Molecular Survey of *Tritrichomonas suis* (=*T. foetus*) ‘Cat’ and ‘Cattle’ Genotypes in Pigs in Japan

Junko DOI1,2), Niichiro ABE3) and Yuzaburo OKU1,2)*

1)Department of Pathological and Preventive Veterinary Science, The United Graduate School of Veterinary Science, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8511, Japan
2)Laboratory of Veterinary Parasitology, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4–101 Koyama-Minami, Tottori 680–8553, Japan
3)Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, 8–34 Tojo-cho, Tennoji-ku, Osaka 543–0026, Japan

(Received 29 August 2012/Accepted 16 November 2012/Published online in J-STAGE 30 November 2012)

**FULL PAPER**

**Parasitology**

**ABSTRACT.** *Tritrichomonas suis* (=*T. foetus*) is a protozoan parasite of pigs, cattle and cats. Based on host range and genetic differences, *T. suis* has been divided into a ‘cat genotype’ and a ‘cattle genotype’, with the latter genotype capable of infecting both cattle and pigs. Since no information is currently available on the genetic characteristics of *T. suis* from pigs in Japan, we conducted a molecular survey of *T. suis* using fecal DNA from pigs in Japan. Of the 64 pigs examined, nested PCR revealed that 36 (56.3%) were positive for *T. suis*. Sequence analysis of 8 positive samples showed that 7 of the pig isolates belonged to the ‘cattle genotype’ and the remaining isolate belonged to the ‘cat genotype’. The findings revealed that *T. suis* infection is common in pigs in Japan and that pigs can be infected by both genotypes.

**KEY WORDS:** genotyping, swine, trichomonosis, *Tritrichomonas foetus, Tritrichomonas suis*.

Trichomonas suis is a protozoan parasite that was originally considered to be restricted to pigs in which it infects the nasal cavity, the stomach and the intestine [28]. Since *T. suis* is only weakly pathogenic in pigs, it has been regarded as being relatively unimportant in veterinary medicine. However, based on morphological studies, cross-transmission experiments, and DNA analyses, it appears that *T. suis* (Gruby and Delamond, 1843) is a synonym of *Tritrichomonas foetus* (Riedmüller, 1928) [19, 28], which is a known pathogen of the reproductive organs in cattle [28]. Because of having a regard for priority of the older specific name *T. suis*, we employed *T. suis* and not *T. foetus* in this paper.

*T. suis* has recently been identified as a causative agent of chronic large-bowel diarrhea in domestic cats in Europe, the U.S.A., Australia and Korea [3, 10, 12, 13, 17, 18, 25]. These epidemiological data show that *T. suis* infection is an important disease in cattle and cats.

Cattle experimentally infected with *T. suis* isolated from pigs presented with similar clinical symptoms (e.g. vaginitis and infertility) as cattle naturally infected with *T. suis* [9]. Furthermore, pigs have been successfully infected with cattle isolates of *T. suis* [8]. Cat isolates of *T. suis* have been shown to be capable of causing endometritis and vaginitis upon experimental infection of heifers; however, the endometrial damage caused by the cat isolates was less severe than that caused by cattle isolates in a parallel experiment [26]. By contrast, cattle isolates could only successfully infect 2 out of 5 cats upon experimental infection [24]. No reports on cross infection of cats and pigs with heterologous trichomonads have been reported in the literature to date. Recent studies have described a conserved single nucleotide polymorphism (SNP) in the internal transcribed spacer-2 (ITS-2) region, as well as other differences between polymorphisms in the TR7/TR8 variable-length repeat, elongation factor 1 alpha, and cysteine protease 8 sequences of cat and cattle/pig isolates [21, 23, 27], which implies the existence of a ‘cat genotype’ in cats and a ‘cattle genotype’ in cattle and pigs.

As in many other countries, *T. suis* infection in cattle is a notifiable disease in Japan. Control measures have been successful in Japan, and no cases of bovine infection have been reported for more than 40 years. However, we previously reported that *T. suis* infection has occurred in cats in Japan with a prevalence rate of 8.8% [5]. Furthermore, although a *T. suis* infection was reported in pigs 2 decades ago in Japan [15], species identification in the paper was based on morphological methods.

Interestingly, no molecular assays of parasites infecting Japanese pigs have been conducted to date, even though genetic assays are considered necessary.

