

## Diagnosis of *Pentatrichomonas hominis* from domestic cats in Southeastern Brazil\*

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**ABSTRACT.** dos Santos C.S., McIntosh D., Berto B.P., de Jesus V.L.T., da Rocha C.N.C., Fernandes J.I., Scott F.B. & Lopes C.W.G. **Diagnosis of *Pentatrichomonas hominis* from domestic cats in Southeastern Brazil.** [Diagnóstico de *Pentatrichomonas hominis* em gatos no sudeste do Brasil.] *Revista Brasileira de Medicina Veterinária*, 37(Supl.1):25-31, 2015. Curso de Pós-Graduação em Ciências Veterinárias, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Campus Seropédica, RJ 23897-970, Brasil. E-mail: carolinespitz@yahoo.com.br

The parabasalid flagellate *Tritrichomonas foetus* is recognized as the causative agent of large bowel diarrhea in domestic cats. A second species of parabasalid flagellate *Pentatrichomonas hominis*, has also been reported in association with domestic cats, albeit almost exclusively as a commensal organism. However, there is growing evidence to suggest that *P. hominis* may also be involved in feline gastro-intestinal disorders including diarrhea and that the incidence of infection with *P. hominis* may have been underestimated due to it being misidentified as *T. foetus*. The aim of the current study was to establish the basis for routine morphological identification of *P. hominis* employing light microscopy and to apply the methodology to the examination of cases of diarrhea in a laboratory population of Brazilian domestic cats (n =39). A detailed morphological description of *P. hominis* isolated from 11 cats with diarrhea was produced and molecular analyses were performed in support of the morphological data and to demonstrate the absence of *T. foetus* in infected cats. All animals with diarrhea were demonstrated to be infected solely with *P. hominis*. The findings of the current study provide a straight forward and validated method for the differential diagnosis of *P. hominis* and contribute to the on-going debate surrounding the pathogenic potential of this parabasalid flagellate.

**KEY WORDS.** *Pentatrichomonas hominis*, Parabasalia, diagnostic, flagellates, cats, diarrhea, Southeastern, Brazil.

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**RESUMO.** O parabasalídeo flagelado *Tritrichomonas foetus* é reconhecido como o agente etiológico causador de diarreia do intestino grosso em gatos domésticos. Uma segunda espécie de flagelados parabasalídeos *Pentatrichomonas hominis*, também tem sido relatada em associação com gatos domésticos, embora quase exclusivamente como um organismo comensal. No entanto, há cada vez mais evidências que indicam que *P. hominis* também pode estar envolvido em distúrbios gastrintestinal de felídeos incluindo diarreia e que a incidência de infecção com *P. hominis* pode ter sido subestimada devido ao fato de ser identificado erroneamente como *T. foetus*. O objetivo do presente estudo foi estabelecer a base para a identificação morfológica rotineira de *P. hominis* empregando microscopia de luz e aplicar a metodologia para a análise de casos de diarreia em uma população de gatos de laboratório no Brasil (n = 40). A descrição morfológica detalhada de *P. hominis* isolado a partir de 11 gatos com diarreia foi produzida e análises moleculares foram realizadas em apoio aos dados morfológicos encontrados e demonstrar a ausência de *T. foetus* nos gatos examinados. Todos os animais com diarreia foram positivos para *P. hominis*. Os resultados do estudo atual fornecem um método simples e validado para o diagnóstico diferencial de *P. hominis* e contribui para o debate em curso em torno do potencial patogênico deste flagelado parabasalídeo.

**PALAVRAS-CHAVE.** *Pentatrichomonas hominis*, Parabasalídeo, diagnóstico, flagelados, gatos, diarreia, Sudeste, Brasil.

## INTRODUCTION

The parabasalid flagellates comprise pathogenic and presumably nonpathogenic species frequently reported in veterinary medicine (Tolbert et al. 2012). These single-celled organisms are obligate protozoan symbionts that are either parasites or commensals, generally living in the digestive or genitourinary tract of humans and animals. In addition, it has been reported that these protozoa can migrate to other sites within their target host, can adapt to new hosts, and are capable of zoonotic transmission (Cobo et al. 2003, Gookin et al. 2007, Dimasuay & Rivera 2013)

To date, two species of parabasalids have been reported in domestic cats. The first is *Pentatrichomonas hominis*, which is generally considered to represent a commensal organism (Da Cunha & Muniz 1922, Kessel 1928, Romatowski 1996), and the second species is *Tritrichomonas foetus* which is recognized as the agent of large bowel diarrhea (Levy et al. 2003).

