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Abstract

Case series summary Infection of the domestic cat by *Tritrichomonas blagburni*, previously referred to as *T foetus*, may lead to a disease called feline trichomonosis, which manifests clinically as large bowel diarrhoea. The disease has a wide geographical distribution, including numerous countries in Europe, North America and Australia/Oceania. Nevertheless, it has occasionally been reported in Asia, South Korea and Japan. A retrospective study was carried out to include all domestic cats with diarrhoea, presented to two veterinary clinics in Hong Kong, China, between 1 July 2009 and 30 June 2014. A total of 29 cats with diarrhoea were diagnosed with *Tritrichomonas* species infection by means of quantitative PCR and direct microscopy. *Tritrichomonas* species was more frequently found in young (median age 10 months), male (66%) and purebred cats (86%). *Giardia* species was found in 31% of *Tritrichomonas* species-positive cats. The recommended dose of ronidazole (30 mg/kg q24h for 14 days) resolved clinical signs in 83% of diagnosed cats.

Relevance and novel information This case series describes *Tritrichomonas* species as a cause for feline chronic diarrhoea in cats in China. The high rate of failure (17%) in those cats receiving a standard regimen of ronidazole might be due to owner or patient compliance rather than protozoal resistance to the drug.

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Introduction

Tritrichomonas foetus is a confirmed aetiology of large bowel diarrhoea in the domestic cat.^{1,2} Nevertheless, a new species, named *Tritrichomonas blagburni*, has been proposed for the protozoan in the domestic cat, in order to distinguish it from the one that infects cattle.³ This is still scientifically debatable as data obtained from comparative transcriptomics failed to confirm the status of the two species. Instead, it strongly suggested them as two isolates of one species.⁴ To avoid further confusion the organism will be referred to as *Tritrichomonas* species. Case reports or survey data on feline trichomonosis have been reported in many geographic regions worldwide including Europe (Austria, Finland, France, Germany, Greece, Italy, the Netherlands, Norway, Poland, Spain, Sweden, Switzerland and the UK), North America (Canada and the USA), Australia/Oceania (Australia and New Zealand) and Asia (Japan and South Korea).⁵ Sampling bias has crept into *Tritrichomonas* species prevalence studies, owing to the variation in the sample population, including either diarrhoeic cats, show cats, cats

housed in catteries or cats presenting to veterinarians and veterinary clinics, varying from 82% of 22 samples tested in New Zealand to 6% reported in a survey in the USA.⁶

Several risk factors are associated with *Tritrichomonas* species infection in the domestic cat with an odds ratio (OR) ≥ 2 . A metadata analysis of previously reported data originating exclusively from European countries concluded that young age is a risk factor.⁵ Another risk

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factor is purebred cats, which had an OR ranging from 26.3 to >999.9; specifically, Siamese, Bengal and Norwegian Forest Cats have an OR of 7.3–25.9.^{7–9} Close and direct contact with a *Tritrichomonas* species-positive cat in the previous 6 months is associated with an OR of 3.2–4.4, and a history of diarrhoea in the previous 6 months has an OR 1.7–3.2.^{5,10} When examining co-infections, *Giardia* species has a greater than random co-occurrence with either *Tritrichomonas* species or *Cryptococcus* species respectively, as well as a greater three-way co-occurrence among these three enteral protozoa.¹¹ A few other factors have been analysed for their association with a *Tritrichomonas* species-positive status, including sex (with a male kitten having a great risk factor; $P < 0.01$), sharing a house with more than five cats (OR 4.6; $P < 0.01$) and food (OR 5.4 for feeding a raw food diet; $P < 0.01$).^{7,12}