We therefore conducted a molecular survey of *T. suis* in pigs and clarified differences in the ITS-2 region of isolates obtained from pigs, cats and cattle in Japan.

**MATERIALS AND METHODS**

A total of 64 fecal samples were examined in the present study.

In a survey of pigs from western Japan, 34 fecal samples were collected from 6 farms in different prefectures in 2002...
The filtrate was then centrifuged at 700 × g for 10 min, and the supernatant was discarded. The pellet was then suspended in sucrose solution (specific gravity: 1.2), mixed thoroughly and centrifuged at 700 × g for a further 10 min. To recover the trichomonads floating on the surface, 1 ml of the supernatant on the top of each tube was collected and washed by centrifugation in physiological saline solution. After centrifugation, the supernatant was discarded, and the pellet was stored at −30°C until PCR analysis.

Of the 30 fecal samples collected in western Japan, 27 (79.4%) were confirmed as positive for *T. suis* by nested PCR (Table 1). The sequences for 4 of these 27 PCR-positive samples were determined, and all 4 belonged to the cattle genotype. PCR-positive samples for *T. suis* were detected in all of the prefectures surveyed; Gifu (n=5), Mie (n=4), Kyoto (n=4), Osaka (n=4), Hyogo (n=4), Okayama (n=4) and Tottori (n=4) prefectures.

Of the 30 samples that were collected in Tottori Prefecture in 2012, 9 (30.0%) were positive for *T. suis* by nested PCR, and 6 and 2 were found to be positive for trichomonads by the fecal direct smear technique and by cultivation method, respectively.

Morphological examination of Giemsa-stained culture smears from 1 sample that was positive for *T. suis* by nested PCR revealed a trichomonad possessing features typical of *T. suis*. Specifically, the trichomonad had 3 anterior flagella and 1 posterior flagellum, an undulating membrane and an axostyle (Fig. 1). However, 5 of the 6 samples showing posi-

---

**Table 1. Details of 64 pig fecal samples used in the study**

<table>
<thead>
<tr>
<th>Production stage of pigs sampled</th>
<th>Farm location (Prefecture)</th>
<th>Year of sampling</th>
<th>No. examined</th>
<th>No. <em>T. suis</em> positive in nested PCR</th>
<th>No. <em>T. suis</em> sequence analyzed (genotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>Gifu</td>
<td>2002</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fattening</td>
<td>Mic</td>
<td>2008</td>
<td>5</td>
<td>1</td>
<td>(cattle genotype)</td>
</tr>
<tr>
<td></td>
<td>Kyoto</td>
<td>2008</td>
<td>5</td>
<td>4</td>
<td>(cattle genotype)</td>
</tr>
<tr>
<td></td>
<td>Osaka</td>
<td>2008</td>
<td>5</td>
<td>4</td>
<td>(cattle genotype)</td>
</tr>
<tr>
<td></td>
<td>Hyogo</td>
<td>2008</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Okayama</td>
<td>2008</td>
<td>5</td>
<td>4</td>
<td>(cattle genotype)</td>
</tr>
<tr>
<td></td>
<td>Tottori</td>
<td>2008</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tottori</td>
<td>2012</td>
<td>8</td>
<td>2</td>
<td>(cattle genotype)</td>
</tr>
<tr>
<td>Nursery</td>
<td>Tottori</td>
<td>2012</td>
<td>22</td>
<td>7</td>
<td>3 (2 were cattle genotype, 1 was cat genotype)</td>
</tr>
</tbody>
</table>

Total 64 36 8 (7 were cattle genotype, 1 was cat genotype)

and 2008 (Table 1). Of these samples, 30 were from fattening pigs (4 to 7-month-old) transferred to the meat inspection center of western Japan. For the remaining 4 samples, the age of the pigs was unknown. The collection method of and DNA extraction method from trichomonads used for the fecal samples were the same as previously described [1, 2]. The extracted DNA was stored at −30°C until PCR analysis.

