



# Intestinal *Tritrichomonas foetus* infection in cats: a retrospective study of 104 cases

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## Abstract

The clinical presentation and response to treatment of cats infected with *Tritrichomonas foetus* have not been sufficiently described in a large number of pet cats. The aim of this study was to collect and analyze clinical data from pet cats diagnosed with intestinal *T foetus* infection. Clinical information was collected for 104 cats that tested polymerase chain reaction-positive for *T foetus*. The most common clinical sign was diarrhea (98%) with a median duration of 135 days (range 1–2880 days). Forty-nine of 83 (59%) cats had diarrhea since adoption. Other clinical signs included anorexia (22%), depression (24%), weight loss or failure to gain weight (20%), vomiting (19%), abdominal pain (9%) and increased appetite (3%). A total of 45 cats had completed treatment with ronidazole, 29 of which (64%) showed a good clinical response to treatment. Sixteen (36%) cats had either partial or no improvement, or a relapse shortly after discontinuation of treatment.

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Over the past decade, the protozoan parasite *Tritrichomonas foetus* has emerged as a new and important cause of feline diarrhea with virtually worldwide distribution.<sup>1–5</sup> Feline *T foetus* isolates were initially believed to be identical to the bovine isolates, but recent genetic studies have shown that *T foetus* from domestic cats and cattle are genetically distinct.<sup>6–8</sup> Furthermore, a recent study suggested that feline intestinal trichomoniasis might be caused by a new *Tritrichomonas* species (*Tritrichomonas blagburni* nova species).<sup>9</sup> Further studies are needed to elucidate the classification, genetics, and properties of the feline *T foetus* isolates and their similarities to *Tritrichomonas* isolates from other species.<sup>9,10</sup>

The pathogenesis of *T foetus* infection in the cat has been investigated in experimental studies and the pathogenicity of the organism in cats is unequivocal.<sup>1,4,11,12</sup> However, it has been shown that many cats, especially those of older age, may be asymptotically infected with *T foetus*.<sup>13–17</sup> Natural *T foetus*-associated intestinal disease has been described mainly in cats from multi-cat environments, such as catteries,<sup>14,16</sup> shelters<sup>18,19</sup> or cats presented at cat shows.<sup>5,17,20</sup> The signalment, history, clinical presentation and response to treatment in a large number of pet cats naturally infected with *T foetus* have not been sufficiently described.<sup>11,13,15,21</sup> Therefore, the aim of the present study was to collect and analyze

clinical data from pet cats that were diagnosed with intestinal *T foetus* infection.

## Materials and methods

### Case enrolment

The database of the Gastrointestinal Laboratory at Texas A&M University (College Station, TX, USA) was searched for the period January 2006 to December 2009 for cats that had tested polymerase chain reaction (PCR)-positive for *T foetus* in fecal samples. The veterinarians who had submitted the fecal samples of the cats that were positive for *T foetus* were contacted and asked to fill

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out a questionnaire for each cat. Questions covered date of birth, sex and sexual status, body weight and body condition score (BCS), medical history, clinical signs, diagnostics performed, concurrent conditions and treatment. Emphasis was given on the onset, severity and duration of gastrointestinal clinical signs that were potentially associated with intestinal *T foetus* infection. For the purposes of the present study, diarrhea was defined as one or more episodes of the passage of feces that contained more water than normal.

