Intestinal Tritrichomonas suis (=T. foetus) Infection in Japanese Cats

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ABSTRACT. Tritrichomonas suis (=T. foetus) has recently been reported to be a causative agent of chronic large-bowel diarrhea in cats. While the disease was previously attributed to Pentatrichomonas hominis, the etiologic agent for feline trichomonal diarrhea was identified as T. suis. Although feline trichomonosis due to T. suis has been reported at prevalences ranging from 14 to 31% in Europe and the U.S., no reports of the pathogen have been published to date in Japan. In 2008, however, we encountered a case of feline trichomonosis at the Veterinary Teaching Hospital of Hokkaido University. The parasite was identified as T. suis by nested PCR amplification of partial internal transcribed spacer region 1 and 5.8S ribosomal RNA gene sequences with T. suis-specific primers and DNA sequencing of the amplified products. We then conducted surveys for feline trichomonosis in three different animal hospitals using either cultivation and/or PCR-based assays. The results revealed that 13 of 147 samples (8.8%) were positive for T. suis, and that 5 of the 13 infected cats, which ranged between 1 month and 7.5 years-old, showed chronic diarrhea. Seven of the infected cats were purebred and 6 were mixed breed. These findings suggested that feline trichomonosis is prevalent in Japan, and that T. suis may play a role as a causative agent of feline chronic diarrhea.

KEY WORDS: chronic diarrhea, feline, trichomonosis, Tritrichomonas foetus, Tritrichomonas suis.


In 2000, feline trichomonosis was classified as a chronic large-bowel diarrhea [18]. At that time, incidence of the disease was tentatively ascribed to the trichomonal Pentatrichomonas hominis, a flagellated organism found in the lumen of the intestine and known to be infectious in a wide variety of hosts, including cats, dogs, and humans [5, 18]. In 2003, however, 18S ribosomal RNA gene, internal transcribed spacer region 1 (ITS 1), 5.8S rRNA gene and ITS2 sequences analysis revealed that feline trichomonal diarrhea was caused by Tritrichomonas suis and not by P. hominis [11]. After demonstrating that T. suis was the etiologic agent for diarrhea in specific-pathogen-free cats [8], T. suis has increasingly become recognized as a cause of large-bowel diarrhea in felines.

Tritrichomonas suis was initially considered to be a parasite of the pig, in which it infects the nasal cavity, stomach and the intestine [17]. Based on morphology, cross-transmission experiments and DNA assays, T. suis is considered to be a synonym of [12], a well known pathogen of cattle in which it infects the reproductive organs and causes abortion [11].

Feline T. suis infection has been reported in numerous countries, with prevalence in Europe and the U.S. ranging from 10 to 31% [2, 3, 9, 10, 15]. However, no reports of this parasite have been published to date in Japan. The aim of this study was therefore to determine whether T. suis occurs in cats in Japan, especially those manifesting with diarrhea, using both cultivation and DNA sequencing techniques.

MATERIALS AND METHODS

A total of 147 feline fecal samples were examined in this study:

Survey I. Forty-nine samples were collected at a local animal hospital in Sapporo, Hokkaido Prefecture, Japan. Fecal samples were frozen until PCR analysis.

Survey II. Forty samples were collected at the Veterinary Teaching Hospital of Hokkaido University in Sapporo, Hokkaido Prefecture, Japan. All samples were cultured in Trichomonas Medium® (Oxoid Ltd., Cambridge, U.K.). PCR analysis was performed on 11 samples.

Survey III. Fifty-eight samples were collected at a local animal hospital in Kounosu, Saitama Prefecture, Japan. Samples were inoculated into the InPouch TF-Feline™ culture system (BioMed Diagnostics, Inc., White City, OR, U.S.A.) [7] and shipped for 2 days to the Graduate School of Veterinary Medicine, Hokkaido University. After ten days cultivation, the culture media was centrifuged at 22,140 × g at room temperature for 3 min. The obtained pellets were subjected to PCR analysis, because it was afraid that trichomonads may have died during shipment of the pouch. The pellets were stored at −80°C before PCR analysis.

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analysis. DNA was extracted from the pellets of all 58 culture samples and subjected to PCR analysis.

By conducting interviews in hospitals, information on the age, breed, and stool characteristics (diarrhea or normal) of the cats were obtained from owners. In addition, the owners were also asked whether the cats were maintained indoors or outdoors.

**Culture methods:** Ninety-eight samples, consisting of 11 fresh stool samples and 88 rectal fecal samples, were used in the cultivation experiments. Briefly, approximately 0.05 g of fresh feces was inoculated into a culture tube containing 1 ml of antibiotic-fortified (10⁶ units of penicillin/l, 15 g of streptomycin/l, 2 mg of amphotericin B/l) Trichomonas Medium®, or into the InPouch TF-Feline™ culture system.