Further 30 fecal samples were collected from a farm in Tottori Prefecture in 2012 (Table 1). Twenty-two of these samples were from nursery pigs (<4-months-old) and 8 were from fattening pigs. Fecal samples were examined by the fecal direct smear technique under a light microscope (BH-2, Olympus, Tokyo, Japan), and all 30 samples were applied to cultivation on Trichomonas Medium® (Oxoid, Cambridge, U.K.) as described previously [5]. Giemsa staining of positive-culture smears was performed for morphological observations of trophozoites. On the same day that fecal samples were collected, the concentration and DNA extraction of trichomonads from the 30 feces samples were performed. Briefly, 10 g of each fecal sample was suspended in 10 ml of physiological saline solution and filtered through 80 mesh sieve. The filtrate was then centrifuged at 700 × g for 10 min, and the supernatant was discarded. The pellet was then suspended in sucrose solution (specific gravity: 1.2), mixed thoroughly and centrifuged at 700 × g for a further 10 min. To recover the trichomonads floating on the surface, 1 ml of the supernatant on the top of each tube was collected and washed by centrifugation in physiological saline. After centrifugation, the supernatant was discarded, and the pellet was stored at −30°C until DNA extraction. Genomic DNA was extracted and purified using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. DNA was stored at −30°C until amplification.

The *T. suis* isolates from the cats that were used in this study were collected previously by the authors [5], and another 2 samples were obtained from Shiga Prefecture in 2011. Because no molecular information of isolates from Japanese cattle is available, an Inui strain of *T. suis* isolated from cattle in Japan was used in this study. This strain was kindly provided by Dr. Yoshisada Yabu (Graduate School of Medical Sciences, Nagoya City University). The ITS-1, 5.8S ribosomal RNA (rRNA) and ITS-2 regions in *T. suis* were amplified by nested PCR as described previously, except that HotStarTaq® Master Mix (Qiagen) was used instead of AmpliTaq Gold® DNA polymerase (Perkin-Elmer, San Jose, CA, U.S.A.) [11]. For the sequence analysis, samples that were confirmed as being positive for *T. suis* by the nested PCR were amplified using the primers TFR3 and TFR4 [7]. The PCR was performed under the same conditions described previously, except that HotStarTaq® Master Mix (Qiagen) was used instead of AmpliTaq® DNA polymerase (Perkin-Elmer) [7]. Amplicons were purified using a Wizard® SV Gel and a PCR Clean-Up System (Promega, Madison, WI, U.S.A.) and sequenced in both directions with the TFR-3 and TFR-4 primer pair on a CE-Q™ 8000 sequencer (Beckman Coulter, Brea, CA, U.S.A.). The sequences of the 348-bp fragments were compared with *T. suis* gene sequences from cats (GenBank accession numbers: AF466749, AF466750 and AF466751) [17], cattle (M81842 and AF339736) [4, 29] and pigs (U85966 and AJ349190) [6, 16].

**RESULTS**

Of the 34 fecal samples collected in western Japan, 27 (79.4%) were confirmed as positive for *T. suis* by nested PCR (Table 1). The sequences for 4 of these 27 PCR-positive samples were determined, and all 4 belonged to the cattle genotype. PCR-positive samples for *T. suis* were detected in all of the prefectures surveyed; Gifu (n=5), Mie (n=4), Kyoto (n=4), Osaka (n=4), Hyogo (n=4), Okayama (n=4) and Tottori (n=4) prefectures.

The sequences of the 348-bp fragments were compared with *T. suis* gene sequences from cats (GenBank accession numbers: AF466749, AF466750 and AF466751) [17], cattle (M81842 and AF339736) [4, 29] and pigs (U85966 and AJ349190) [6, 16].
ive in fecal smears were negative by nested PCR. In addition, the number of samples that were successfully cultivated was low.

In total, 36 of the 64 samples (56.3%) were positive for *T. suis* by nested PCR (Table 1). Of these 36 samples, 28 were collected from infected pigs in the fattening stage and 7 from pigs in the nursery stage.

TFR3/4 PCR was conducted on 8 of the samples that were positive by nested PCR. The 348 bp fragments containing the ITS-1, 5.8S rRNA and ITS-2 regions were all amplified and sequenced. Comparison of the obtained sequences against *T. suis* sequences deposited in GenBank revealed that the isolates collected in this study showed complete genetic identity with published *T. suis* sequences from the cat (AF466749, AF466750 and AF447651), cattle (M81842 and AF339736) and pig (U85966 and AY349190). As in previous studies, the 7 pig isolates and the *T. suis* Inui strain from cattle differed from the cat isolates by a single SNP (T to C transition) at the ITS-2 locus (Fig. 2) [21, 23]. Of the 7 isolates, 6 were identical to the published sequences and one isolate had a SNP (T to C transition) at the ITS-2 locus.