#### Diagnosis of intestinal *T foetus* infection

The diagnosis of *T foetus* infection was based on detection of the microorganism's DNA in fecal samples, using a single-tube nested PCR, as described previously.<sup>13</sup> Fecal samples had been submitted to the Gastrointestinal Laboratory for evaluation of potential *T foetus* infection. DNA for PCR analysis was extracted from fecal samples within 3 days of receipt (during which time they were kept frozen at -4°C) using a commercially available DNA extraction kit (ZR Fecal DNA kit; Zymo Research), which has been validated for the extraction of DNA for the detection of *T foetus* DNA in fecal samples from cats.<sup>22</sup> Fecal samples from all cats were extracted in duplicate fashion, and negative controls (sterile water) were extracted with each batch to ensure that contamination of extraction reagents was not present. The quality of DNA and presence of endogenous PCR inhibitors in each DNA sample were assessed by PCR amplification of a 876 base pair sequence of the bacterial 16S ribosomal RNA gene using universal bacterial primers (517F [5' GTGCCAGCAGCCGCGGTAA 3'] and 1391R [5' GACGGGCGGTGAGTGCA 3']), as described previously.<sup>23</sup> The single-tube nested PCR was carried out using two sets of specific primers targeting the *T foetus* internal transcribed spacer region (ITS1 and ITS2) and the 5.8S rRNA gene.<sup>23</sup> Positive *T foetus* DNA purified from a positive culture specific for this parasite (In PouchTF; Biomed Diagnostics)<sup>24</sup> and negative (sterile water) controls were run with each batch to identify failure of the assay (positive control), or reagent contamination (negative control) respectively. PCR amplicons were verified by electrophoresis on 2% agarose gels (E-Gels 2%; Invitrogen) and visualized under ultraviolet light.

#### Statistical analysis

All statistical analyses were conducted using a commercially available statistical software program (Prism 5; GraphPad). Continuous data were tested for normality using the Kolmogorov–Smirnov test. Descriptive statistics were performed for all variables. Variables with a Gaussian distribution were reported as mean ± standard deviation (SD), while variables with a non-Gaussian distribution were reported as median and range. The frequencies of clinical signs were reported as percentages.

Ninety-five percent confidence intervals (95% CI) were reported for all variables.

## Results

#### Study population

Questionnaires for 104 cats living in the USA and Canada were completed and returned to the Gastrointestinal Laboratory. The number of cats per household was reported for 92 cases; the median number of cats per household was two (range 1–50). Ninety-one (90%)/101 for which information was available were exclusively indoor, while 10/101 cats (10%) were both indoor and outdoor.

#### Signalment

Fifty-four of 104 cats (52%) were purebred. A total of 24 breeds were represented, the most common being domestic shorthair (DSH) (33/104; 32%), Bengal (13/104; 13%), Persian (6/104; 6%), Abyssinian (6/104; 6%), Ragdoll (5/104; 5%), Maine Coon (4/104; 4%), domestic longhair (4/104; 4%), domestic mediumhair (4/104; 4%) and Siamese (4/104; 4%).

Forty-six (44%) cats were female and 58 (56%) were male. The median age of the cats was 1 year, with a range of 1 month to 16.3 years. Fifty-eight (56%) cats were 12 months of age or older at the time of *T foetus* diagnosis (Figure 1). The mean BCS was 2.9/5.

