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The first report of clinical case of intestinal trichomoniasis caused by *Tritrichomonas foetus* in a cat with chronic diarrhea in Iran

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Abstract

Background: *Tritrichomonas foetus* is recently found to be one of the causes of chronic diarrhea in domestic cats but there is no report of feline intestinal trichomoniasis in Iran. **Case description:** A 9 months female domestic short-haired cat was referred to our hospital with a history of chronic diarrhea over the past two months, and with an unsuccessful previous antibiotic therapy in another clinic. **Findings/treatment and outcome:** Clinical examination revealed 39.8°C rectal temperature. Complete blood count (CBC) showed moderate inflammatory leukogram. By the wet-mount faecal assessment, moving organisms were observed. Rectal scarping dry-mount cytology revealed many inflammatory cells along with huge numbers of trophozoites with typical morphologic characteristics including central axostyle, undulating membrane, flagella, and one laterally located nucleus. Polymerase chain reaction (PCR) was performed and confirmed the diagnosis of *T. foetus*. After treatment with metronidazole and probiotics, the cat was completely recovered. **Conclusion:** This study represents the first case of intestinal trichomoniasis in a cat with chronic diarrhea in Iran.

Key words: Cat, Chronic diarrhea, Iran, *Tritrichomonas foetus*

Introduction

Trichomonads are protozoans with flagella that belong to Parabasalia class (Tolbert and Gookin, 2016). Although there are many known trichomonads, only a few species are regarded as pathogens (Tolbert and Gookin, 2016). These highly motile trophozoites have a 1-stage life cycle and asexual binary fission reproduction (Felleisen, 1999; Tolbert and Gookin, 2016). They are most likely found close to the mucosal surface rather than in the lumen of colonic crypts (Yaeger *et al.*, 2005). The colonic trichomonads may cause mild-to-moderate lymphoplasmacytic and/or neutrophilic colitis, atrophy of the superficial colonic mucosa, goblet cells depletion, crypt epithelial cell hypertrophy, crypt microabscess formation, and increased mitotic activity of crypt epithelium (Yaeger *et al.*, 2005). *Tritrichomonas foetus* is a flagellated protozoan with determined undulating membrane flagella (Derek *et al.*, 2004), known to be an obligate parasite of the cats' gastrointestinal tract (Chaoqun *et al.*, 2015). *T. foetus* has been recently found to be one of the causes of chronic diarrhea in domestic cats (Derek *et al.*, 2004; Chaoqun *et al.*, 2015). *T. foetus*-associated diarrhea is common among cats in shelters,

and purebred cats (Derek *et al.*, 2004; Gookin *et al.*, 2004). Increasing the risk of re-infection or higher stress level of cats under dense housing conditions prolongs the interval between relapses and recovery (Foster *et al.*, 2004). Besides cats, other species may be infected with *T. foetus* through oral-faecal transmission (Chaoqun *et al.*, 2015). *T. foetus* causes a variety of clinical signs such as faecal incontinence, relapsing malodorous diarrhea, hematochezia, rectal prolapse, and proctitis (Tolbert and Gookin, 2016), however, it may sometimes be asymptomatic (Tolbert and Gookin, 2009). The infected cats are often misdiagnosed with *Giardia* sp. (Derek *et al.*, 2004). The diagnosis is based on the history of chronic diarrhea and microscopic examination of faecal smear (Derek *et al.*, 2004). Trichomonad is rarely found in a diarrhea of a cat and is related to an overgrowth of the endogenous fauna (Michael *et al.*, 2003). *T. foetus* is refractory to antiprotozoal drugs, such as fenbendazole and metronidazole (Derek *et al.*, 2004). Moreover, prednisolone and antibiotics are not considered as effective drugs in infection with *T. foetus* (Jody *et al.*, 2001). Actually, antimicrobials diminish diarrhea and decrease the number of flagellates in the faeces but they cannot cure the infection. In addition,

right after discontinuing the antibiotics, diarrhea will intensify again (Gookin *et al.*, 1999).