Upon arrival at the Graduate School of Veterinary Medicine of Hokkaido University, aliquots of the media were examined at 100 × magnification under a light microscope (BH-2, Olympus, Tokyo, Japan). The cultures were then maintained at 37°C and examined 2 days later under a light microscope for the presence of motile trophozoites. Negative cultures were maintained for 10 days, and examined under a microscope every 2 days. Giemsa staining of positive-culture smears was performed for morphological observations of trophozoites.

**PCR analysis:** Of the 147 samples, 118 (fecal samples or pellets of cultured samples) were subjected to PCR analysis. Sixty of the 118 samples were fecal samples, 49 of which from Survey I and 11 from Survey II. The rest were 58 pellets of culture from Survey III. DNA was extracted from 200 mg of the fecal samples or the pelleted cultures using a QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD, U.S.A.). A 208-bp fragment containing *T. suis* ITS1 and 5.8S rRNA gene was amplified using *T. suis*-specific nested primers as described previously [4]. Amplified gene sequences from each positive sample were then compared with gene sequences in GenBank from *T. suis* (=*T. foetus*) isolated from felines (AF466749, AF466750 and AF466751; all identifying numbers are GenBank accession numbers) [11], bovine (AF339736) [19] and swine (U859661) [1]. We also compared with *P. hominis* sequence (U86616) [1].

**RESULTS**

Light microscopic examination of Giemsa-stained culture smears revealed that each trichomonad possessed 3 anterior flagella and 1 posterior flagellum, an undulating membrane, and an axostyle (Fig. 1).

In survey I, the PCR analysis performed using partial ITS1 and 5.8S rRNA gene revealed that 2 of the 49 fecal samples (4.1%) were positive for *T. suis*. In survey II, an examination of cultures revealed that 3 of the 40 (7.5%) samples were positive for *T. suis* and these 3 samples were also positive for the PCR assay. In survey III, PCR analysis revealed that 8 of the 58 (13.8%) pelleted samples were positive for *T. suis*.

Cultivation and PCR methods revealed that a total of 13 of the 147 samples (8.8%) were positive for *T. suis* (Table 1). Five of the thirteen infected cats had chronic diarrhea, and this association between chronic diarrhea and *T. suis* infection was significant (*P*=0.0035, Fisher’s exact test). In addition, in 4 of these infected cats, the chronic diarrhea first appeared when the cats were a few weeks or a few months old (Table 2).

The infected cats were aged between 1 month and 9 years-old. Seven of the infected cats were purebred and six were mixed breed. No differences in pathogen occurrence were observed with respect to age, breed and whether cats were maintained indoors/outdoors between infected and uninfected cats (Fisher’s exact test) (Table 3).

DNA was successfully extracted from the feces of all 13 infected cats and a 208-bp fragment containing ITS1 and 5.8S rRNA gene was amplified and subjected to DNA sequencing. Comparison of the sequenced products against known trichomonad and *P. hominis* sequences deposited in GenBank revealed that the isolates obtained in this study showed 98 to 100% sequence homology with published feline (AF466749, AF466750 and AF447651), bovine (AF339736) and swine (U859661) *T. suis* (=*T. foetus*) isolates. Furthermore, the feline trichomonad isolates obtained in this study shared a low degree of sequence identity (63–64%) with the *P. hominis* sequence (U86616).

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**Table 1.** Positive rates of *Tritrichomonas suis* in cultivation and PCR methods in 3 surveys

<table>
<thead>
<tr>
<th></th>
<th>Cultivation methods</th>
<th>PCR analysis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey I</td>
<td>—</td>
<td>2/49</td>
<td>2/49</td>
</tr>
<tr>
<td>Survey II</td>
<td>3/40</td>
<td>3/11</td>
<td>3/40</td>
</tr>
<tr>
<td>Survey III</td>
<td>1/58</td>
<td>8/58</td>
<td>8/58</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4/98</td>
<td>13/118</td>
<td>13/147</td>
</tr>
</tbody>
</table>

Number of *T. suis* infected cats/ number of surveyed cats.
DISCUSSION

This study is the first report of feline *T. suis* infection in Japan. Cats infected with this trichomonad were not only found in Hokkaido Prefecture, but also Saitama Prefecture in the mainland of Japan.

Interestingly, trichomonads have not been reported as a possible causative agent of diarrhea in cats to date. This may be because *P. hominis*, which is not considered to be a pathogenic trichomonad, is common in Japan. Precise morphological comparisons of *T. suis* and *P. hominis* show that they differ with respect to the number of anterior flagella; *T. suis* possesses 3 anterior flagella while *P. hominis* has 5. However, when viewed under a light microscope, both species are relatively similar in size, shape and movement. Therefore, *T. suis* has been confused with *P. hominis*. Indeed, this may explain why feline *T. suis* infection has not been reported before this study.