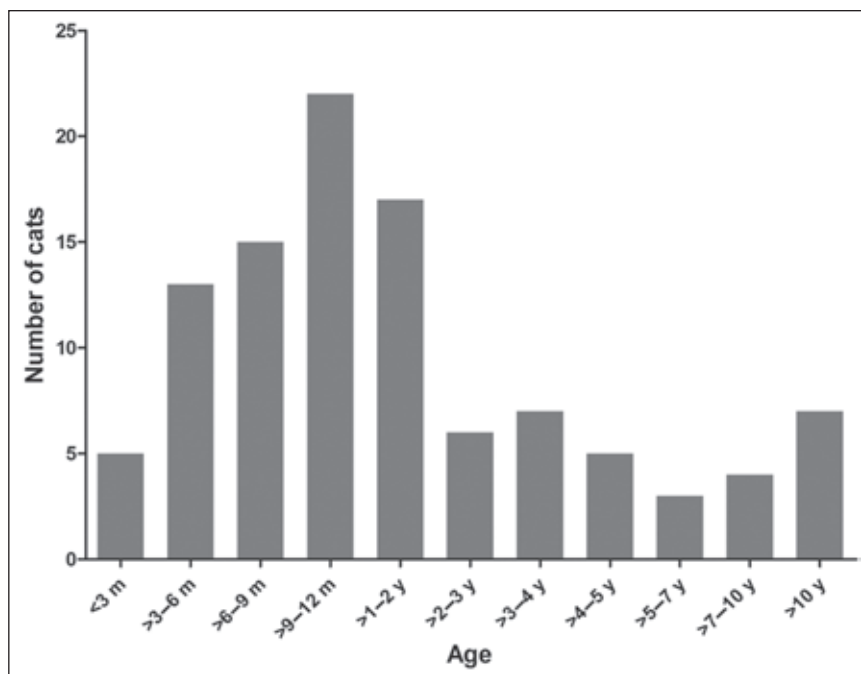
#### Clinical signs

The most common clinical sign was diarrhea, which was reported in 101/103 cats (98%). The median duration of the diarrhea was 135 days, with a range of 1 day to 7.9 years. Forty-nine of 83 cats (59%) were reported to have had diarrhea since adoption. The fecal consistency was reported in 86 cases; 36 (42%) cats had soft stool, 29 (34%) had watery stool, and 21 (24%) cats were reported to have both watery diarrhea and soft stool. Haematochezia was reported in 45/99 cats (46%), tenesmus in 42/98 cats (43%) and mucus in 59/100 cats (59%). The frequency of episodes of diarrhea ranged from 1 to 8 times per day. The fecal characteristics, the clinical signs related to the diarrhea and their frequencies are shown in Table 1.

Other clinical signs reported included depression (24/102 cats; 24%), decreased appetite or anorexia (22/103 cats; 22%), weight loss (20/101 cats; 20%), vomiting (20/103 cats; 19%), abdominal pain (9/103 cats; 9%) and increased appetite (3/103 cats; 3%).

#### Other intestinal parasites

Fecal flotation had been performed in 85 cases and three of those cats (4%) were found to be positive for coccidia. Testing for *Giardia* species had been performed in 67 cats using different test methods (eg, SNAP *Giardia*, zinc flotation); 15 (22%) of those cats were positive for *Giardia* species.



**Figure 1** Age distribution of cats diagnosed with intestinal *Trichostrongylus axei* infection. m = months; y = years

**Table 1** Fecal characteristics, clinical signs related to the diarrhea and their frequencies in cats with diarrhea and intestinal *Trichostrongylus axei* infection

Fecal characteristics and clinical signs related to the diarrhea	Percentage of cats
Semi-formed feces	41
Watery feces	33
Semi-formed/watery feces	24
Mucus	59
Hematochezia	46
Tenesmus	43
Melena	7

### Treatment

A total of 79/102 cats (78%) were treated with ronidazole. Of these, 30 cats were either still early on the treatment cycle with ronidazole at the time of the study and therefore the effect of treatment could not be appreciated or were lost to follow-up. In four cats, the treatment was discontinued owing to neurological signs ( $n = 3$ ) and/or anorexia ( $n = 3$ ). Of the remaining 45 cats, 29 (64%) had a good clinical response to treatment, defined as complete or close to complete resolution of clinical signs. Sixteen cats (36%) were reported to have either partial or no improvement, or a relapse shortly after discontinuation of treatment. Ten of those cats (22% of 45 cats) had been treated with the currently recommended dosages of ronidazole (30 mg/kg, q24h, for 2 weeks;  $n = 4$ ) or higher ( $n = 6$ ). Five cats had been treated with doses lower than

those currently recommended, while one cat had been treated for only 1 week.

### Discussion

To our knowledge, this is the largest study investigating the clinical picture and response to treatment of pet cats naturally infected with *T. foetus*. While a plethora of other studies have described the clinical picture of cats with intestinal *T. foetus* infection, the vast majority of them<sup>5,14,16-20</sup> have enrolled cats living in multi-cat environments, such as catteries and shelters, which may not reflect the average pet cat population living at homes alone or in small numbers.

Diarrhea was by far the most common clinical sign in the present study, which was present in almost all cats. Because diarrhea has been described as a major clinical

sign associated with clinical trichomonosis in cats,<sup>4,11,15,16,19–21</sup> the submitting veterinarians might have been biased in testing mainly for *T foetus* cats that had diarrhea. However, in agreement with our findings, studies that have investigated the prevalence of *T foetus* infection in cats both with and without diarrhea, have reported that diarrhea typically represents the most common clinical sign.<sup>16</sup> More importantly, a study that investigated pet cats in the USA reported that all 17 cats that were positive for *T foetus* (100%) presented with chronic diarrhea.<sup>21</sup> The duration of the diarrhea in the present study ranged from 1 day to several years. Previous studies have shown that *T foetus*-induced diarrhea typically resolves within 2 years in most cats.<sup>11</sup> However, as suggested by the present study, diarrhea might persist for much longer in some cats.