Assessment of feces for the presence of parabasalids can be made using a number of different methods (Gookin et al. 2004): visual examination for motile trophozoites in fresh fecal smears with saline dilution, isolation using specific culture systems (Gookin et al. 2002), or by detection of ribosomal DNA using the polymerase chain reaction (PCR) (Gookin et al. 2002). The first method is not specific and may result in the misidentification of *T. foetus* as *P. hominis* (Romatowski 1996, Romatowski 2000). Detection by PCR using *Tritrichomonas*-specific primers (Gookin et al. 2002) is considered to be the method with the highest specificity and sensitivity (Gookin et al. 2004). Accurate differentiation of these organisms is crucial, since in veterinary practices misdiagnosis would lead to the ineffective initiation of therapy for *T. foetus* using ronidazole, a potentially neurotoxic compound (Cepelcha et al. 2013).

In light of the existing difficulties encountered in obtaining a definitive diagnosis, the current study aimed to produce a detailed morphological description of *P. hominis* isolated from domestic cats in Brazil. Molecular methods were subsequently employed to confirm the morphological diagnosis and to establish that the samples were free of *T. foetus*.

## MATERIAL AND METHODS

### Ethical considerations

All aspects of the study involving animals were processed in strict accordance with the recommendations approved by the Ethics committee of The Universidade Federal Rural do Rio de Janeiro (UFRRJ). (CEUA/IV/UFRRJ; Process # 006/2014). We declare that the animals were not harmed in any way during the procedure.

### Sample collection and processing

Fecal samples were collected from 40 domestic cats with or without diarrhea, maintained at the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária at Universidade Federal Rural do Rio de Janeiro (UFRRJ), located in the Municipality of Seropédica, in the State of Rio de Janeiro, Brazil. Samples were collected immediately after defecation or via rectal infusion of warm saline and were transported without delay to the Laboratório de Patologias da Reprodução located at UFRRJ. Thereafter, they were diluted in Hanks solution supplemented with 10% inactivated horse serum, incubated at 28-33°C, and examined microscopically at 48h intervals. Fecal samples considered as positive upon direct examination were inoculated into peptone broth, incubated at 35°C, and sub cultured at 72h intervals.

### Observation, measurement and illustration of trophozoites

Microscope slides were prepared using smears of culture fixed using 2.5% glutaraldehyde followed by

eosin or Heidenhain's iron hematoxylin staining. Morphological observations, line drawings, photomicrographs and measurements, given in micrometers, were made using a Primo Star Zeiss® microscope coupled to an AxioCamERc 5s digital camera. Size ranges are provided in parentheses following the mean values.

