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Pentatrichomonas hominis in laboratory-bred common marmosets

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Abstract: Trichomonadid protozoa have been found in the intestinal tracts of common marmosets (*Callithrix jacchus*). However, there is little information available on species identification and the pathogenicity of these trichomonads. In this study, we conducted a fecal survey of a common marmoset colony maintained as laboratory animals in Japan and identified the trichomonad species. Screening using a fecal smear examination revealed that 66% (58/88) of the marmosets had trichomonadid trophozoites in their feces. The trichomonads were found in both normal feces (31/49, 63%) and diarrhea (27/39, 69%), with no significant difference in frequency. The protozoa were identified as *Pentatrichomonas hominis* using morphological characters and the 100% identity of the nucleotide sequence of the partial 18S rRNA gene (297 bp). The intraspecific genetic variability between *P. hominis* from the marmosets in this study and *P. hominis* from other reported mammal hosts was ≤1% in the nucleotide sequence, including the internal transcribed spacer (ITS)-1, 5.8S rRNA gene, and ITS-2 (293 bp). *P. hominis* inhabits the large intestine of various mammalian hosts, including primates, and is considered nonpathogenic. These results suggest that *P. hominis* is transmitted among marmosets and other mammals but is not a primary cause of bowel disease in marmosets.
Key words: marmoset, *Pentatrichomonas hominis*, protozoa, *Trichomonas*

Introduction

The common marmoset (*Callithrix jacchus*), a New World primate, has been increasingly used for biomedical and preclinical research in recent decades. This laboratory nonhuman primate has the advantages of small body size, easy handling, high fertility, and low zoonotic risk compared with other nonhuman primates and has become popular in various research fields including neuroscience, regenerative medicine, infectious disease, and drug testing [24, 26, 35]. Recent progress in transgenic and developmental engineering technology has expanded the research use of this laboratory animal [31, 36]. In this situation, improved management of marmoset health is necessary, and more information on spontaneous diseases and pathogens is needed. Diarrhea and gastrointestinal diseases are important health problems in marmoset colonies worldwide, but the exact cause of the diarrhea remains unknown; chronic diarrhea is associated with wasting marmoset syndrome, which is characterized by impaired weight gain, weight loss, muscle atrophy, alopecia, and diarrhea, and the etiology of this syndrome is poorly understood [3, 23, 29].

Trichomonad protozoa have been found in the large intestines of some callitrichid species in several colonies [8, 27]. However, there is little information available on species identification and the pathogenicity of these trichomonads. Intestinal trichomonad species include *Pentatrichomonas hominis*, which has been found and is considered nonpathogenic in a wide variety of mam-

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malian hosts, including cats, dogs, rodents, and primates [18, 33, 38]. Nevertheless, several studies have indicated that some trichomonad species are associated with bowel diseases. *Tritrichomonas suis* is a causative agent of chronic large-bowel diarrhea in cats [11, 21]. Reports of trichomonad-induced lesions in nonhuman primates are rare, but invasive trichomoniasis involving the gastric mucosa and the colonic mucosa suspected of being caused by *Tritrichomonas mobilensis* has been reported in rhesus macaques (*Macaca mulatta*) infected with simian immunodeficiency virus (SIV) [20] and a titi monkey (*Callicebus* sp.) [4], respectively. There have been no detailed reports of intestinal trichomonads in common marmosets. Therefore, this study conducted fecal screening and identified the trichomonad species in a facility-bred common marmoset colony to investigate the relationship between parasitism and bowel disease.

Materials and Methods

Animals

This survey was performed at the Central Institute for Experimental Animals (CIEA) in Kawasaki, Japan. The animal experimental procedures were reviewed by the Institutional Animal Care and Use Committee and approved according to the Regulations for Animal Experiments in CIEA established based on the Basic Policies on Animal Experiments conducted in Research Institutions (Notice No. 71 of the Ministry of Education, Culture, Sports, Science and Technology, June 2006) and the Guidelines for the Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

The common marmosets used in this survey were from a commercial breeder, CLEA Japan (Tokyo, Japan), or were born in the CIEA facility. Ultimately, all of the animals trace back to a breeding colony that has been maintained for more than 20 years in the indoor facilities of CLEA Japan. The animals were kept in stainless steel wire cages on a 12 h:12 h light:dark cycle at 25–28°C and 40–60% humidity. They were given a New World primate diet (CMS-1M; CLEA Japan) with added vitamins and tap water *ad libitum*.

