

A survey of feline trichomonosis suggests a low incidence of *Tritrichomonas blagburni* among cats in the Czech Republic

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ABSTRACT: *Tritrichomonas blagburni* (previously called *T. foetus*) has been implicated as an aetiological agent of long-term large-bowel diarrhoea in cats in many countries worldwide. The aim of this study was to determine the presence of, and risk factors for *T. blagburni* among a cohort of cats living in different conditions in the Czech Republic. Samples were collected from 170 cats living in different environments. The InPouch™ TF-Feline medium method was used for diagnosis of feline trichomonosis. A single case (0.6%) with motile trichomonads identified as *Pentatrichomonas hominis* was found in a cat from a multi-cat household. Our study suggests that trichomonads and in particular, *T. blagburni*, infection may be much less common in the Czech Republic than in neighbouring countries, despite the inclusion of cats that were likely to be from higher-risk groups. A review of studies of the association of trichomonads and feline diarrhoea carried out in different countries revealed variation in the frequency of trichomonads detected. Different combinations of PCR or culture methods for screening or confirmation have been utilised, with or without species differentiation; however, this could not solely account for the variation in the occurrence between countries. From those studies where differentiation was performed, we calculated from the combined studies that *T. blagburni* occurred in six cats without diarrhoea (1.1%) and 47 cases with diarrhoea (5%). This finding supports an association with diarrhoea as well as the occurrence of asymptomatic cases. We note that in many studies, including our own, the occurrence of *T. blagburni* may well be underestimated and suggest that future studies use a combination of PCR screening of both faeces and faecal cultures, with differentiation of trichomonad species.

Keywords: *Tritrichomonas foetus*; *Pentatrichomonas hominis*; InPouch™ TF-Feline medium; PCR; feline trichomonosis; occurrence

Trichomonads are a group of single-cell anaerobic flagellates. *Tritrichomonas foetus* is a cause of bovine trichomonosis (Suchodolski 2008). Recent molecular studies have shown that the trichomonads in cats differ from those found in bovine cases, and the feline trichomonads have now been named *Tritrichomonas blagburni* nova species

(Walden et al. 2013). However, a clear separation of feline from bovine isolates was not shown (Yao and Koster 2015). *T. blagburni* has been reported in cats around the world and is associated with clinical signs of varying severity, ranging from asymptomatic to chronic intermittent diarrhoea (Gookin et al. 2004; Xenoulis et al. 2010; Tysnes

et al. 2011), often in young (less than 12 months), purebred cats from catteries and those living in households with a high density of cats (Gookin et al. 1999; Foster et al. 2004; Bell et al. 2010). Prevalence rates range from 0% to 31% (Gookin et al. 2004; Bissett et al. 2009), the highest (32%) being found in non-pedigree shelter cats (Holliday et al. 2009) suggesting that a multi-cat environment is likely to be the main risk factor.

To the authors' knowledge, no data exists on the frequency of feline trichomonosis in the Czech Republic. The aim of this study was to gain new insights into the presence and risk factors for *T. blagburni* among cats in this region.

MATERIAL AND METHODS

Cats. Faecal samples were collected from 181 cats from September 2010 to September 2012. The cats were from four groups: (1) catteries, (2) private owners, (3) in-patients at the Small Animal Clinic, University of Veterinary and Pharmaceutical Sciences, Brno and (4) shelter cats.

Detection of trichomonads. The "gold standard" for the detection of different species of trichomonads and for the diagnosis of feline trichomonosis is considered to be the culture method: the InPouch™ TF-Feline medium (Bio-Med Diagnostics, White City, USA). An in-house positive control (laboratory culture of *T. blagburni*) was also utilised. A client questionnaire and physical examination by the veterinarian were completed followed by collection of a rectal faecal sample using a faecal loop (Jorgensen Laboratories, Loveland, USA). Representative samples of approximately 30 mg of faeces were placed in the InPouch™ TF-Feline medium, and incubated according to the manufacturer's instructions. Media with bacterial overgrowth or gas production were ruled out from further examination according to the manufacturer's instructions, a necessary step because bacterial overgrowth can interfere with the propagation of trichomonads in the InPouch™ medium (Clothier et al. 2015). The faecal flotation method was used concurrently to identify other parasitic infections.