### Molecular analyses

DNA was extracted from approximately  $1 \times 10^6$  trophozoites (enumerated microscopically), as follows; Cells in peptone broth were harvested by centrifugation ( $16,000 \times g$  for 5 min), in 1.5mL screw-capped micro-centrifuge tubes. Supernatants were discarded and the cell pellets were washed once with 1mL of phosphate buffered saline (PBS; pH 7.2). Washed cells were resuspended in 100 $\mu$ L of Instagene™ matrix (BIO RAD), with incubation at 56°C for 30 minutes then boiled for 10 minutes. The tubes were centrifuged ( $16,000 \times g$  for 5 min), to sediment cell debris together with the chelex matrix, and 60 $\mu$ L of each supernatant were transferred to new tubes, with storage at -20°C. A Polymerase chain reaction (PCR) assay specific for Trichomonadidae and Tritrichomonadidae families was performed as described by Felleisen (Felleisen et al. 1997), using the primers TFR1 and TFR2. In addition, an assay specific for *T. foetus* was carried out, using the primers TFR3 and TFR4 according to the methods of Felleisen et al. (1998). Finally, samples were examined using an assay specific for *P. hominis*, employing the primers Th3 and Th5 as reported by Crucitti et al. (2004). The K strain of *T. foetus* isolated by H. Guida (Embrapa, Rio de Janeiro, Brazil), from the urogenital tract of a bull was used as a positive amplification control in the first two assays. An aliquot (5 $\mu$ L) of each PCR reaction was examined by agarose gel electrophoresis to confirm the presence of amplicons with the predicted molecular weight. The PCR products (339 base pairs), from the *P. hominis* specific assay were digested using 10 units of the restriction endonucleases *Hae*III (Promega) and *Hinf*I (Invitrogen) for 2 h at 37°C. Digestion products were separated by electrophoresis on 12% polyacrylamide gels followed by staining with ethidium bromide. The resulting banding patterns were compared to those predicted by *in silico* digestion (using the program NEB-cutter V 2.0; New England Biolabs) of the amplified region present in the 18S rDNA, GenBank accession number KC594038, of a feline isolate of *P. hominis* (Cepulecha et al. 2013). Sequencing of *P. hominis* 18S rDNA amplicons was performed as follows; 10  $\mu$ L of PCR products were treated with Exo-Sap-IT (GE Healthcare), according to the manufacturer's protocol and sequenced in both directions, employing the amplification primers, by use of the BigDye Ready Reaction mix (ABI Corp); reaction products were analyzed on a 3500 automated genetic analyzer (ABI Corp). Sequence alignments were performed using Sequencher (Version 5.1, Genecodes Corporation). All sequences were entered into the BLAST search algorithm (Altschul et al. 1990) and the NCBI nucleotide database to determine gene identity.

### Statistical analyses

Differences were analyzed by the Fisher's exact test, according to Sampaio (2002).

## RESULTS

Wet mounts of stools from 40 cats were observed by light microscopy. Parabasalid-like organisms were evident in samples from 11 of the 40 cats (27.5%). Four of the eleven cats were male and other seven were females. The infected animals had an average age of 36 months-old (10-60). Diarrhea was the main feature observed among the examined cats and they have 14.667 more chance, of having *P. hominis* as observed in this work (Table 1).

### In vitro cultivation

The trophozoites observed in the feces presented progressive and irregular movement characteristic of parabasalids (Diamond 1957). All microscopy positive samples were also culture positive.

Table 1. The importance of *Pentatrichomonas hominis* in naturally infected cats

Diarrhea	Animals		p value	OR	95% IC <sup>a</sup>
	Fecal examination	Values			
Yes	Positive:	11 (28) <sup>b</sup>	0.0053	14.667	1.658 to 129.77
	Negative:	12 (30)			
No	Positive:	1 (3)			
	Negative:	16 (40)			
Total		40 (100)			

<sup>a</sup> Using the approximation of Woolf, <sup>b</sup> Perceptual values.

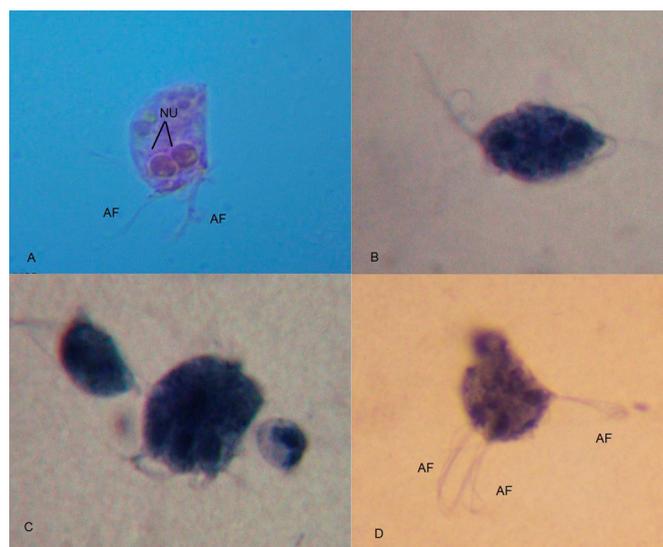


Figure 1. Forms of division of *Pentatrichomonas hominis* recovered from domestic cats. (A) Trophozoite in binary division, with two nuclei and two groups of flagella Eosin. (B) Trophozoite in cytokinesis. (C-D) Multiple division with formation of three / four organisms. Heidenhain's iron hematoxylin. NU= nucleous, AF= anterior flagela. Obj. 1000X. Scale bar = 5 $\mu$ m.

