Whole-genome shotgun sequencing from a canine fecal sample reveals rare canine infection by the parasitic protist *Tritrichomonas foetus*.

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Abstract

A fecal sample from a 1.5 year-old Labrador retriever. Shotgun community-wide whole-genome sequencing was performed using a fecal sample from the canine. The analysis revealed the presence of the parasitic protist, *Tritrichomonas foetus*. This is the first case reporting the presence of *T. foetus* in canines using whole genome sequencing.

Keywords:

Tritrichomonas foetus, whole-genome sequencing, microbiome

Introduction:

Tritrichomonas foetus is a parasitic protist that primarily colonizes gastrointestinal tract in cats and reproductive tract in cattle (Dabrowska et al. 2019). The parasite exists almost exclusively in the trophozoite (feeding) stage in the gastrointestinal tract where it disrupts the host mucosal barrier and induces epithelial apoptosis (Pereira-Neves & Benchimol, 2009). In recent years, the host range of *T. foetus* has expanded to include canines (Jiang et al. 2025). Although its pathogenic role in canines is contentious, some suggest that *T. foetus,* in conjunction with *Pentatrichomonas hominis,* may contribute to trichomoniasis characterized by diarrhea, anorexia, and enteropathy (Gookin et al. 2005; Tolbert et al. 2012; Tuska-Szalay et al. 2024). Occasionally, infected canines cohabitate with cats, suggesting a transmission route between feline and canine (Franchi et al. 2020).

T. foetus is commonly associated with gastrointestinal disease in naturally infected cats where it is spread through fecal to oral routes (Stockdale et al. 2009; Yao et al. 2015; Arranz-Solis et al. 2016). Trophozoites are then shed in the cat's feces and remain infectious if consumed by another cat. *T. foetus* can survive for up to seven days at room temperature (Hale et al. 2009), which increases the chances of exposure to and infection

of the parasite, despite the parasite's inability to form resistant cysts. *T.foetus* also causes bovine trichomoniasis, which is a notifiable disease in the World Organization for Animal Health (WOAH) and causes enormous economic losses due to a 15-25% drop in conception rates (Hermadi et al. 2024). Bovine trichomoniasis is typically sexually transmitted and can cause infertility and embryonic death in infected cows. There are 3 cases of *T. foetus* infection reported in humans, all in immunocompromised or immunosuppressed individuals (Suzuki et al. 2015). Together, the severity of trichomoniasis in bovines, its virulence in cats, its threat to immunocompromised humans, and more recent identification in canine infections warrant adoption of sophisticated means to identify *T. foetus* even in low parasite burdens in less widely recognized host species.

T. foetus infections have been historically diagnosed through culture-based techniques and real time PCR, however both methods require intact *T. foetus* cells while bacterial competition can prevent the growth of *T. foetus* in culture tests (Clothier et al. 2015; Rae et al. 2006; Parker et al. 2001). Diagnosis with metagenomic sequencing does not require viable cells and enables us to examine relative abundance of different organisms in the sample, and detect atypical or emerging pathogens missed by targeted diagnostics. This case report describes the first known case of *T. foetus* infection in a canine diagnosed through metagenome shotgun sequencing.

Case Presentation:

Patient information:

A 1.5-year-old female yellow Labrador retriever was presented on 11/19/2024 for routine examination and vaccination in preparation for boarding. The patient was overall in good health. *Giardia duodenalis* was detected at low levels using Antech's Keyscreen GI Parasite PCR Panel during the initial visit. At a follow-up examination on 12/17/2024, Giardia and all other parasites tested via the same PCR panel were no longer detected. Fecal samples collected during the follow-up visit were submitted for metagenomic shotgun sequencing for detection of pathogens not reported on Antech's Keyscreen GI Parasite PCR panel.

Diagnostic Approach:

Fecal samples from the patient were collected in a microbial DNA preservative buffer (DNA/RNA Shield TM, Zymo Research Corp. Cat. No. R1108, Irvine, CA, USA) and sent to MiDOG LLC for DNA extraction and sequencing. DNA was purified using a commercial kit (ZymoBIOMICSTM-96 DNA kit, Ca. No. D4304, Zymo Research Corp., Irvine, CA, USA). Sequencing libraries were prepared with the Illumina DNA Prep Kit (Illumina, San Diego,

CA) following the manufacturers protocol using 10 bp unique dual indexes. All libraries were quantified with Qubit (Thermo Fisher Scientific) and then pooled together by equal abundance. The final pool was quantified using qPCR. The metagenomic DNA was sequenced using the Illumina Novaseq X platform, generating 151bp paired end reads. The library produced 45,330,984 reads, which were trimmed with Trimmomatic v.0.33 (Bolger et al., 2014) to remove low-quality fractions and adaptors. Quality trimming by a sliding window with 6bp window size and a quality cutoff of 20 and reads with size lower than 70bp were removed. Low-diversity reads were detected and removed with sdust (Li, 2018). The microbial composition of the sample was identified using Sourmash v.4.8.11 (Irber et al., 2024) with the k=51 option to generate a profile based on 51 base pair (bp) kmers, short DNA fragments representing the microbial community. These kmers were then compared against a collection of curated and decontaminated genomes of bacteria, fungi, and protists to identify microbial taxa present in the sample. To reduce potential false positives, only species with a total kmer coverage exceeding 50 kilobases (kb) were retained. Abundances of individual microbial taxa were calculated using the abundance-weighted number of kmers unique to each taxon. This analysis detected *Tritrichomonas foetus* at low abundance compared to the bacterial flora in the fecal sample; however, the parasitic protist was the only eukaryote species (Figure 1). This analysis captured the entire microbiome including bacteria and protists, while also screening for other eukaryotic parasites, including nematodes and tapeworms, and DNA viruses.