Materials and Methods

A 9 months female domestic short-haired cat with chronic diarrhea over the past two months was referred to our hospital with a history of antibacterial treatment (a quarter of 80/400 mg Co-Trimoxazole every 12 h for 2 weeks). Clinical examination, complete blood count (CBC), and wet-mount faecal assessment were performed to rule out other common causes of diarrhea such as feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), feline panleukopenia virus (FPV), and *Giardia*. Feline leukemia virus, FIV, and FPV were also tested using rapid kits. In addition to wet-mount faecal microscopy, a rectal scraping dry-mount cytology was performed for further evaluation of motile organisms. Furthermore, a voided faecal sample was collected in a sterile tube and frozen at -20°C to perform conventional polymerase chain reaction (PCR) with the following primers to detect a 208-bp fragment of *T. foetus* internal transcribed spacer region 1 (ITS1) as previously described (Gookin *et al.*, 2002); forward primer: CTG CCG TTG GAT CAG TTT CG, reverse primer: GCA ATG TGC ATT CAA AGA TCG. For this purpose, DNA was extracted from the faeces using QIAamp stool mini kit (Qiagen, Hilden, Germany) based on the manufacturer's instructions. Extracted DNA was then subjected to PCR as the template together with PCR mastermix (Taq PCR MasterMix, Amplicon), primers, and distilled water in a total volume of 25 μL . DNA amplification was performed with a thermal cycler (master cycle, Eppendorf, Germany) at the following temperature profiles: initial denaturation at 95°C for 5 min, DNA denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s for 60 cycles, followed by a single final extension at 72°C for 5 min. Gel electrophoresis of PCR products was performed by 1% agarose gel. The gel was then stained with ethidium bromide and viewed under UV Fluorescent Gel Documentation System (Biorad, Hercules, CA, United States). DNA band was then extracted using Qiagen Gel Extraction kit (Qiagen, Hilden, Germany) to confirm the sequence.

Results

Physical examination

Clinical examination revealed rectal temperature of 39.8°C with a very poor body condition. The animal had mucosal diarrhea (yellowish jelly-like stool). No more abnormalities were detected.

Clinical pathology findings

Complete blood count showed a moderate inflammatory leukogram. Moving organisms were observed in the wet-mount faecal, and they were assumed as flagellated protozoa according to their size, shape, and type of movements. Rectal scraping dry-

mount cytology revealed many inflammatory cells consisting of degenerated and non-degenerated neutrophils, and few eosinophils along with huge numbers of *Tritrichomonas* trophozoites with typical morphologic characteristics including central axostyle, undulating membrane, flagella, and one laterally located nucleus which all were differentiable (Figs. 1A and B).

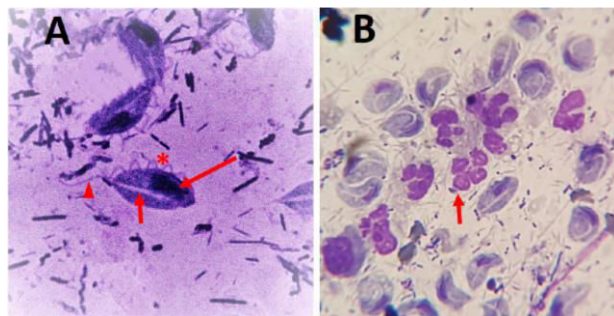


Fig. 1: Microscopic evaluation of rectal cytology ($\times 100$). (A) Typical features of *T. foetus*; nucleus (long arrow), undulating membrane (asterisk), axostyle (short arrow), and flagellum (arrow head), and (B) Presence of neutrophils (arrow) as the evidence of colitis

PCR result

A PCR product of 208-bp was amplified, and the sequence analysis of gel-extracted PCR product revealed full sequence homology with that of *T. foetus* (GenBank accession no. AF466751) (Fig. 2).