Fecal examination and species identification of trichomonads

Fresh feces of common marmosets were collected from 88 cages. These cages housed 123 animals (53 females, 70 males) individually (53 cages) or in pairs (35 cages). The ages of the animals ranged from 6 months to 9 years old.

The feces were examined using a direct smear method; feces mixed with a drop of saline on a slide glass were covered with a cover slip and examined under a microscope. Four samples of trichomonad-positive feces were examined morphologically using Giemsa-stained fecal smears and were cultured on *Trichomonas* medium (CM0161, Oxoid, Basingstoke, UK). After a 5-day culture, medium containing the protozoa was used for DNA extraction with a MagExtractor Genome kit (Toyobo, Tokyo, Japan). Extracted DNA samples were used for the following analyses.

A species-specific PCR assay to identify *P. hominis* was performed using a primer pair, 5′-TGTAAACGATGCCGACAGAG-3′ (Th3) and 5′-CAACACTGAAGCCAATGCGAGG-3′ (Th5) designed to amplify a 339-bp sequence of the 18S ribosomal RNA (rRNA) gene [7]. PCR was performed in a 20-μl reaction volume containing 0.5 U of PrimeSTAR HS DNA Polymerase (Takara, Tokyo, Japan), 4 μl of 5× PrimeSTAR Buffer, 200 μM of each dNTP, 0.3 μM of each primer, and 1 μl of DNA extract. DNA amplification consisted of 30 cycles of 98°C for 10 s, 55°C for 5 s, and 72°C for 20 s. The PCR products were confirmed by electrophoresis on an agarose gel with ethidium bromide staining. After purifying the PCR products with DNA Clean & Concentrator-5 (Zymo Research, Irvine, CA, USA), the nucleotide sequences of the reaction products were determined using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and ABI Prism 310 Genetic Analyzer (Applied Biosystems).

To investigate intraspecies variation between *P. hominis* from these common marmosets and *P. hominis* from other mammals, a 338-bp fragment consisting of the internal transcribed spacer region (ITS)-1, 5.8S rRNA gene, and ITS-2 of the obtained DNA samples was also amplified by PCR using a primer pair, 5′-TGCTTCAGTTCAGCGGGTCTTCC-3′ (TFR1) and 5′-CGGTAGGTGAACCTGCCGTTGG-3′ (TFR2) [13] and the nucleotide sequences were determined by sequencing. Homology between the obtained nucleotide sequences and known sequences from the GenBank database was analyzed using a BLAST search [2] on the website of the DNA Data Bank of Japan (DDBJ). The phylogenetic relationships among *P. hominis* from marmosets and *P. hominis* from other hosts were inferred using the neighbor-joining method [30]. The evolution-

Table 1 Detection of trichomonad trophozoites in the feces of common marmosets

	No. positive / no. examined (%)		
	Normal feces	Diarrheal feces	Total
Young (< 2 years)	6/7 (86)	2/3 (67)	8/10 (80)
Adult (2–9 years)	25/42 (60)	25/36 (69)	50/78 (64)
Total	31/49 (63)	27/39 (69)	58/88 (66)

ary distances were computed using the Kimura two-parameter method [19], and they are given as the number of base substitutions per site. The evolutionary analyses were conducted in MEGA5 [37].

Statistical analysis

The difference in positive rates of trichomonad trophozoites was analyzed using Fisher's exact probability test.

Results

Trichomonadid trophozoites were detected in 58 (66%) of the 88 samples. There was no significant difference in positive rate between normal feces (31/49, 63%) and diarrheal stools (27/39, 69%) (Table 1). According to age, the positive rate in samples from young (0–1 years old) animals was high (8/10, 80%), but it was not significantly different from that in adults (2–9 years old) (50/78, 64%). There was also no significant difference in the positive rate between females (16/25, 64%) and males (27/43, 63%), excluding the 20 samples from male-female pairs. The trichomonadid trophozoites were similar morphologically in all samples, and no other protozoans were detected. The detected trophozoites were piliform with multiple flagella and an undulating membrane. In Giemsa-stained specimens, the trophozoites had a nucleus, axostyle, four or five anterior flagella, and a posterior flagellum that aligned along the undulating membrane and extended freely beyond the body (Fig. 1). Their bodies were 10–15 μm long by 7–12 μm wide according to measurements of ten trophozoites from each of the two specimens. These morphological characteristics of the detected trichomonadid trophozoites conformed to those of *P. hominis*.

