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FULL PAPER

Parasitology

Prevalence of *Cryptosporidium*, *Giardia*, *Blastocystis*, and trichomonads in domestic cats in East China

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ABSTRACT. The cat is a reported reservoir for several zoonotic pathogens, including *Cryptosporidium* spp., *Giardia duodenalis*, and *Blastocystis* sp. These parasites represent a significant, but often neglected, threat to humans and animals. Furthermore, *Tritrichomonas foetus* has been described inhabiting the digestive tract of cats, and may be causative agents of gastrointestinal symptoms. However, scant data are available concerning the molecular epidemiology of these parasites in domestic cats in China. This study examined fecal samples from domestic cats in Eastern China to unravel the molecular epidemiology of four protozoans. Of the 346 samples examined, 47 (13.6%) were positive for the detected pathogens, including 8 (2.3%), 5 (1.4%), 2 (0.6%), and 35 (10.1%) samples positive for *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus*, respectively. Co-infection with *Cryptosporidium* spp. and *T. foetus* was detected in three cats, no other mixed infections were observed. No age, sex or fecal condition predisposition was observed with any of the four pathogens. The species/assemblages/subtypes/genotypes were C. *felis*, Assemblage A and F, ST1, and cat genotype for *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus* detected in this study, respectively. The presence of zoonotic species/assemblages/subtypes/genotypes poses a threat to public health. These findings provide useful information for the design of prevention and control strategies to reduce the burden of protozoal infections in cats.

KEY WORDS: *Blastocystis* sp., cat, *Cryptosporidium* spp, *Giardia duodenalis*, *Tritrichomonas foetus*

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The genera *Cryptosporidium*, *Giardia*, *Blastocystis*, and *Tritrichomonas* have been identified in human and diverse animals [22]. Some are commensal, others are related to acute or chronic diarrhea and other gastrointestinal symptoms in hosts and recognized as a significant, though often neglected, threat to public health [30].

Cryptosporidium spp. and *G. duodenalis* are the primary causative agents of zoonotic diarrhea worldwide [30]. They are transmitted via fecal-oral routes [37]. Currently, over 34 species and 40 genotypes of *Cryptosporidium* have been established and most of these are host-adapted [12]. *C. felis* is the most common species infecting cat, although *C. parvum*, *C. muris*, *C. ryanae*, *Cryptosporidium* rat genotype III, and a novel genotype related to *Cryptosporidium* rat genotype III have also been observed in cats [38]. Unlike *C. parvum*, which is globally recognized as the most important species infecting humans, *C. felis* has a limited host range with limited zoonotic potential [8]. *G. duodenalis* is regarded as a multispecies complex and displays high genetic diversity among isolates from various hosts. Eight genetically distinct assemblages (A to H) have been characterized, including the zoonotic assemblages A and B and the host-adapted assemblages C through H [37]. Assemblage F is the most common in cats globally [9]. however, sporadic infections with zoonotic assemblages A and B have been documented in cats in some countries [17, 37, 40].

Blastocystis sp., a single-celled parasite belonging to the stramenopiles, is often detected in fecal samples from humans and animals [30]. Extensive intra-genetic variation among *Blastocystis* sp. isolates has been described, leading to the identification of at least 17 distinct subtypes (STs). The STs related to infections in humans and animals are 1–9 and 12, while STs 10, 11, and 13–17 have only been observed in animals [32].

Trichomonads, such as *T. foetus*, are frequently identified in veterinary clinics. *T. foetus*, traditionally recognized as the agent responsible for bovine trichomoniasis, has been determined to be an important cause of chronic diarrhea in cats [25, 39].