Gastrointestinal disease has been demonstrated in cats experimentally infected with *Tritrichomonas* species as early as 2–7 days after orogastric inoculation.^{1,13,14} *Tritrichomonas* species is limited to the ileum, caecum and colon in experimentally infected cats.¹⁴ Clinical signs, if present, include chronic or intermittent large bowel diarrhoea, malodorous yellow–green faeces and the hallmark features of colitis, including fresh blood, mucous, faecal incontinence, increased urgency and frequency, tenesmus and flatulence.^{1,9,14,15} One-fifth of infected cats demonstrate systemic clinical signs, which include, anorexia, depression, vomiting and weight loss, and less commonly, vomiting and fever.^{15–17} Clinical signs are reported to persist for 5–24 months, with a median of 9 months, from the time of diagnosis, but despite clinical remission >50% will have persistent trichomonosis and will frequently relapse with diarrhoea.¹⁸

Diagnosis of a trichomonad infection is confirmed by several methods, but they vary in their sensitivity and include demonstration of the trophozoite on a saline-diluted direct faecal smear (14.7% sensitivity); culture of the organism by inoculating modified Diamond's fluid (26.4% sensitivity) or the commercially available InPouch TF medium (InPouch TF; BioMed Diagnostics [58.8% sensitivity]); and the extraction of DNA in faeces followed by the amplification of *Tritrichomonas* species rDNA by PCR.^{5,10,19} Faecal samples should be fresh and include either a voided stool or one collected by manual extraction with the aid of faecal loops or by a colon flush technique, described elsewhere.⁵

Ronidazole is the current recommended treatment at a dosage of 30 mg/kg, administered once daily for 14 days, although it is not registered for human or veterinary use and informed consent is necessary prior to use in the domestic cat.²⁰ Therefore, it should only be prescribed in confirmed cases. Relapse is common and cats with resolved clinical signs can continue to carry and

shed the organism. Side effects reported include, neurological signs, lasting 1–4 weeks.²¹

Case series description

This retrospective study examined the data of cats treated by a large hospital and a cat clinic in Hong Kong, China, for diarrhoea with confirmed trichomonosis for a 5 year period. For this study, the medical records of all cats presented between 1 July 2009 and 30 June 2014 were searched for cases of cats diagnosed with diarrhoea consistent with large bowel diarrhoea that was confirmed to be caused by *Tritrichomonas* species infection on quantitative PCR (qPCR). The search criteria used included 'cat' and 'diarrhoea' or 'large bowel' or 'colitis'. Medical records were reviewed in detail and only those cases with complete medical records, including history and clinical examination, and details regarding the diagnostic testing, were included. The stool was scored based on the Purina Veterinary Diet faecal scoring system.²² Data were entered using Excel spreadsheets (Microsoft). The signalment was determined by the written medical record. Historical findings, including the presence of diarrhoea, clinical signs that were consistent with small or large bowel diarrhoea, presence of in-contact cats with diarrhoea and the use of antibiotics in the preceding 14 days, were recorded. The diagnosis of *Tritrichomonas* species was made by a combination of faecal microscopy (direct faecal smear) and PCR of faeces. PCR diagnosis was contracted to two commercial diagnostic laboratories (Hong Kong University and VetPath Asia Laboratory, Hong Kong SAR, China) that performed real-time PCR as previously described.^{23,24} Faeces were screened for *Giardia* species infection in many cases by either a direct faecal smear or a commercially available antigen test (SNAP Giardia Test; IDEXX) or a commercially available PCR test offered by IDEXX Laboratories (IDEXX). Descriptive statistics were used. Excel (Microsoft) was used to calculate the medians and a 25th–75th interquartile range (IQR) of the continuous data.

A total of 29 domestic cats had confirmed *Tritrichomonas* species infection over this period of time. All cats had *Tritrichomonas* species organism DNA confirmed in the stool sample by means of qPCR testing. In the 14 samples that were subjected to microscopic examination, 13 cats had detectable parasites (93%). Four of the cats were spayed females, six were intact females, seven were neutered males and 12 were intact males. The majority of the infected cats were purebred (86%, $n = 25$). The most frequently reported breeds included British Shorthair (26%; $n = 8$), Exotic Shorthair (17%; $n = 5$) and American Shorthair (10%; $n = 3$) with only one or two cats of other breeds, including Abyssinian, Chinchilla, Munchkin, Persian, Ragdoll and Scottish Fold. The median age of the cats that tested positive was 10 months (IQR 6.5–26.5 months). Thirteen cats

