Application of molecular diagnostics to investigate the prevalence of *Tritrichomonas* infection in cats

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Introduction

- *Tritrichomonas* is a protozoan parasite that infects cows, cats, and pigs, and causes pathology in the reproductive and digestive systems.[3,4]
- In cats, *Tritrichomonas* infects the large intestines and causes chronic large-bowel diarrhea.[1,4]
- The parasite can infect cats of any age, but younger cats are symptomatic more often.[1]
- Cats in high-density environments (e.g. shelters) have higher risk for infection.[1]
- While *Tritrichomonas* occurs worldwide, only one study has examined the prevalence in cats in California, with a focus on owned (non-shelter derived) cats (prevalence 3-4%).[5]
- Fecal smear, fecal culture, and PCR can be used for *Tritrichomonas* detection with increasing likelihood of sensitivity, respectively.[1,3]

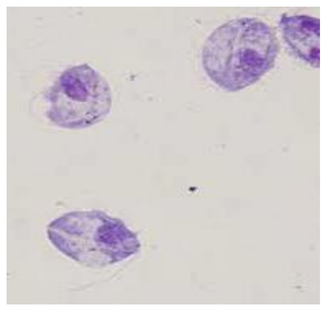

Figure 1. *Tritrichomonas* trophozoites.

Hypothesis & Aims

Hypothesis:

Molecular testing, or PCR, is a more sensitive detection method than fecal culture and *Tritrichomonas* is more prevalent in shelter-derived cats than previously assumed.

Aims:

1. Collect fecal samples from 100 diarrhetic or non-diarrhetic shelter-derived cats.
2. Extract DNA from fecal samples pre- and post-fecal culture and detect *Tritrichomonas* DNA using a nested PCR assay.
3. Data Analysis: Compare PCR and fecal culture in their ability to detect *Tritrichomonas* and establish a prevalence of infection in the study population.

Materials & Methods

Aim 1:

Recruit fosters

Train fosters and provide fecal collection kits

Collect history forms and demographic data about each cat

Fosters collect fresh fecal samples and inoculate fecal cultures

Fecal samples and inoculated InPouches received at VMTH

Figure 2. One of the sampled cats (Ronnie, 4 months old).

Aim 2:

Pre-culture PCR

Subsample 200 µL of fecal samples in triplicate

Extract DNA using Qiagen DNeasy Blood & Tissue Kit

Perform non-nested and nested PCR assays as previously described[2]

Post-culture PCR

InPouch fecal cultures are examined under a microscope daily while being incubated for 12 days

After incubation period, 200 uL of culture media is subsampled in duplicate

Perform DNA extraction & PCR

Amplification products are purified and submitted to the UC Davis Sequencing Facility. Sequences are analyzed using Basic Local Alignment Search Tool (BLAST) to determine organism identity.

Figure 3. InPouch being observed under a microscope.

Results

Table 1. Sampled cats categorized by age, gender, and health status (symptomatic vs. asymptomatic).

Classifications		# of Cats
Age	Kitten (>1 year)	32
	Adult (<1 year)	2
Gender	Female	16
	Male	18
Health Status	Symptomatic (Diarrhetic)	25
	Asymptomatic	9
Total	**Collected**	**34**
	Collected + Banked	**58**

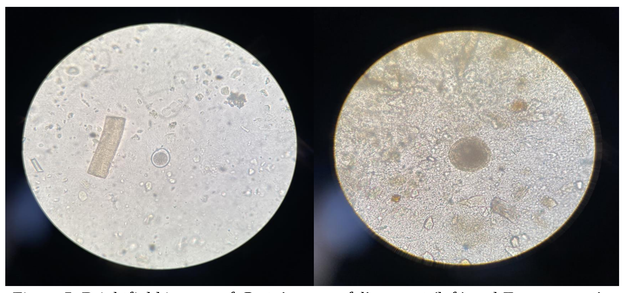

Figure 5. Brightfield images of *Cystoisospora felis* oocyst (left) and *Toxocara cati* ovum (right) within InPouches.