Interestingly, 59% of 83 cats were reported to have diarrhea since adoption. This might suggest that the majority of pet cats acquire the infection in catteries, shelters, pet shops, etc, where cats live in densely populated environments. Although epidemiological differences may occur in this disease depending upon the geographical region, these environments, especially catteries, have been shown repeatedly to represent important risk factors for *T foetus* infection in cats.<sup>15</sup> The present study suggests that cats might be living in single-cat environments for years and still display clinical signs of an infection that they acquired during early stages of their life while they were still living in multi-cat environments. Therefore, *T foetus* infection should not be considered unlikely in cats with chronic diarrhea that live in single-cat environments.

About 20% of cats each had complete or partial anorexia, depression, vomiting and/or weight loss. These clinical signs have not been commonly associated with *T foetus* infection in previous studies,<sup>16</sup> although there are some exceptions, especially in young cats and kittens.<sup>15,19</sup> These systemic clinical signs might be the result of concurrent intestinal infections or other diseases and not *T foetus* infection per se. Although enteric co-infections have frequently been documented in *T foetus*-infected cats, their association with clinical signs has not been well established.<sup>5,21</sup> In recent studies, co-infection with *Giardia* species or coccidia was not associated with the prevalence or severity of diarrhea in *T foetus*-positive cats, and clinical signs of *T foetus* infection were not exacerbated by co-infection with other intestinal parasites.<sup>16,17</sup> Regardless, the findings of the present study might suggest that in cats without the expected typical clinical signs (ie, chronic or intermittent large bowel diarrhea without systemic signs), *T foetus* infection should not necessarily be ruled out without testing because it may contribute to the clinical signs caused by other diseases.

The exact association between feline *T foetus* infection, the development of clinical signs associated with *T foetus*

infection, and age has not been fully elucidated. Although the majority of studies have suggested that *T foetus* primarily infects and causes disease in kittens and young cats,<sup>11,15,16,21,25,26</sup> it is increasingly recognized that older cats are also often infected with *T foetus*.<sup>13,14,17,19</sup> In one study at a rescue colony in Italy the majority of infected cats (67% of 24 cats infected) was found to be older than 1 year of age.<sup>19</sup> In another study that investigated healthy cats presented at cat shows in Norway, the mean age of *T foetus*-infected cats was 20.1 months, and 55% of these cats were >1 year of age.<sup>17</sup> Similar to the findings of the two above-mentioned studies, most cats (nearly 56%) in our study were 1 year of age or older and about 7% of cats were more than 10 years of age. The reason for the discrepancy among studies regarding the age of affected cats is not known, but differences in study populations (which may be related to differences between cats living in catteries and cats kept as pets) and previous exposure to *T foetus* may have played a role. Regardless of the reason, these data suggest that *T foetus* should not be considered an unlikely diagnosis in older cats with a compatible history and compatible clinical signs.

Reports regarding predisposition of different cat breeds to *T foetus* infection have also been puzzling. Different studies have identified different breeds as being at high risk for *T foetus* infection, and results from one study are not typically verified by subsequent studies.<sup>15,16,21,26,27</sup> In general, a genetic predisposition of pure-bred cats to *T foetus* infection has previously been suggested by some, but the observed over-representation of pure-bred cats might be a result of high-risk husbandry conditions of many catteries rather than true genetic predisposition.<sup>5,11,15</sup> In the present study, although several breeds were over-represented, DSH cats were by far the most commonly affected. This finding is in accordance with the findings of another study at a rescue colony in Italy.<sup>19</sup> However, the results of our study might be simply because DSH cats are very commonly kept as pets, while previous studies have mainly evaluated cats from catteries and cat shows where cats are exclusively pure-bred. Inclusion of a reference feline population could have provided more accurate interpretations of breed predispositions in the present study, but, unfortunately, one was not available. The susceptibility of certain feline breeds or pure-bred cats, in general, to *T foetus* infection requires further study. However, any cat, regardless of breed, could be infected with *T foetus*.