All four cultured samples from trichomonadid trophozoite-positive feces were positive for *P. hominis* species-specific PCR of the partial 18S rRNA gene (339 bp) (Fig. 2). The nucleotide sequence of a 297-bp product without primers was determined for two cultured samples; these two sequences were identical (GenBank no. AB773865). The sequence showed 100% identity to known *P. hominis* sequences from dogs [10], cats [6, 16], cattle [12], and nonhuman primates in zoos, including common marmosets [34], and 99.7% identity to those from dogs [15] and humans [9]. The nucleotide sequence of a 293-bp fragment containing ITS-1, the 5.8S rRNA gene, and ITS-2 without primers was determined for two cultured

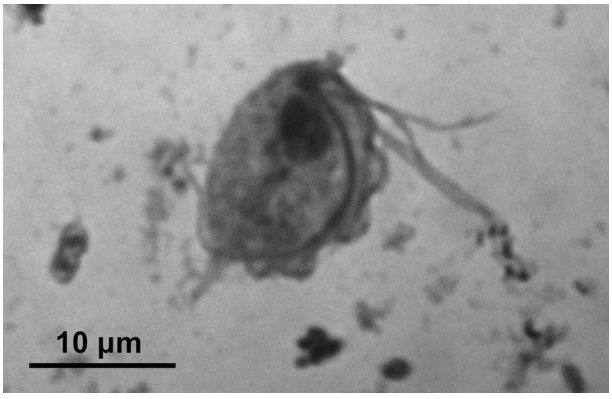

Fig. 1. A trichomonad trophozoite in a Giemsa-stained fecal smear from a common marmoset.

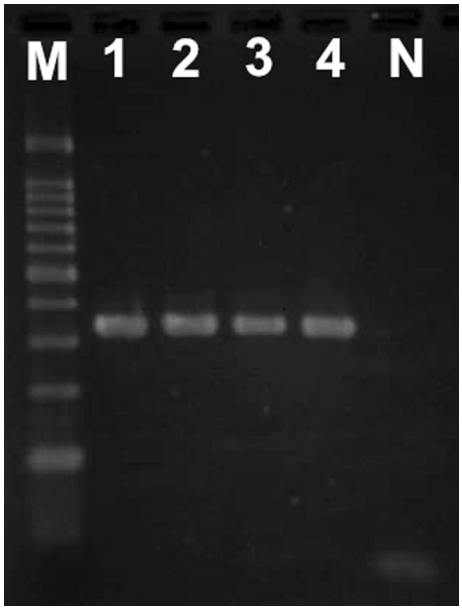

Fig. 2. Agarose gel electrophoresis of the products (339 bp) of *Pentatrichomonas hominis* species-specific PCR. M, 100-bp ladder; 1-4, DNA samples of cultures from trichomonad trophozoites-positive feces; N, water (negative control).