With the rapid socioeconomic development, increasing numbers of cats are raised in China. The large number of cats and high

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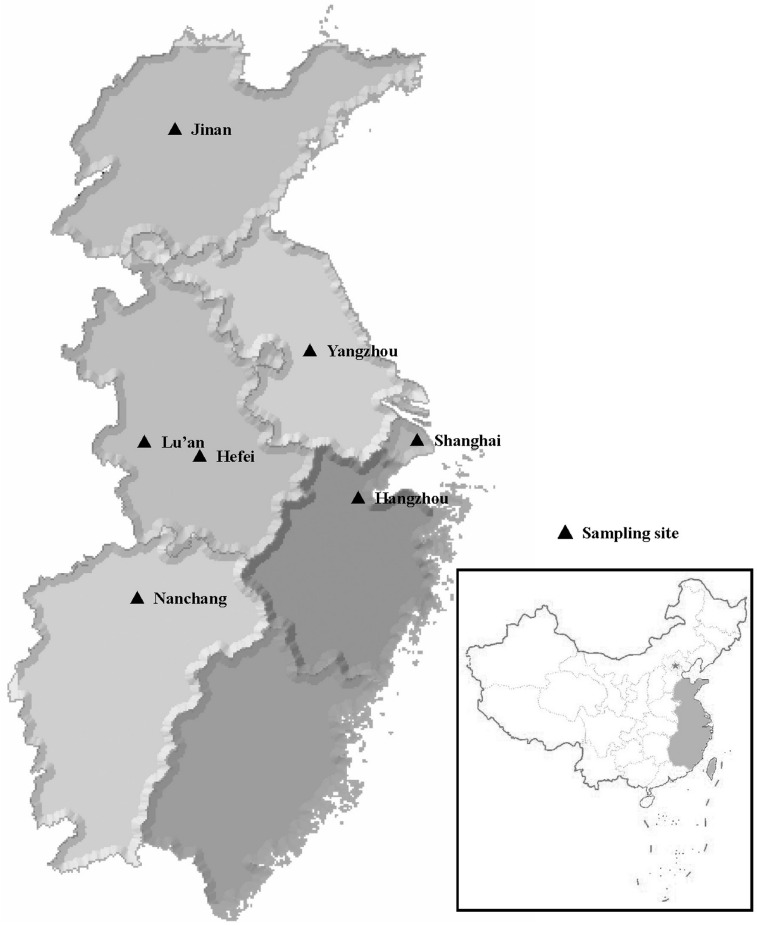

Fig. 1. Location of the sampling areas.

human population density may increase the spread of zoonotic and potentially zoonotic diseases, including cryptosporidiosis, giardiasis, blastocystosis and so on. Therefore, the aim of this study was to determine the prevalence and genetic diversity of *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus* in cats in Eastern China.

MATERIALS AND METHODS

Study population and specimen collection

During Mar 2015 to December 2018, 346 fecal samples were collected from cats in seven veterinary hospitals in Zhejiang Province (Hangzhou), Anhui Province (Lu'an and Hefei), Shanghai city (Minhang), Jiangsu Province (Yangzhou), Shandong Province (Jinan), and Jiangxi Province (Nanchang) in eastern China (Fig. 1). Freshly voided fecal samples were collected by the owners, who were willing participants in this study, and submitted to the laboratory with a questionnaire concerning the age, sex and fecal condition (normal vs. soft vs. diarrhea). These cats, including 151 males and 195 females, were divided into two age groups: ≤12 months (n=60) and >12 months (n=286). This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences and the Animal Care and Welfare Committee of Anhui Science and Technology University.

DNA isolation, molecular examination and analysis

Genomic DNA was isolated from a 0.2 g sample of each fecal specimen, using the Stool DNA Kit (Tiangen, Beijing, China) and following manufacturer-recommended procedures. Isolated DNA was stored at −20°C until examination by PCR. PCR were performed for *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus* as previously described [1, 6, 33, 36]. All PCRs at each locus were performed in duplicate. Positive secondary PCR amplicons were directly sequenced on both strands. The sequences obtained were aligned using BioEdit v7.0.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and then subjected to the GenBank database to confirm their identity using the basic local alignment search tool (BLAST). Additionally, *Blastocystis* 18S allele calling and confirmation of ST were done using a sequence query at the *Blastocystis* 18S database (http://pubmlst.org/blastocystis/). The representative sequences obtained in this study have been deposited in GenBank under accession number MH115431 (for *Cryptosporidium* spp.), MH115434 (for *G. duodenalis*), MH115432–MH115433 (for *Blastocystis* sp.), and MH115435 (for *T. foetus*).