In culture, the trophozoites presented rounded shapes, and multiplication by binary or multiple divisions (Figure 1). Organisms generally survived well for at least 10 days in feces, with some surviving for as long as 3 weeks. Cultures grew well at 33°C and could be continually passaged in peptone broth medium. All cultures showed heavy bacterial contamination that could not be eliminated.

**Morphology**

Parabasalids were clearly visible by light microscopy in unstained, and in eosin and iron-hematoxylin stained preparations (Figures 1A and 2D), but visualization of and the ability to enumerate flagella was better in the wet mount method than in stained preparations. Trophozoite shape (n=50) was piriform to rounded. Body size, without the axostyle protrusion was 10.7 (7-15) µm long and 8.2 (5-13) µm wide. Five unequal anterior flagella demonstrated lengths greater than or equal to that of the body, with one flagellum frequently observed to be orientated towards the vent (Figure 2 A). Trophozoites with four anterior flagella were infrequent. A recurrent flagellum extended throughout the body with three to four long waves (undulating membrane) ending in a free posterior flagellum (~ 9 µm) (Figure 2 B and C). Costa is slender, with

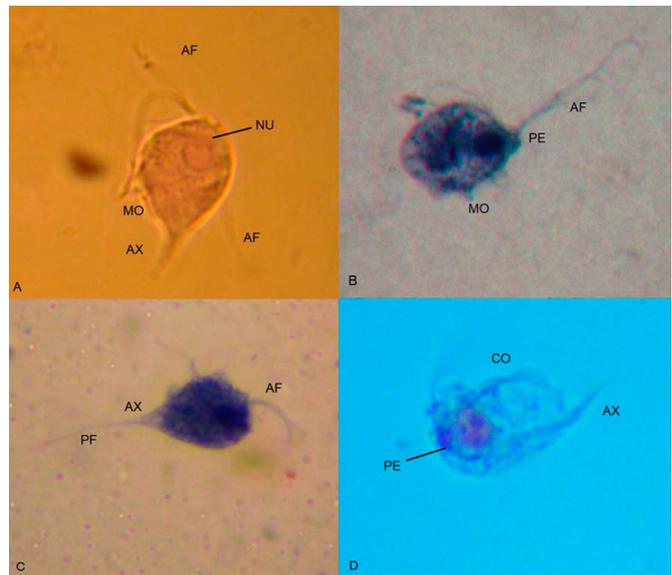


Figure 2. *Pentatrichomonas hominis* recovered from domestic cats. (A) Trophozoites present five anterior flagellum visible, round nucleus and typical axostyle. Eosin. (B) *P. hominis* showing undulating membrane, anterior flagella group and pelta. Heidenhain's iron hematoxylin. (C) Trophozoite present anterior flagella group, undulating membrane and a long posterior flagella. Heidenhain's iron hematoxylin. (D) Trophozoite showing the visible costa below the undulating membrane and for its entire length, round nucleus, thin pelta, and visible axostyle in its entire length. Eosin. NU = nucleus, FA= anterior flagella, AX = axostyle, CO = costa, Pe = pelta, UM = undulating membrane, PF= posterior flagella. Obj. 1000X. Scale bar = 5µm.

Table 2. Comparative morphology of *Pentatrichomonas hominis* trophozoites isolated from cats and humans.

Authors	Wenrich (1944)	Kirby (1943)	Da Cunha & Muniz (1922)	Kessel (1928)	Present study
Hosts:	Human	Human	Cat	Cat	Cat
Size (Average and limits in µm):					
Number 1	Equal to or greater than the length of the body	Equal to or greater than the length of the body	Equal to or greater than the length of the body	-	13.50
Number 2	-	-	-	-	13.48
Number 3	-	-	-	-	13.30
Number 4	-	-	-	-	13.00
Number 5	-	-	-	-	13,00
Posterior Flagella	-	5	-	2-4	8,67 (5,25-12,05)
Axostyle Protusion	-	-	-	-	4,15 (3,42-5,04)
Waves Membrane Undulating	3-5	-	-	-	3-5
Structure (Average and limits in µm):					
Body Shape					
	Fusiforme/ piriform Piriform/rounded	Piriform	Piriform/ globular	Rounded	Fusiform/ Piriform
Body	Length 8.3 (6 -13.5)	8-20	7.5	8-15	10.69 (6.87-14.87)
	Width -	3-14	6	5-8	8.22 (5.5-12.45)
	Shape Round / oval	-	elliptic	-	round
Nucleus	Length -	-	-	-	3.51 (2.66-5.19)
	Width -	-	-	-	3.29 (2.61-5.02)
	Shape -	ellipsoid	-	-	round
Parabasal Body	Length -	small	-	-	1.36
	Width -	-	-	-	1.07
Nº Anterior Flagella	majority 5 (4 - 3)	majority 5 (4)	4	4	5