presented with large bowel diarrhoea; the remaining 16 cats had clinical signs consistent with both small and large bowel diarrhoea. Nineteen of the cats (66%) had been on antibiotics for at least 2 weeks preceding the diagnosis of trichomonosis. Twelve of these cats were tested for feline immunodeficiency virus and all were negative. Stool for diagnostic testing was collected on freshly voided samples in 20 cats and by colonic flush in nine cats. The median faecal score for the 20 voided samples was 6 (IQ 6–7). Of the 29 cats diagnosed and treated for *Tritrichomonas* species infection with the recommended dose and course of ronidazole (30 mg/kg, once daily for 14 days), five cats did not have resolution of clinical signs, two of which came from multi-cat households.

Among the 29 *Tritrichomonas* species-positive cats, in addition to the 14 cats that had direct faecal smears, 13 had *Giardia* species antigen testing, and one had *Giardia* species PCR testing recorded on their medical record, and three cats had *Giardia* species screening provided by the referring veterinarian, but the specific details were not documented. A total of nine cats were co-infected with *Giardia* species (31%). Six and four of the cats were found to be positive by direct faecal smear and antigen testing, respectively, with one of these cats positive by both faecal smear and antigen testing.

Discussion

It is difficult to compare these results with data from other geographical regions, as the criteria of inclusion in previous studies are different. The age of diagnosis (10 months; 6.5–26.5 months) and higher numbers of male cats (66%) compared with female cats with trichomonosis is consistent with previous reports.⁵ It is impossible to determine the relative risk of various epidemiological factors as the results were not compared with those of the hospital population and it is also possible that breeds including the British, Exotic and American Shorthairs are overrepresented as they are popular breeds in Hong Kong. It was apparent that the clinical signs often overlapped with small intestinal diarrhoea and thus trichomonosis should be considered in cats with both large and mixed small and large bowel diarrhoea. The use of supportive care, including dietary manipulation such as low-residue prescription diets and probiotics, may have contributed to the resolution of comorbid small intestinal disease. Antibiotic use in the 2 weeks prior to diagnosis in 66% *Tritrichomonas* species-positive cats is not surprising as it most likely represents an attempt to treat the clinical sign of large bowel diarrhoea with symptomatic therapy or an antibiotic trial for suspected *Giardia* species. Nevertheless, antibiotic treatment did not eliminate infection. A freshly voided stool sample is apparently a viable sample option, as 69% of cases were diagnosed

using this sampling technique, despite the recommendation to collect a faecal sample using colon flush. The median faecal score of 20 cats was 6, which, according to the Purina Veterinary Diet faecal scoring chart, is a stool that is described as having texture but no defined shape; present as pile or spots; and leaves residue when picked up. This is consistent with previous reports of feline trichomonosis, although a different faecal scoring system was used in our case series.¹⁸ Direct microscopic examination used for faecal screening was positive in 93% of all cats that were diagnosed with trichomonosis using PCR, although the numbers analysed are very small and it is questionable if statistical inferences can be made. This represents a much higher percentage than in previous reports.^{10,19} Co-infection with intestinal protozoan *Giardia* species was reported in 31% of the cats, similar to previous reports.^{9,25–27} The high number of relapsing cats, despite appropriate treatment, could be due to reinfection from their contaminated environment. The cause of treatment failure in the remaining three cats was unknown; owner or patient compliance was not documented, but resistance of the *Tritrichomonas* species organism, which has been reported previously, could also be considered.²⁸

Conclusions

Tritrichomonas species infection should be considered as a differential diagnosis, although uncommon, in any domestic cat living in Hong Kong, China, with clinical signs of chronic large or combined small and large bowel diarrhoea, particularly, young, purebred, male cats. Co-infections with *Giardia* species is not uncommon.

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