Discussion:

This study was the first to use metagenome shotgun sequencing to examine *T. foetus,* quantifying the relative abundance of *T. foetus* compared to other organisms in fecal samples, which the methodologies used in previous studies could not achieve. In this study, *T. foetus* was identified from fecal samples of a healthy canine, and *T. foetus* was the only eukaryote species detected. In cattle and cats, the parasite is typically identified using culture, PCR, and histopathologic analysis.

However, these traditional methods have limitations. Detection from culture is time-consuming, requires viable trophozoites, and has low sensitivity. It is also difficult to morphologically distinguish trophozoites of *T. foetus* from those of other trichomonads. While Gel-PCR and qPCR are highly specific and sensitive, detection accuracy can be reduced by PCR inhibitors in fecal samples, and a comprehensive view of the entire

microbiome is unachievable (Yao 2013). Motile trophozoites are used for identification of *T. foetus* from culture and histopathology, while PCR commonly targets ITS, 18S, and/or 5.8S rRNA genes (Yao 2013, Bastos et al. 2019).

T. foetus primarily infects cattle and cats and has generally been considered clinically irrelevant to dogs, especially when detected within healthy individuals. However, there are a few cases of *T. foetus* associated with disease in canines. *T. foetus* has been identified from both healthy and diarrheic fecal samples of canines using cytology and PCR (Tolbert et al. 2012, Gookin et al.2005, Tuska-Szalay et al. 2024). One study found *T. foetus* was marginally more prevalent in dogs with diarrhea (14.89%) than dogs without diarrhea (6.11%) although the association did not reach statistical significance (Jiang et al.2025). Additionally, a unique case of *T. foetus* infection was diagnosed within a subcutaneous mammary mass in a dog with ongoing immunosuppression therapy, marking the only known case of *T. foetus* infection outside the gastrointestinal tract in canines (Franchi et al. 2020). These reports suggest that *T. foetus* may act as an opportunistic pathogen of canines, causing disease in immunocompromised hosts or high parasite load, while existing asymptomatically in other cases. The evidence also supports the likelihood of fecal-oral transmission between cats and dogs.

Metagenome shotgun sequencing overcomes many limitations of traditional detection methods. It does not require viable trophozoites, is less affected by PCR inhibitors, and enables detection of a broad range of microbial taxa simultaneously. Notably, *T. foetus* was not identified by the GI parasite PCR panel performed for the patient at the initial examination, highlighting the potential of metagenome sequencing to reveal organisms missed by targeted diagnostics. To our knowledge, this is the first report of *T. foetus* detection by metagenome shotgun sequencing in any host species.

In conclusion, this case provides further evidence of *T.foetus* infection in dogs, suggesting that canines even when asymptomatic, can deposit the parasite in feces and pass on to more susceptible animals or even humans. It also highlights the value of metagenomic sequencing as a sensitive and unbiased tool for detecting *T. foetus* and identifying additional parasites in veterinary diagnostics. Metagenome sequencing enables detection of emerging, atypical, or low-abundance pathogens that are missed by traditional methods. Its application may expand diagnostic capabilities, improve pathogen surveillance, and contribute to better management of infectious diseases in companion animals.

Data Availability:

The raw sequence data has been deposited to NCBI under the bioproject accession number PRJNA1308134, with associated BioSample ID SAMN50684447. The data is available under the Sequence Read Archive accession number SRR35037681.

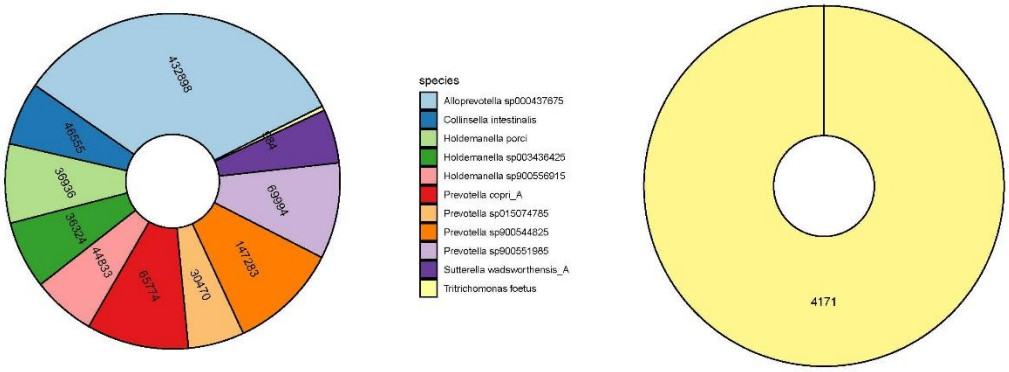

Figure 1: Two doughnut plots are shown depicting the relative abundances of 11 taxa from the canine fecal sample. Colors indicate the microbial species while numeric values represent the number of sequences that aligned to each taxon. **A)** The relative abundances of the top 10 most abundant bacterial taxa and *T. foetus* are shown. **B)** The weighted abundance of *T. foetus* unique kmers are indicated. While *T. foetus* composes a relatively small portion of the microbiome, a substantial number of reads were still detected within the sample.

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