diameter similar to the anterior flagellum and extends throughout the body. Pelta is thin, short and barely discernible. The axostyle gradually attenuated toward its end and protruded for 4.2 (2-7)  $\mu\text{m}$  from the cell body (Figure 2 D). All measures of trophozoites are in Table 2 and were compared to other morphological studies done about the species (Da Cunha & Muniz, 1922, Kessel 1928, Kirby 1943, Wenrich 1944). Based on the observed morphological characters a schematic drawing was produced with the main characteristics that define the species *P. hominis* (Figure 3)

### DNA analysis

All culture positive samples yielded amplicons of approximately 360 bp using the primers TRF1 and TRF2, indicating the presence of parabasalid DNA. Conversely, no amplicons were observed using the *T. foetus* specific assay. Importantly, the positive control (DNA equivalent to 100 trophozi-

tes of strain K of *T. foetus*) showed strong amplification of a band of 350 bp in accordance with the results of Felleisen et al. (1998). All of the 11 samples were recorded as positive, based on the presence of a 339 bp product, in the *P. hominis* PCR assay. In all cases, digestion of the 339 bp amplicons with *Hae*III resulted in bands of 245 bp and 94 bp, and in the generation of three bands of 155bp, 126bp and 58bp with the enzyme *Hinf*I. The observed banding patterns matched those predicted by *in silico* digestion. Four of the 11 amplicons were sequenced and each showed 100% nucleotide similarity over 297 bases (=amplicon after removal of the primer sequences), with sequences deposited in the GenBank as originating from feline (accession number KC594038.1) or canine (accession number KC953860.1) isolates of *P. hominis*. The sequence of a representative isolate was deposited in the GenBank with the accession number KF953914.

### DISCUSSION

In the current study, only *P. hominis* was identified in fecal samples, which matches the findings of Mostegl et al. (2012), where *P. hominis* but not *T. foetus* was observed in feline intestinal tissue sections examined by chromogenic *in situ* hybridization, with the absence of *T. foetus* confirmed by PCR. Moreover, our findings correlate with the isolation of pure cultures of *P. hominis* from a single cat with diarrhea in the Czech Republic, using In-Pouch<sup>TM</sup>TF-Feline medium (Ceplecha et al. 2013). In contrast, the study of Gookin et al. (2007), reported that *P. hominis* was detectable by PCR in the feces of a limited number of the animals examined, and that *P. hominis* was not detected in any fecal samples that were not also positive for *T. foetus* infection.

Traditionally, *P. hominis* is presumed to be a commensal; in contrast, *T. foetus* is recognized as an important pathogen of cats with diarrhea (Gookin et al. 2005). In this sense, reliable diagnosis of parabasalid infections of cats is important to ensure the most effective clinical treatment.

Alternatively, some authors have suggested the ability of this species to cause gastrointestinal disturbance in cats, based on the presence of parabasalids morphologically consistent with *P. hominis* in fecal samples (Romatowski 1996, 2000, Gookin et al. 1999), although other workers contest this assumption (Levy et al. 2003). The current study reports the first identification of *P. hominis* associated with cats with diarrhea in Brazil.