In accordance with previous studies,<sup>5,15,16,20,21</sup> a relatively large percentage of cats (more than 20%) tested were found to have concurrent *Giardia* species infection. A smaller portion of cats (4%) was found to have concurrent infection with coccidia. These percentages should be interpreted with caution because different methods were used by the submitting veterinarians to test for each parasite. Ideally, the submitted fecal samples should have



been tested using the same methods, but this was not possible owing to the retrospective nature of the study. Although *T foetus* and *Giardia* species co-infections seem to occur frequently, a recent study that investigated naturally infected cats did not find any association between co-infection with other enteric parasites (eg, *Giardia* species or coccidia) and *T foetus*.<sup>16</sup> In the same study,<sup>16</sup> and in agreement with another study,<sup>15</sup> *T foetus*-associated diarrhea was not exacerbated by infection with *Giardia* species or coccidia, and infection with *T foetus* alone was sufficient to cause clinical signs. Therefore, the clinical picture of the cats described in the present study was unlikely to have been affected by co-infection with other enteric parasites.

Sixty-four percent of cats treated with ronidazole were judged to have a good response to treatment, while about 36% of cats had an inadequate response to or a relapse shortly after treatment. This is in disagreement with the results of a smaller study in Australia, in which ronidazole was effective in almost 100% of treated cats.<sup>15</sup> About 60% of the cats that had an inadequate response to ronidazole or a relapse shortly after treatment was only treated for one 2-week cycle with ronidazole at a commonly recommended therapeutic regimen (30 mg/kg, q24h PO), which might suggest that this therapeutic regimen might not be effective in all cats. This is supported by the findings of another study,<sup>19</sup> in which some of the cats treated with ronidazole continued to have occasional loose stools. In a study by Gookin et al,<sup>28</sup> it was recommended that cats that have a relapse should be treated with higher dosages of ronidazole (30–50 mg/kg PO q12h for 14 days).<sup>28</sup> Also, lower ronidazole dosages (10 mg/kg q24h) were associated with relapses in this study.<sup>28</sup> Alternatively, the relapses in this group of cats might have been associated with worsening or inadequate control of concurrent diseases (including co-infection with other enteric pathogens), resistance to ronidazole<sup>29</sup> and/or poor owner compliance. Another possible explanation for poor response to ronidazole treatment in the present study is problems with compounding of the drug or the use of inappropriate ronidazole preparations. Owing to the retrospective nature of the study, information on compounding and specific preparations was not available. About 40% of the cats that had an inadequate response to or a relapse shortly after treatment had actually been treated with ronidazole dosages that are considered inadequate. This means that treatment failure is often due to the prescription of inadequate dosages of ronidazole. Ideally, the response to treatment should be judged in the light of a follow-up PCR examination of fecal samples. However, this is often not done owing to financial constraints, as was the case in the present study, in which the vast majority of the submitting veterinarians had not submitted a follow-up sample. The effectiveness of ronidazole in the treatment of feline intestinal *T foetus*

infection requires further investigation in prospective and controlled clinical trials.

Neurotoxicity is the most commonly reported and serious side effect of ronidazole treatment in cats.<sup>15,28,30</sup> In the present study, neurological signs were observed in 3/49 cats (6%) treated with ronidazole. Anorexia was another relatively common clinical sign associated with ronidazole treatment in the present series.

## Conclusions

In this study, the majority of cats infected with *T foetus* were DSH cats, and slightly more than 50% of cats were over 1 year of age. Also, the majority of infected cats were not from multi-cat environments. Diarrhea was the predominant clinical sign, and most cats had diarrhea since adoption. Other clinical signs, such as anorexia, depression, vomiting and weight loss, were less commonly present. In most cats, ronidazole treatment at the currently recommended dosage was efficacious in ameliorating clinical signs. However, in some cats clinical signs persisted despite use of ronidazole at the currently recommended dosages.

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