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SHORT COMMUNICATION
Detection of *Tritrichomonas foetus* in cats in Greece

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Intestinal infection of cats with *Tritrichomonas foetus* has been reported in the USA, Canada, several European countries, and Australia. However, *T foetus* has not been previously reported in cats in Greece. The aim of this study was to test fecal samples from cats living in Greece for the presence of *T foetus* DNA. Feces were collected from 31 cats living in Greece. DNA was extracted from the fecal samples and the presence of *T foetus* DNA was detected by a single-tube nested polymerase chain reaction (PCR). *T foetus* specific DNA was detected in the feces of 6/30 (20.0%) cats. All six cats were reported to have normal fecal quality at the time of sample collection and five of them were adults. The present study confirms for the first time the presence of *T foetus* in cats in Greece and suggests that *T foetus* infection is often asymptomatic in older cats.

Tritrichomonas foetus has been recently recognized as an important cause of diarrhea in cats in many countries around the world. *T foetus* is a flagellated protozoan parasite that was first described as a venereal pathogen in cattle causing infertility, abortions and pyometra.[1] *T foetus* is identical to *Tritrichomonas suis*, which is commonly found in the nasal cavity and gastrointestinal tract of pigs, and those two names are now considered to be synonyms as they represent the same organism.[2] The presence of trichomonads in the intestinal tract or feces of domestic cats was first described in several countries in the 1920s, but gastrointestinal signs associated with these organisms were generally not reported.[2] The first report of gastrointestinal disease (lethargy, weight loss, diarrhea) caused by a trichomonad in a cat was in 1956.[1]

In 1996 and 2000, Romatowski reported several cases of large-bowel diarrhea in cats that were associated with an intestinal trichomonad infection.[3,4] The causative organism in these reports was identified as *Pentatrichomonas hominis*. However, in 2003, Levy et al showed that *T foetus* and not *P hominis* was the etiological agent of feline trichomonal diarrhea.[2] *T foetus* has been shown to fulfil Koch's postulates and is currently recognized as a primary intestinal pathogen in cats.[2,5] *T foetus* primarily colonizes the surface of the distal ileal and colonic mucosa, leading to chronic or intermittent large-bowel diarrhea.[5] Although *T foetus* was initially described as an intestinal pathogen in cats in the USA, it has since also been reported in Canada, several European countries (including the UK, Germany, Austria, Switzerland and Italy) and Australia.[2,6–10] Intestinal *T foetus* infection in cats has not been previously reported in Greece. The aim of this study was to test cat feces collected from cats living in Greece for the presence of *T foetus* DNA.

Feces were collected from 31 cats living in Greece. Twenty-six cats were client-owned, three belonged to the cattery of the School of Veterinary Medicine, University of Thessaly, and two were stray cats. Twenty-five cats were domestic shorthairs, three were Siamese, two were Persian and one was a Persian-cross. The median age of the cats enrolled was 3 years (range: 0.1–12 years). The majority of cats (28/31) were older than 1 year of age. A total of 25 cats (80.6%) were exclusively indoor cats and 20 cats (64.5%) were housed with at least one other cat. Six cats had a history of current diarrhea at the time of sample collection and four more cats had a past history of diarrhea; the remaining 21 cats were reported to have normal stools (19 cats) or an unknown fecal quality (two stray cats).

DNA was extracted from all fecal samples using a commercially available DNA extraction kit (ZR Fecal DNA kit, Zymo Research), which has been validated

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for the extraction of DNA for the detection of *T foetus* DNA in fecal samples from cats.[11] Fecal samples from all 31 cats were extracted in duplicate fashion, and negative controls (using sterile water) were extracted with each batch. The quality of DNA and presence of endogenous polymerase chain reaction (PCR) inhibitors in each DNA sample were assessed by PCR amplification of a 876 base pair (bp) sequence of bacterial 16S ribosomal RNA gene using universal bacterial primers (517F [5′ GTGCCAGCAGCCGCGGTAA 3′] and 1391R [5′ GACGGGCGGTGAGTGCA 3′]) as previously described.[11] The presence of *T foetus* DNA was detected by a single-tube nested PCR as previously described[12] with some modifications (HotStar Taq MasterMix kit, Qiagen). 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. Positive (*T foetus* DNA purified from a positive culture specific for this parasite [In PouchTF [Biomed Diagnostics, Inc, White City, OR, USA]]) 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 separated by electrophoresis on 4% agarose gels (E-Gels 48 4%, Invitrogen) and visualised under ultraviolet light.

Statistical analysis was performed using a commercially available statistical software package (Prism5, GraphPad, San Diego, CA, USA). Age was compared between groups using a Mann−Whitney test and comparison of proportions was performed using a Fisher's exact test.

Based on the results of the PCR using universal bacterial primers, 1/31 samples was excluded because no amplification product was identified. *T foetus* specific DNA was detected in the feces of 6/30 (20.0%) cats. None of the six cats had diarrhea at the time of sample collection, while 2/6 cats (33.3%) had a known past history of diarrhea (approximately 12 months before sample collection). All six cats that were positive for *T foetus* were exclusively indoor cats. Three of the six cats (50%) were housed with at least one other cat. Of the remaining 24 cats that were negative for *T foetus*, 7 (29.2%) cats had a current or previous history of diarrhea and two had an unknown fecal quality. There was no difference in the proportion of cats with a history of diarrhea between *T foetus*-infected and uninfected cats ($P = 0.82$). The age was not significantly different between cats infected with *T foetus* (median: 3 years) from those that were not (median: 3 years; $P = 0.53$). Five of the six cats infected with *T foetus* were adults (>1 year of age; age range: 6 months−9 years).

The present pilot study confirms for the first time the presence of *T foetus* in cats in Greece. The proportion of *T foetus*-positive cats (20.0%) was similar to that of previous studies (10−32% depending on the location and population sampled).[7,9,13–15] This percentage might seem higher than expected, however, if one considers the fact that the majority of cats in the present study did not belong to high-risk populations (eg, young cats, pure-bred cats and cats living in multi-cat households).[14]

The results of the present study also suggest that *T foetus* infection is often asymptomatic in older cats. The importance of asymptomatic *T foetus* infection and its role in the epidemiology of the disease are not clearly defined. Published studies have mostly focused on the association between intestinal *T foetus* infection and current diarrhea,[7,9,13,15] or a recent history of diarrhea.[14] Due to the small number of cats with diarrhea in the present study it is difficult to draw conclusions regarding the association between *T foetus* and diarrhea in this specific cat population. However, the pathogenicity of *T foetus* in cats has been confirmed by other studies.[2,5]

In conclusion, the present study has confirmed cases of intestinal *T foetus* infection in cats in Greece. Thus, *T foetus* should be considered as a differential diagnosis for cats with diarrhea living in Greece. Infected but asymptomatic older cats might be an important source of infection for other cats. Further studies are needed to more accurately determine the prevalence of *T foetus* in the general cat population in Greece as well as the importance of asymptomatic cats in the epidemiology of this infection.

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