDonough. 2001. Prevalence of enteric zoonotic agents in cats less than 1 year old in central New York State. J. Vet. Intern. Med. **15**:33–38.
18. Tachezy, J., R. Tachezy, V. Hampl, M. Edinova, J. Flegr, and J. Kulda. 2002. Cattle pathogen *Tritrichomonas foetus* (Riedmuller, 1928) and pig commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) belong to the same species. J. Eukaryot. Microbiol. **49**:54–163.