Statistical analysis

The χ^2 test or Fisher's exact test in SPSS standard version 17.0 (SPSS Inc., Chicago, IL, U.S.A.) was used to compare differences in infection rates. Differences with a *P* value below 0.05 were considered statistically significant.

RESULTS

Prevalence of several protozoas in cats

Table 1 showed the infection rates of four pathogens considered in cats from various cities. Forty-seven cats were positive for one or more parasites by PCR detection, 299 were negative by molecular methods in this study. *T. foetus* infections (10.1%) was predominated, followed by *Cryptosporidium* spp. (2.3%), *G. duodenalis* (1.4%), and *Blastocystis* sp., (0.6%) in the 346 feline specimens. *T. foetus* is also the most distributed parasite in this study, and was found in the other six sample areas except Lu'an. Jinan recorded the highest prevalence of *T. foetus* (17.8%; 8/45), followed by Hangzhou (14.8%; 12/81), Nanjing (10.2%; 10/98), Minhang (7.1%; 1/14), Hefei (5.0%; 3/60), and Yangzhou (2.6%; 1/39). *Cryptosporidium* spp. were found in Hangzhou, Hefei, and Nanjing, and the prevalence rates were 2.5% (2/81), 3.3% (2/60) and 4.1% (4/98), respectively. *G. duodenalis* were observed in cats from Hangzhou (1.2%), Nanjing (2.0%) and Jinan (4.4%), and *Blastocystis* sp. was only observed in cats from Lu'an (22.2%; 2/9). Three co-infections with *Cryptosporidium* spp. and *T. foetus* was found in one cat (0.7%) in Hangzhou and two cats (2.0%) in Nanjing. No other mixed infections were observed in this study. Regarding the total infection rate (all four pathogens considered), the highest prevalence was detected in Lu'an (22.2%) and Jinan (22.2%), followed by Hangzhou (17.3%), Nanjing (14.3%), Hefei (8.3%), Minhang (7.1%), and Yangzhou (2.6%). Furthermore,Three pathogens were found in cats from Hangzhou and Nanjing, two pathogens were detected in cats from Hefei, and Jinan, and only one pathogen was found in cats form the other three sample areas.

Infection rates of *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus* from different age, sex and fecal condition groups are shown in Table 2. There was no significance difference in age or sex groups for any of the pathogens ($P>0.05$). There was also no significant difference in the prevalence of *Cryptosporidium* spp., *G. duodenalis*, and *Blastocystis* sp., with regard to fecal condition ($P>0.05$). Among the cats with soft feces and diarrhea, the infection rate of *T. foetus* was both 12.5% instead of 9.7% in cats with normal feces. However the difference was also not statistical significant ($P>0.05$).

Species identification and assemblage/subtyping/genotyping

Cryptosporidium species, *G. duodenalis* assemblages, *Blastocystis* sp. STs and alleles, and *T. foetus* genotypes detected in cats are displayed in Table 1. DNA sequencing of eight samples positive for *Cryptosporidium* spp. were successful, with both identified as C. *felis* with more than 98% sequence identity to *C. felis* homologous sequences in GenBank. Assemblage A was determined in the *Giardia* isolates from Hangzhou, and Assemblage F was seen in the four *Giardia* isolates from Nanjing and Jinan by sequencing of the *gdh* gene of *G. duodenalis*. Sequence alignment of Assemblage A obtained from this study and reference sequences demonstrated that the feline specimen was similar to subtype A2 (KT235917) with one SNP (G to C substitution at position 81). The four Assemblage F in this study showed 100% sequence identity to the isolate (LC341552) from cat in Japan.