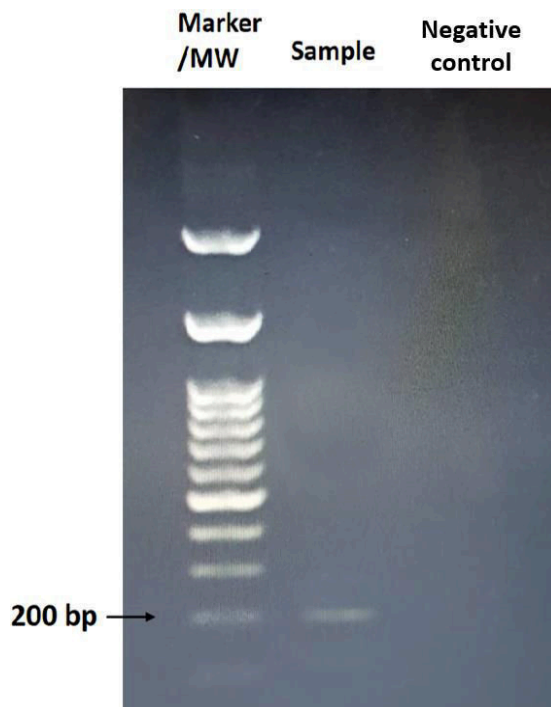


Fig. 2: PCR amplification product with 208 molecular weight (MW) compared with the marker

Treatment and outcome

Metronidazole (METROMAX, Tehran Chemistry, Tehran, Iran), 250 mg every 8 h p.o., together with 70 ml ringer-lactated serum, every 12 h were administered for 7

days. Considering the effects of the drug on natural microflora of the intestine, Biokid Probiotic Sachets (TAKGENE, Tehran, Iran) were also added to the cat's diet for 2 weeks. After 4 days of treatment, diarrhea was stopped and the patient was fully recovered at day 7.

Discussion

Tritrichomonas foetus is recently recognized as a pathogenic agent of feline enteric trichomonosis, although this protozoan was detected in kittens several decades ago, as early as 1928 (Levy *et al.*, 2003; Yao *et al.*, 2015). Animals with chronic gastrointestinal signs were significantly more infected by *T. foetus* in comparison to cats with no gastrointestinal distresses. However, there is no association between *Tritrichomonas* infection and age, sex, breed, or management of the cats (Tan *et al.*, 2020; Crisi *et al.*, 2021). Trophozoites of *T. foetus* may be detected via direct faecal smear or a wet mount under light microscopy (Manning, 2010). However, as aforementioned, the motile trophozoites may be mistaken with *Giardia* spp. and *Pentatrichomonas hominis*, because of the size of the trophozoite form of these species that are similar (Manning, 2010). Polymerase chain reaction is currently the most sensitive and specific tool in the detection of *T. foetus* in cats' faecal samples (Gookin *et al.*, 2002; Manning, 2010). To the best of our knowledge, only case reports of feline trichomonosis in Asia are from Korea and Malaysia (Lim *et al.*, 2010; Tan *et al.*, 2020). This study represents the first case of intestinal trichomoniasis causing chronic diarrhea in a cat in Iran. Although treatment with various antimicrobials may improve the faecal consistency, diarrhea recurs and sometimes gets worse, when treatment is discontinued (Mobile, 1999). Except medicine, several special diets were administered to the infectious cats, but it does not seem to have any effective results (Derek *et al.*, 2004).

Recent studies and observations suggest that pathogenesis of *T. foetus* is related to the disturbance of colonic microflora (Gookin *et al.*, 2007), potential effects of probiotics on infectious cats; it is suggested to be examined in next studies. It is reported that cats with *T. foetus* infection has a median time of 2.5 months before the diagnosis (Derek *et al.*, 2004), consistent with our case with a 2-month history of chronic diarrhea. According to the importance of this organism as one of the differential diagnosis of chronic diarrhea in cats, it is necessary to be detected in Iran prior to prevalence studies.

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Conflict of interest

The authors declare no conflict of interest.

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