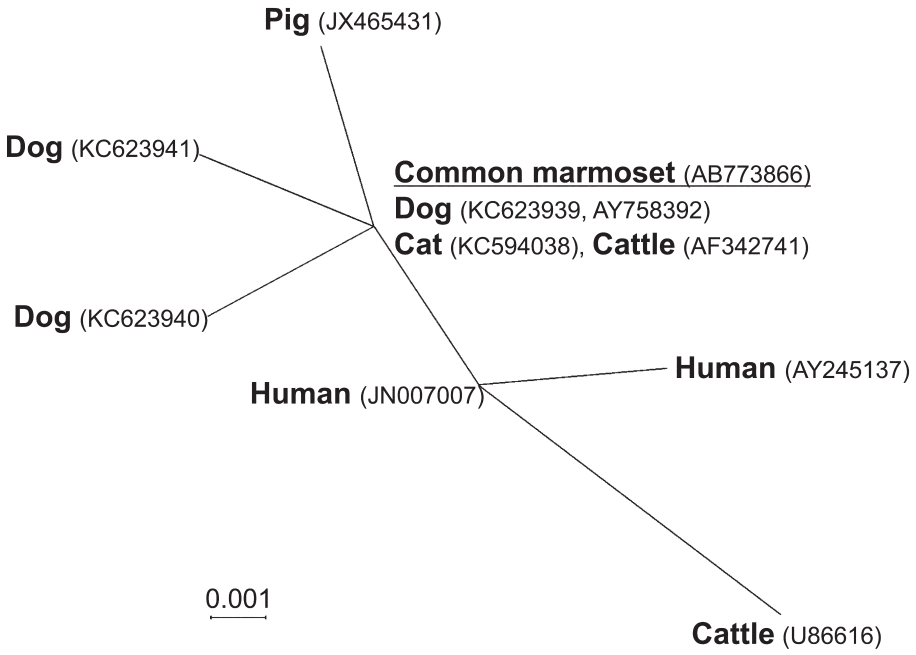

Fig. 3. Phylogenetic relationship of *Pentatrichomonas hominis* from common marmosets and other mammal hosts using the nucleotide sequence of the internal transcribed spacer region (ITS)-1, the 5.8S rRNA gene, and ITS-2 (297 bp) and the neighbor-joining method. The information shown for each taxon includes the host names and GenBank accession numbers. The sequence data for *P. hominis* from common marmosets obtained in this study are underlined. Data for other hosts were from previous reports [5, 6, 13, 15, 17, 22, 28, 39]. The genetic distances were computed using the Kimura two-parameter method.

samples; these two sequences were identical (GenBank no. AB773866). The sequence showed 100% identity to known sequences of *P. hominis* from dogs [15, 17], cats [6], and cattle [39] and 99.0–99.7% identity to those from dogs [17], pigs [22], cattle [13], and humans [5, 28]. Fig. 3 shows the phylogenetic relationships among these sequences.

Discussion

This fecal screening and genetic species identification revealed that *P. hominis* was prevalent in a colony of facility-bred common marmosets in Japan. *P. hominis* is regarded as a nonpathogenic opportunist in the large intestines of various mammalian hosts, including nonhuman primates [18, 38]. Our survey found similar positive rates for the trophozoites in normal and diarrheal feces, implying that *P. hominis* infection was not the primary cause of the diarrhea or colitis in marmosets. In histopathological examinations, no invasive or inflammatory lesions involving trichomonads have been found in the intestinal mucosa of marmosets (data not shown). This supports the assertion that *P. hominis* is nonpathogenic in marmosets. Nevertheless, there is no evidence that refutes any relationship between *P. hominis* infection and disease in mammalian hosts. Our data were not sufficient to make conclusions regarding whether or not *P. hominis* is nonpathogenic in marmosets and further investigations, such as experimental infection and antiparasite treatment experiments, are needed to clarify this.

Our analysis showed that the genetic variation of *P. hominis* was low, even in the ITS regions, which are highly variable sites. There was no difference in the analyzed nucleotide sequences between *P. hominis* from marmosets in this survey and those reported from other nonhuman primates, dogs, cats, and cattle. This suggests that isolated protozoa have been transmitted among various mammal hosts. From the perspective of animal facility management, managers should consider the possibility that *P. hominis* spreads from marmosets to other animals. The protozoa have been found in most laboratory animal species, including dogs, mice, rats, and hamsters [17, 32]. *P. hominis* from a beagle dog was transmitted experimentally to mice and rats by oral ad-

ministeration of the trophozoites, and they were detected in the rodents 2 months later [14]. The protozoa are transmitted by oral ingestion of trophozoites or pseudocysts excreted in feces. To prevent transmission, good hygiene is important. There was a slight difference between the protozoa isolated from marmosets and those reported from humans. This genetic difference suggests that there is a low risk of *P. hominis* transmission from marmosets to humans. The zoonotic potential of *P. hominis* is controversial [25], but *P. hominis* occurs infrequently in humans [1, 33], and there is no clear evidence of its pathogenicity. Therefore, the zoonotic risk of *P. hominis* from marmosets to humans is quite low.

In conclusion, this survey found that laboratory-bred common marmosets harbored *P. hominis* but that was not a primary cause of diarrhea and had low intraspecific genetic variation among various mammal hosts.

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