The two *Blastocystis* isolates obtained in this study, with 99–100% sequence identity to homologous GenBank sequences, were identified as ST1. Regarding the ST1 alleles retrieved from the 18S database, one was allele 4 and the other had no match with alleles in the database. Sequencing of PCR products with primer TRICHO-FBIS/ TRICHO-RBIS yielded 30 identical sequences with 100% homology to *T. foetus* isolates from several countries (JX960422 in France, KX267765 in Brazil, JN006994 in Switzerland, HM856630 and EF165538 in Norway, GU170216 in Australia, AF466749, AF466750, and EU569309 in U.S.A.). All of these feline isolates, differing by a single-nucleotide polymorphism (SNP) in the ITS2 region from those isolated from cattle (JN106456, GU170220, AF339736, AY485677-79, AY349189, M81842 and U85967), belonged to the so-called cat genotype (Table 1, and Fig. 2).

DISCUSSION

Cats are intimate companions of humans and may harbor human pathogens such as *Cryptosporidium*, *Giardia*, and *Blastocystis*. Cats can also transmit *T. foetus*. There are limited molecular epidemiological surveys of *Cryptosporidium*, *Giardia*, and *T. foetus* in cats in China, notably for the latter pathogen [14, 17, 37, 40]. To our knowledge, this is the first report regarding the prevalence and genetic characteristics of *Blastocystis* sp., in domestic cats in China.

Cryptosporidium infection in cats has been investigated worldwide, but there were only two reports in Heilongjiang and Shanghai in China [17, 37]. Studies globally have reported feline prevalences of *Cryptosporidium* spp. ranging from 0 to 29.4% [38]. The present study showed that cats in Eastern China have a prevalence of 2.3% (8/346), in accordance with observations in some earlier studies in Australia (1.2 and 2.2%), Thailand (2.5%), Japan (1.4%), Heilongjiang (2.2%), and Shanghai (3.8%) in China [10, 13, 17, 24, 31, 37]. The eight isolates in this study were all genetically identified as *C. felis*. This species is the most common in cats, and it has also been frequently identified in humans in developing countries, indicating that zoonotic transmission of this species is possible [29].

G. duodenalis infection in cats has been reported in many countries, including Heilongjiang, Guangdong, and Shanghai in China [3, 17, 37, 38, 40]. The infection rate of *G. duodenalis* in this study was 1.4%, which is similar to the study in Heilongjiang (1.9%), but lower than another study in Shanghai (13.1%) [17, 37]. Sequence analysis identified the one isolate as assemblage A and the other four isolates as assemblage F, which is consistent with the previous conclusion that assemblage F was the most common

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Table 1. Prevalence and species/ assemblages/ subtypes/ genotypes of *Cryptosporidium*, *Giardia*, *Blastocystis*, and *Tritrichomonas foetus* in cats in Eastern China

Region		No. of samples	*Cryptosporidium*		*Giardia*		*Blastocystis*		*T. foetus*		Total (Prevalence %)[a)]
			No. positives (Prevalence %)	Species	No. positives (Prevalence %)	Assemblages	No. positives (Prevalence %)	Subtypes (allele)	No. positives (Prevalence %)	Genotypes	
Zhejiang	Hangzhou	81	2 (2.5)	*C. felis*	1 (1.2)	Assemblage A	0 (0)	-	12 (14.8)	Cat genotype	14 (17.3)
Anhui	Lu'an	9	0 (0)	-	0 (0)	-	2 (22.2)	ST1 (allele 4), ST1 (no match)	0 (0)	-	2 (22.2)
	Hefei	60	2 (3.3)	*C. felis*	0 (0)	-	0 (0)	-	3 (5.0)	Cat genotype	5 (8.3)
Shanghai	Minhang	14	0 (0)	-	0 (0)	-	0 (0)	-	1 (7.1)	Cat genotype	1 (7.1)
Jiangsu	Yangzhou	39	0 (0)	-	0 (0)	-	0 (0)	-	1 (2.6)	Cat genotype	1 (2.6)
	Nanjing	98	4 (4.1)	*C. felis*	2 (2.0)	Assemblage F	0 (0)	-	10 (10.2)	Cat genotype	14 (14.3)
Shangdong	Jinan	45	0 (0)	-	2 (4.4)	Assemblage F	0 (0)	-	8 (17.8)	Cat genotype	10 (22.2)
Total		346	8 (2.3)	*C. felis*	5 (1.4)	Assemblage A, and F	2 (0.6)	ST1 (allele 4), ST1 (no match)	35 (10.1)	Cat genotype	47 (13.6)