Methods used to detect trichomonads in cat feces include (1) direct microscopic examination of feces diluted in saline, (2) cultivation methods, e.g., Trichomonas Medium® or the InPouch TF-Feline™ culture system, and (3) PCR analysis of the feces. Given the low sensitivity associated with direct fecal examination (≤14%) [9], we selected two cultivation methods in our study. In addition, to identify *T. suis* using frozen fecal samples, we employed PCR analysis. No differences were observed in the sensitivity of the 2 culture systems used in this study (data not shown). However, compared to the Trichomonas Medium® method, the InPouch TF-Feline™ culture system has several practical advantages. Specifically, the pouch system is commercially available, there is no need to prepare sterile media, and the ability to view the contents of the sealed pouch directly under a light microscope reduces the exposure of personnel to large numbers of trichomonads. Taken together, these advantages are particularly well suited to in-clinic diagnosis of *T. suis* infection in cats. In survey III, of the 58 samples assayed, 8 were found to be positive for *T. suis* by PCR analysis and one sample by cultivation methods. This relatively low incidence suggests that trichomonads died during shipment of the pouch. Accordingly, if shipped media is negative for *T. suis*, then PCR analysis should be performed on pellets of cultured samples to avoid false-negative results.

Of the 13 infected cats identified in this study, 5 cats presented with chronic diarrhea, which was occasionally associated with blood or mucus. The significant association
between chronic diarrhea and *T. suis* infection \((P = 0.0035, \text{Fisher's exact test})\) suggests that *T. suis* could be a causative agent of feline chronic diarrhea.

A previous study demonstrated that, by having the ability to act as a primary enteric pathogen in cats, *T. suis* fulfilled Koch's postulates [8]. However, the current understanding of factors mediating the pathogenicity of *T. suis* in feline diarrhea is unknown. Furthermore, it remains to be clarified whether *T. suis* is the single cause of large-bowel diarrhea in infected cats, or if an impaired or immature immune system leaves younger cats more vulnerable to *T. suis* infection [15]. Indeed, further investigations are therefore required in order to clarify the pathogenic mechanism of *T. suis* in feline diarrhea.

We found that both purebred and mixed-breed cats were infected, corroborating the findings of studies conducted in other parts of the world [10, 15]. While these results indicate that feline *T. suis* infection is not restricted to pedigreed cats, the possibility that these cats are at greater risk should be investigated further. Because a high housing density (i.e., small areas per cat) is a likely risk factor for *T. suis* infection [9], pedigreed cats bred in catteries may have a greater risk of infection than mixed-breed cats.

Our results show that veterinarians in Japan should consider the possibility of cats being infected by *T. suis* if they present with diarrhea. At present, the only known treatment for *T. suis* is ivermectin, one of the 5-nitroimidazole drugs [6]. However, since relapses of *T. suis* infection and diarrhea in infected cats, or if an impaired or immature immune system leaves younger cats more vulnerable to *T. suis* infection [15], further investigations are therefore required in order to clarify the pathogenic mechanism of *T. suis* in feline diarrhea.

RESOLVING THE ISSUES RELATED TO THE TAXONOMIC CLASSIFICATION

*T. suis* is considered important because *T. foetus* is a notifiable disease of cattle in Japan and no case of bovine infection has been reported for more than 40 years. The results of the present study strongly suggest that *T. suis* may be fairly common in diarrheic cats in Japan. In addition, a recent study demonstrated that a *T. suis* isolate from a cat was capable of causing endometritis and vaginitis upon experimental infection of heifers; however, the endometrial damage caused by the cat isolate was less severe than that caused by *T. suis* isolated from cattle in a parallel experiment [16]. Conversely, *T. suis* isolated from cattle could only successfully infect two out of five cats upon experimental infection [14]. Slapeta et al. (2010) examined the phylogenetic relationships among *T. suis* isolated from domestic cats and cattle using DNA sequences of the TR7/TR8 variable-length repeat [13]. The results showed that *T. suis* isolated from domestic cats is genetically distinct from *T. suis* (=*T. foetus*) in cattle, prompting the authors to suggest the existence of a ‘cat genotype’ and a ‘cattle genotype’ in *T. suis*. They also reported that a review of public nucleotide databases revealed that the ‘cat genotype’ has not been isolated from cattle and that the ‘cattle genotype’ has not been recovered from cats. It is therefore suggested that the *T. suis* isolated from the cats in the present study is only prevalent in feline population and not in the bovine population.

Although, according to the rules established by the International Commission on Zoological Nomenclature, *T. suis* (Gruby and Delamond, 1843) should take precedence over *T. foetus* (Riedmüller, 1928) and thus *T. foetus* is an invalid name, most of the studies that have been conducted to date on feline trichomonosis have employed the name *T. foetus* and not *T. suis*. Furthermore, given that the majority of the published literature has used the name *T. foetus*, several researchers have proposed suppressing the older specific name *suis* and to continue using the junior synonym *foetus* as a nomen protectum [17]. However *T. suis* has been used to refer to this parasite in pigs since 1843, *T. suis* should not become a nomen oblitum.

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