It is of value to note that *P. hominis* is euryxe-

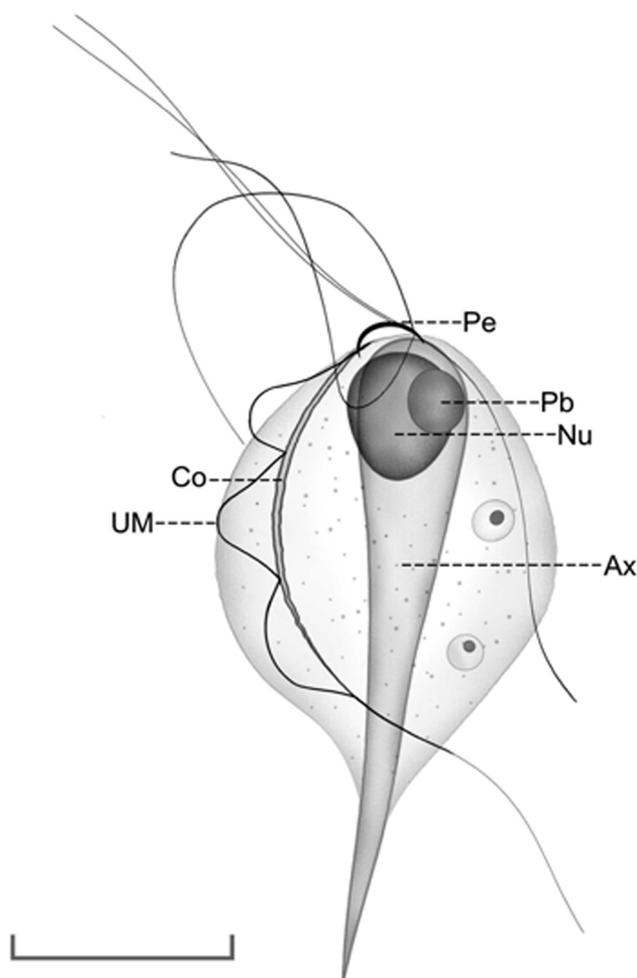


Figure 3. Composite line drawing of a representative trophozoite of *Pentatrichomonas hominis* recovered from domestic cats. Ax = axostyle, Co = costa, Nu - nucleous, Pb = parabasal body, Pe = pelta, UM = undulating membrane. Scale bar = 5 $\mu\text{m}$ .

nic, with reports of this parasite colonizing the gastrointestinal tract of several mammalian species including dogs, cats, cattle, mice, monkeys and humans (Meloni et al. 2011). Interestingly, the lack of host specificity for *P. hominis*, raises questions concerning the zoonotic potential of this species, particularly in light of reports from companion animals. More recently, additional hosts including the Philippine scops-owl *Otus megalotis* and the eastern racer *Coluber constrictor* have been reported in the Philippines. These observations may be linked to the dietary habits of these predators and clearly demonstrate the ease with which this parabasalid adapts to different hosts (Dimasuay & Rivera 2013).

A growing number of human cases of trichomoniasis involving *P. hominis* and *T. foetus* have emerged recently. Specifically, *P. hominis* has been considered as a causative agent of gastrointestinal illness in humans, with clinical presentation of irritable bowel syndrome (Meloni et al. 2011), and was also found in both stool and exudative pleural effusion from a woman with lupus erythematosus who died after 18 days of drug treatment (Jongwutives et al. 2000). More recently, a man with fever, mucous diarrhea, tenesmus, and arthralgia, who recovered after treatment with metronidazole, was shown to be *P. hominis* positive (Compaoré et al. 2013). Likewise, *T. foetus* was identified in peritoneal fluid from a 52-year-old man with common variable immunodeficiency, rheumatoid arthritis, splenectomy, and cryptogenic cirrhosis, who lived on a farm and had contact with swine, horses, and cats (Zalonis et al. 2011).

Regardless of the uncertainty in relation to the pathogenic and zoonotic potential of *P. hominis*, it is clear that more studies should be conducted on this topic. In particular diagnostic methods should be available to assist in the specific identification of these parabasalids. The majority of recent studies on the identification of parabasalids have employed molecular methods without presenting any morphological data that would enable a reliable diagnosis in clinical laboratories, which may lack access to molecular techniques. In contrast, in this study, the relatively simple morphological analysis served to provide satisfactory differentiation between *P. hominis* and *T. foetus*. Characteristics including the number of flagella, and the shape and size of the axostyle were demonstrated to be useful for the purpose of differentiation. Subsequently, molecular methods were employed to sequentially demonstrate the presence of parabasalids, the absence of *T. foetus* and finally the recovery of pure cultures of *P. hominis*.