a) Including multiple infections.

Table 2. Prevalence of *Cryptosporidium*, *Giardia*, *Blastocystis*, and *Tritrichomonas foetus* in cats in different ages, sexes and fecal condition

Factor		No. of specimens	No. of positives (Positive rate/%)			
			Cryptosporidium	*Giardia*	*Blastocystis*	*T. foetus*
Age	≤12	60	2 (3.3)	1 (1.7)	0 (0.0)	7 (11.7)
	>12	286	6 (2.1)	4 (1.4)	2 (0.7)	28 (9.8)
Sex	Male	151	3 (2.0)	2 (1.3)	1 (0.7)	14 (9.3)
	Female	195	5 (2.6)	3 (1.5)	1 (0.5)	21 (10.8)
Fecal condition	Formed	298	7 (2.4)	4 (1.3)	2 (0.7)	29 (9.7)
	Soft	40	1 (2.5)	1 (2.5)	0 (0)	5 (12.5)
	Diarrhea	8	0 (0)	0 (0)	0 (0)	1 (12.5)

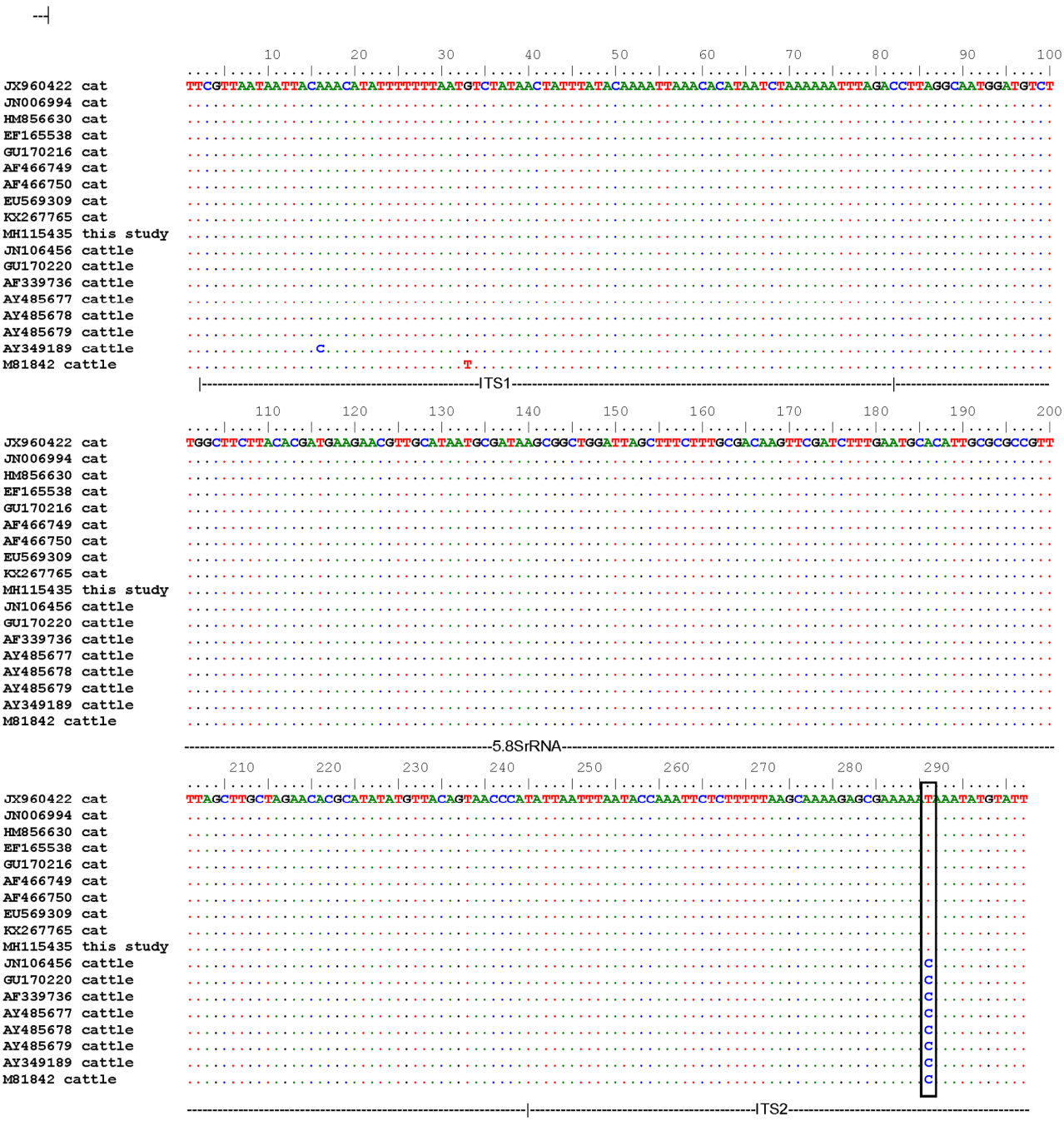

Fig. 2. Alignment of the ITS1-5.8S rRNA-ITS2 sequences of the *Tritrichomonas foetus* isolates. The black-boxed residues indicate differences in the ITS2 region.

genotype in cats around the world [9]. Assemblage F has been found in cats in Heilongjiang, Guangdong, and Shanghai in China [17, 37, 40]. Assemblage A has also been reported in cats in Guangdong and Shanghai in China [37, 40]. In addition, assemblage A has been found to be dominant in human and waste-water in Shanghai, suggesting a possibility of zoonotic transmission of giardiasis [16, 35].