PCR analysis. PCR was performed on faecal samples only from cats testing positive for the motile trophozoites of trichomonads identified in the InPouch™ TF-Feline medium test (Ceplecha

et al. 2013). *T. blagburni* genomic DNA was used as a positive control.

RESULTS

Samples from 181 cats were prospectively collected during the study; however, because of bacterial overgrowth in 11 samples (6%) and the consequent possibility of interference with the culture of trichomonads, only the remaining 170 cats were included in the analysis. The distribution of cats between the four groups was: (1) catteries (32.7%), (2) private owners (35.7%), (3) in-patients at the Small Animal Clinic, Brno (23.4%) and (4) shelter cats (8.2%). The characteristics of the cohort of cats used in this study were the following: male (46%), female (54%); under 12 months of age (38%), over 12 months (62%); indoor (43%), outdoor (57%); history of diarrhoea (55%); no history of diarrhoea (45%); number of cats in the household, two or less (26%), three cats (7%), four or more (67%).

Participating cats belonged to the following breeds: 22 British shorthairs (12.9%), 16 Persians (9.4%), nine Maine coons (5.3%), seven Snowshoes (4.1%), six European shorthairs (3.5%), five Siberians (2.9%), five Devon rex (2.9%), four Ragdolls (2.3%), two Birmans (1.2%), one Turkish van (0.6%) and one Bengal (0.6%). Ninety-two cats were mixed-breeds (54.1%).

Motile trophozoites of trichomonads were observed in the InPouch™ TF-Feline medium in only one case (0.6%). This sample was from an 8-month-old intact male, outdoor cat of mixed-breed that lived together with four other cats belonging to the same private owner. During faecal collection, a cow-pat stool (grade 6/7) was observed. Other cats from the same household did not show clinical signs of trichomonosis and had negative faecal cultures. The density of trichomonads in the InPouch™ TF-Feline medium from this case, was comparable to the in-house positive control (laboratory culture of *T. blagburni*). However, PCR analysis revealed that the trichomonads were commensal *P. hominis* and not *T. blagburni* (Ceplecha et al. 2013).

A repeat examination of faeces (InPouch™ TF-Feline medium) in the cat positive for trichomonads was performed after 12 weeks and was found to be negative for the presence of motile trophozoites of trichomonads. Treatment with ronidazole was not instituted in this case.

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DISCUSSION

The InPouch™ TF-Feline medium method of detection of trichomonads used in our study is commercially available, relatively cheap and easy-to-use in-house, and has been reported to be four times more sensitive than native microscopy on faecal smears (Gookin et al. 2004). Both *T. blagburni* and *P. hominis* can be successfully cultured using this method, thus a positive finding for trichomonads requires species-specific PCR for differentiation (Ceplecha et al. 2013). In the current study, the single positive case detected using InPouch™ TF-Feline medium was *P. hominis*.

The numbers of cases of trichomonads and *T. blagburni* vary greatly between studies and countries, likely in part due to differences such as the numbers of cats living together, exposure, hygiene and husbandry. Similarly low frequencies of trichomonads were reported by others also using the InPouch™ culture detection method (see Table 1; Bissett et al. 2009; Tysnes et al. 2011; Queen et al. 2012) from Australia, Norway and the USA. Species differentiation of positive cultures by PCR was not performed