The number of flagella is constant in some parabasalids, including *T. foetus*, and has emerged as a key characteristic used for species identification. Yet in the past, reliance upon this phenomenon has led to the publication of a number of dubious descriptions of new genera and species (Flick 1954). Kirby (1943) defined five anterior flagella as a stable and definitive characteristic feature for *P. hominis*. However in the study of Flick (1954), 80% of the trophozoites of *P. hominis* from human and primates had five anterior flagella; 15% had four anterior flagella; and 5% had three anterior flagella. This variation was attributed to factors including culture age and the presence of specific bacteria. More recently, difficulties in differentiating *P. hominis* from *T. foetus* using a limited number of morphological criteria, primarily flagella number, have been reported (Levy et al. 2003, dos Santos et al. 2015).

The majority of the isolates examined in the current study demonstrated the presence of five anterior flagella or infrequently four. We are of the opinion that the use of this feature, which was readily observable using the staining scheme employed herein, provided a clear identification of the feline isolates as *P. hominis*. These characteristic features were also highlighted by (Cepicka et al. 2010), for the morphological identification of trophozoites of Parabasalia.

The morphological data used for identification of *P. hominis* agree with the findings of the study conducted by Li et al. (2014), in which *P. hominis* isolates from swine presented five flagella with four of the structures having the same origin while a fifth, independent, flagellum originated separately from the others. In contrast to the findings of those authors, we observed forms of binary and multiple divisions with protozoa dividing into up to four new trophozoites. It is pertinent to note that this feature was also observed by Wenrich (1944) with *P. hominis* isolated from man, monkeys, cats and dogs.

In conclusion, the current study highlighted some characteristic features that may serve to ensure reliable identification and accurate diagnosis of *P. hominis* in routine diagnostic laboratories and to improve our knowledge of this versatile flagellate.

