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

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### Introduction

- *Trichomonas* is a protozoan parasite that infects cows, cats, and pigs, and causes pathology in the reproductive and digestive systems.  
- In cats, *Trichomonas* infects the large intestines and causes chronic large-bowel diarrhea.  
- The parasite can infect cats of any age, but younger cats are symptomatic more often.  
- Cats in high-density environments (e.g., shelters) have higher risk for infection.  
- While *Trichomonas* 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%).  
- Fecal smear, fecal culture, and PCR can be used for *Trichomonas* detection with increasing likelihood of sensitivity, respectively.  

### Hypothesis & Aims

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

**Aims:**
1. Collect fecal samples from 100 diarhetic or non-diarhetic shelter-derived cats.
2. Extract DNA from fecal samples pre- and post-fecal culture and detect *Trichomonas* DNA using a nested PCR assay.
3. Data Analysis: Compare PCR and fecal culture in their ability to detect *Trichomonas* 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

**Aim 2:**

#### Pre-culture PCR
- Subsample 200 µL of fecal samples in triplicate
- Extract DNA using Qagen DNAeasy Blood & Tissue Kit
- Perform non-nested and nested PCR assays as previously described
- 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.

#### Post-culture PCR
- InPouch fecal cultures are examined under a microscope daily while being incubated for 12 days
- After incubation period, 200 µL of culture media is subsampled in duplicate
- Perform DNA extraction & PCR

### Results

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

<table>
<thead>
<tr>
<th>Classifications</th>
<th># of Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>Kitten (&gt;1 year)</td>
<td>32</td>
</tr>
<tr>
<td>Adult (≤1 year)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
</tr>
<tr>
<td><strong>Health Status</strong></td>
<td></td>
</tr>
<tr>
<td>Symptomatic (Diarrhetic)</td>
<td>25</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
<tr>
<td>Collected</td>
<td>34</td>
</tr>
<tr>
<td>Collected + Banked</td>
<td>58</td>
</tr>
</tbody>
</table>

**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 *Trichomonas* DNA. However, contamination in our reagents has hindered data interpretation.

### 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 *Trichomonas* 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.

**Trichomonas Detection:**
- Although no parasites were detected in samples to date, additional sampling will be required to rule out the presence of *Trichomonas* 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.
- 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.

### Acknowledgements

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### References