in some sub-sets of cats in several studies (Gookin et al. 2004; Brigui et al. 2007; Burgener et al. 2009; Holliday et al. 2009; Tysnes et al. 2011; Queen et al. 2012). We used data from studies where the species of trichomonads were identified by PCR, and calculated that *T. blagburni* was found in 1.1% of cats without diarrhoea, from a Greek study (Xenoulis et al. 2010) and in 5% of cats with diarrhoea from a combination of reports including the current study (Gun-Moore et al. 2007; Bissett et al. 2009; Xenoulis et al. 2010). These data are consistent with a causative association with diarrhoea and also support the occurrence of asymptomatic cases. However, the combined data is likely an underestimate of *T. blagburni* occurrence because not all studies differentiated trichomonad species by PCR. Also, the data reported in some studies enabled only limited interpretation as the consistency of stools was not reported (Gookin et al. 2004; Tysnes et al. 2011). The total frequency of cats with trichomonads of any species was 551 cases (3%) without diarrhoea and 944 cases (17%) with diarrhoea.

It is also possible that an initial screen of samples using InPouch™ culture followed by PCR on those

Table 1. Comparison of trichomonad (T) and *T. blagburni* (Tb) detection in cohorts of cats in different countries

Country	Setting	Number of cats (% trichomonads)	Presence of T and Tb in cats		Reference
			without diarrhoea <i>n</i> (%)	diarrhoea <i>n</i> (%)	
Australia	catteries, shelters	134 (0)	134 (0 ^{Tb})	0 (0 ^{Tb})	Bissett et al. (2009)
Czech Republic	catteries, private owners, inpatients, shelters	170 (0.6)	76 (0)	94 (1, 0 ^{Tb})	current study
France	catteries	141 (10.2)	98 (7)	43 (18.6)	Brigui et al. (2007)*
Germany	cat shows	230 (15.7)	91 (4.4)	139 (23)	Kuehner et al. (2011)*
Great Britain	na	111 (14)	0 (0 ^{Tb})	111 (14 ^{Tb})	Gun-Moore et al. (2007)
Italy	shelter	74 (32)	0 (0)	74 (32, 1 ^{Tb})	Holliday et al. (2009)*
Greece	private owners	30 (20)	24 (25 ^{Tb})	6 (0 ^{Tb})	Xenoulis et al. (2010)
USA	patients	146 (9.8)	69 (0)	77 (22, 15.6 ^{Tb})	Stockdale et al. (2009)*
USA	patients, shelters	223 (2.2) 131 (3.8)	53 (0) 6 (0 ^{Tb})	170 (2.9) 125 (4 ^{Tb})	Queen et al. (2012)*
Switzerland	patients	105 (26)	0 (0)	105 (26, 12.5 ^{Tb})	Burgener et al. (2009)*
Total		1495 (10%)	551 (3, 1.1 ^{Tb})	944 (17, 5 ^{Tb})	

^{Tb}*T. blagburni* nova species (previously reported as *T. foetus*) confirmed or diagnosed using PCR

*Studies which did not use PCR method for differentiation and confirmation of *T. blagburni* in some sub-sets of cats

samples positive for trichomonads could underestimate *T. blagburni* occurrence if trichomonads did not multiply. Some authors found PCR to be approximately two-fold more sensitive than culture alone (Gookin et al. 2004). DNA extraction from cultures of InPouch™ TF-Feline medium was recommended by Vermeulen (2009) who proposed this to be the most sensitive method. In hindsight, for greater detection efficiency, we recommend that PCR be carried out on saline smears and faecal cultures.

The low frequency of trichomonads, including *T. blagburni* in the current study precluded statistical analysis of the questionnaires collected and therefore it was not possible to assess risk factors such as breed, age, sex, cat density or environmental setting. The identification, albeit transient, of one case of infection with *P. hominis*, raises concerns for possible zoonotic transmission to humans (Meloni et al. 2011).

Further studies including greater numbers of cats from different environments and risk groups will indicate whether our findings from the 170 cats are representative of the greater cat population in the Czech Republic. Should the low numbers of *T. blagburni* in the Czech Republic be confirmed, these data would be useful in providing information on factors that have led to these low numbers, such as possible successful hygiene and husbandry practices or other factors limiting spread.

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