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## REFERENCES

- Cepicka I., Hampl V. & Kulda J. Critical taxonomic revision of parabasalids with description of one new genus and three new species. *Protist*, 161:400-433, 2010.
- Cepička V., Svoboda M., Cepička I., Husník R., Horáčková K. & Svobodová V. InPouch™ TF-Feline medium is not specific for *Tritrichomonas foetus*. *Veterinary Parasitology*, 196:503-505, 2013.
- Cobo E.R., Campero C.M., Mariante R.M. & Benchimol M. Ultrastructural study of a tetratrichomonad species isolated from prepucial smegma of virgin bulls. *Veterinary Parasitology*, 117:195-211, 2003.
- Compaoré C., Lekpa F.K., Nebie L., Niamba P. & Niakara A. Pentatrichomonas hominis infection in rheumatoid arthritis treated with adalimumab. *Rheumatology*, 52:1534-1535, 2013.
- Crucitti T., Abdellati S., Ross D.A., Chagalucha J., Dyck E. & Buve A. Detection of *Pentatrichomonas hominis* DNA in biological specimens by PCR. *Letters in Applied Microbiology*, 38:510-516, 2004.
- Da Cunha A.M. & Muniz J. Trabalhos do Instituto Oswaldo Cruz: Sobre um flagelado parasito do gato. *Brasil Médico*, 36:285-286, 1922.
- Diamond L.S. The establishment of various *Trichomonas* of animals and man in axenic cultures. *Journal of Parasitology*, 43:488-490, 1957.
- Dimasuy K.G.B. & Rivera W.L. Molecular characterization of trichomonads isolated from animal hosts in the Philippines. *Veterinary Parasitology*, 196:289-295, 2013.
- Dos Santos C.S., De Jesus V.L.T., McIntosh D., Berto B.P. & Lopes C.W.G. Co-infection by *Tritrichomonas foetus* and *Pentatrichomonas hominis* in asymptomatic cats. *Pesquisa Veterinária Brasileira*, 35:981-989, 2015.
- Felleisen R.S.J. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. *Parasitology* 115:111-119, 1997.
- Felleisen R.S.J., Lambelet N., Bachmann P., Nicolet J., Müller N. & Gottstein B. Detection of *Tritrichomonas foetus* by PCR and DNA Enzyme Immunoassay Based on rRNA Gene Unit Sequences. *Journal of Clinical Microbiology*, 36:513-519, 1998.
- Flick E.W. Experimental analysis of some factors influencing variation in the flagellar number of *Trichomonas hominis* from man and other primates and their relationship to nomenclature. *Experimental Parasitology*, 3:105-121, 1954.
- Gookin J.L., Breitschwerdt E.B., Levy M.G., Gager R.B. & Benrud J.G. Diarrhea associated with trichomonosis in cats. *Journal of the American Veterinary Medicine Association*, 10:1450-1454, 1999.
- Gookin J.L., Birkenheuer A.J., Breitschwerdt E.B. & Levy M.G. Single-tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. *Journal of Clinical Microbiology*, 40:4126-4130, 2002.
- Gookin J.L., Foster D.M., Poore M.F., Stebbins M.E., Levy M.G. Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats. *Journal of the American Veterinary Medicine Association*, 222:1376-1379, 2003.
- Gookin J.L., Stebbins M.E., Hunt E., Burlone K., Fulton M., Hochel R., Talaat M., Poore M. & Levy M.G. Prevalence of and risk factors for feline *Tritrichomonas foetus* and *Giardia* infection. *Journal of Clinical Microbiology*, 42:2707-2710, 2004.
- Gookin J.L., Birkenheuer A.J., St John V., Spector M. & Levy M.G. Molecular characterization of trichomonads from feces of dogs with diarrhea. *Journal of Parasitology*, 91:939-943, 2005.
- Gookin J.L., Stauffer S.H. & Levy M.G. Identification of *Pentatrichomonas hominis* in feline fecal samples by polymerase chain reaction assay. *Veterinary Parasitology*, 145:11-15, 2007.
- Jongwutiwes S., Silachamroon U. & Putaporntip C. *Pentatrichomonas hominis* in empyema thoracis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94:185-186, 2000.
- Kessel J.F. Trichomoniasis in kittens. *Transactions of Royal Society Tropical Medicine Hygiene*, 22:61-80, 1928.
- Kirby H. Observations on a trichomonad from instestine of man. *Journal of Parasitology*, 49:422-423, 1943.
- Levy M.G., Gookin J.L., Poore M., Birkenheuer A.J., Dykstra M.J., R. & Litaker W. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the Etiologic Agent of Feline Trichomonal Diarrhea. *Journal of Parasitology*, 89:99-104, 2003.
- Li W., Li W., Gong P., Meng Y., Li W., Zhang C., Li S., Yang J., Li H., Zhang X. & Li J. 2014. Molecular and Morphologic Identification of *Pentatrichomonas hominis* in Swine. *Veterinary Parasitology*, 202:241-247, 2014.
- Meloni D., Mantini C., Goustille J., Desoubieux G., Maakaroun-Vermesse Z., Chandener J., Gantois N., Duboucher C., Fiori P.L., Dei-Cas E., Duong T.H. & Viscogliosi E. Molecular identification of *Pentatrichomonas hominis* in two patients with gastrointestinal symptoms. *Journal of Clinical Pathology*, 64:933-935, 2011.
- Mostegl M. M., Wetscher A., Richter B., Nedorost N., Dinhopf N. & Weissenböck H. Detection of *Tritrichomonas foetus* and *Pentatrichomonas hominis* in intestinal tissue specimens of cats by chromogenic *in situ* hybridization. *Veterinary Parasitology*, 183:209-214, 2012.
- Romatowski J. An uncommon protozoan parasite (*Pentatrichomonas hominis*) associated with colitis in three cats. *Feline Practice*, 24:10-14, 1996.
- Romatowski J. *Pentatrichomonas* infection in four kittens. *Journal of the American Veterinary Medicine Association*, 216:1270-1272, 2000.
- Sampaio I.B.M. *Estatística aplicada à experimentação animal*. FEPMVZ Editora, Belo Horizonte, 2002.
- Tolbert M.K., Leutenegger C.M., Lobett R., Birrell J. & Gookin J.L. Species identification of trichomonads and associated coinfections in dogs with diarrhea and suspected trichomonosis. *Veterinary Parasitology*, 187:319-322, 2012.
- Wenrich D. H. Morphology of the intestinal trichomonad flagellates in man and of similar forms in monkeys, cats, dogs, and rats. *Journal of Morphology*, 74:189-211, 1944.
- Zalonis C.A., Pillay A., Secor W., Humburg B. & Aber R. Rare Case of Trichomonal peritonitis. *Emerging Infection Diseases*, 17:1312-1313, 2011.