---

Fig. 1. Giemsa-stained *Tritrichomonas suis* isolated from a pig fecal sample positive for *T. suis* by nested PCR (scale bar=10 µm).

Fig. 2. Alignment of the internal transcribed spacer (ITS)-1–5.8S ribosomal RNA–ITS-2 region of *Tritrichomonas suis* from this study and from GenBank (*). The conserved single nucleotide polymorphism (SNP) in the ITS-2 locus is enclosed by a box. Seven pig isolates and the cattle *T. suis* Inui strain differed from the cat isolates (T to C transition) at the SNP in the ITS-2 locus. However, 1 pig isolate had the same SNP as the cat isolates.
‘cattle genotype’ pig isolates, 1 was from Mie Prefecture, 1 was from Okayama Prefecture, 1 was from Osaka Prefecture, and 3 were from Tottori Prefecture (Table 1). Conversely, a swine isolate from Tottori Prefecture shared the same SNP at the ITS-2 locus with the cat isolates, but not with the other pig isolates or the cattle isolate (Fig. 2 and Table 1).

DISCUSSION

Tritrichomonas suis has been divided into a ‘cat genotype’ and a ‘cattle genotype’ since 2010 [23]. Cattle genotype and cat genotype infects cattle and pigs, and cats, respectively. However, no information is currently available on the genetic characteristics of T. suis from pigs and clarified differences in the ITS-2 region of several isolates obtained from pigs.

While previous studies have reported the existence of several nonpathogenic trichomonads, Tettratrichomonas buttreyi [22], Trichomitus rotunda [14] and Hypotrichomonadidae sp. [20] in the digestive tracts of pigs, this is the first molecular survey of pigs for the trichomonad, T. suis. The findings of this study revealed that T. suis infection is fairly common in Japanese pigs from the nursery stage to the fattening stage. Interestingly, the 5 samples positive for trichomonads using the fecal direct smear technique were negative for T. suis by the PCR assay. These findings imply that trichomonads other than T. suis were present in pigs in Japan, and that further genetic analyses are required to better clarify the identity of these trichomonad species.

Recent studies on SNPs in the ITS-2 locus have shown that T. suis isolates from the cat, cattle, and pig can be divided into 2 genotypes; the ‘cat genotype’ for cat isolates and ‘cattle genotype’ for cattle and pig isolates [21, 23]. In addition to isolating T. suis with a ‘cattle genotype’ in this study, we also isolated T. suis with a ‘cat genotype’ from a pig fecal sample. These findings imply that pigs may be carriers of both bovine and feline pathogenic T. suis. According to our knowledge, only 3 ITS-2 locus sequences for T. suis from pigs have been deposited in GenBank, and all of these sequences belong to the ‘cattle genotype’ [6, 16]. Consequently, further genetic investigations of T. suis in pigs from the world are needed to be undertaken in the ITS-2 region in combination with a variety of genotyping markers. Furthermore, in order to identify suitable genetic markers for screening parasite virulence, the pathogenicity of T. suis isolates from pigs in cattle and cats must be examined in greater detail.

The mode of natural transmission of T. suis from pigs to cattle remains unknown. However, the experimental infection studies described above suggest that T. suis is likely transmitted from pigs to cattle [8, 9]. In this study, we found that pigs can be infected with the cat genotype T. suis. Therefore, future studies should examine the possible modes of transmission among cattle, pigs, and also cats.

ACKNOWLEDGMENTS. We thank Dr. Kanae Fukushima (Kohnosu Animal Hospital) and Dr. Naoaki Fukunaga (Kusatsu Dog and Cat Hospital) for providing feline fecal samples.

REFERENCES

6. Felleisen, R. S. 1997. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitology 115: 111–119. [Medline] [CrossRef]
15. Kitano, Y., Makinoda, K., Furukawa, M., Toyomitsu, Y., Fuku-


Stockdale, H., Rodning, S., Given, M., Carpenter, D., Lenz, S., Spencer, J., Dykstra, C., Lindsay, D. and Blagburn, B. 2007. Experimental infection of cattle with a feline isolate of *Trichomonas foetus*. J. Parasitol. 93: 1429–1434. [Medline] [CrossRef]