Fecal Cultures

- Of the 32 fecal cultures that have been analyzed to date, no *Tritrichomonas* was observed microscopically.
- *Cystoisospora felis* oocysts were observed in 5 fecal cultures (Figure 5, left).
- *Toxocara cati* ovum was observed in 1 fecal culture (Figure 5, right).

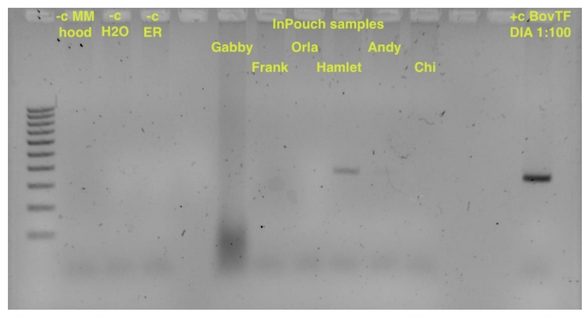

Figure 4a. Gel image depicting external (non nested) PCR products from 6 InPouch samples.

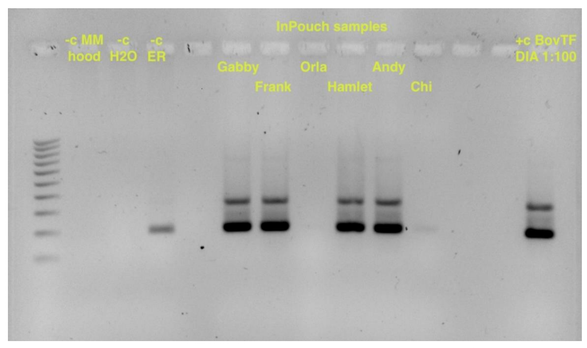

Figure 4b. Gel image depicting internal (nested) PCR products from 6 InPouch samples. Note possible contamination in extraction control (-cER).

PCR

- Of the 58 samples collected, 51 were extracted and 49 have undergone molecular analysis via PCR.
- Of the 34 cultures collected, 13 were extracted and 11 have undergone PCR.
- To date, out of the 60 samples processed via molecular analysis, none have been confirmed to contain *Tritrichomonas* DNA. However, contamination in our reagents has hindered data interpretation (Figure 4b).

Discussion

Sample Collection:

- Collected samples from 58 individual cats.
  - 94% of sampled cats were kittens (< 1 year old) and 73.5% were diarrhetic at the time of collection (Table 1).
  - Although cats that fit the demographic of being infected with *Tritrichomonas* were not specifically targeted, most of the samples came from cats that were most at risk for infection.
- Challenges with a prospective study design over a short effort period hindered our goal of collecting 100 samples during the STAR program duration.
  - Specific issues were identified with communication, collecting fresh samples (within 2 hours of voiding), distinguishing feces in large litters/multi-cat homes, and inoculating InPouches with the right amount of feces
- However, notable progress was made in making connections with local shelters and rescue organizations and in refining the process of recruiting and training fosters for sample collection.

***Tritrichomonas* Detection:**

- Although no parasites were detected in samples to date, additional sampling will be required to rule out the presence of *Tritrichomonas* in this population
- Molecular results have been delayed due to reagent contamination.
  - The non-nested and nested PCR assays were both successful, with amplification of positive controls (Figure 4a), but there were issues with contamination in the primers and extraction reagent blanks (Figure 4b).
  - Despite replacing the contaminated primers, extraction reagents, and PCR reagents and establishing new protocols to reduce further contamination, the issues persisted through the month of July.
  - Sequenced amplification products showed 100% identity with *T. foetus*, however, the conserved nature of the ITS1 locus between cat and cow isolates prevents us from distinguishing between true positive samples and contamination.
  - Therefore, any amplification products consistent with the positive control could not be interpreted as true positives.

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