Blastocystis sp. is a remarkably successful parasite with a vast array of host species. However, few studies have been conducted on the prevalence and subtype distribution of *Blastocystis* sp. in cats worldwide, as shown in Table 3 [7, 11, 18, 19, 21, 28]. Only two cats (0.6%) were found to be infected with *Blastocystis* sp. in this study. It is lower than that has previously been reported in cats worldwide. So far, only two documents globally showed the existence of STs 1, 3, 4, and 10 in cats [21, 28]. In our study, we identified ST1, which has been recognized as the second most predominant subtype in humans worldwide [2]. When the results of the 18S alleles were retrieved, we found ST1 identified in this study possessed one known allele 4 and one unmatched allele. The allele 4 has been described in humans in Colombia, Brazil, and other countries in South America [23, 26, 27]. The observation of

Table 3. Prevalence and subtypes of *Blastocystis* sp. found in cats in reported studies

Location	Methods	No. positive/No.examined (%)	Subtypes identified	References
Australia	Light microscopy	67.3 (35/52)	–	[7]
Chile	Light microscopy	37.0 (85/230)	–	[18]
Iran	Light microscopy	16.8 (19/113)	–	[19]
Australia	Faecal smears, cultures and PCR	Faecal smears: 33.3 (1/3) Cultures: 0.0 (0/3) PCR: 100.0 (3/3)	STs 1, and 4	[21]
Iran	Light microscopy	14.3 (20/140)	–	[11]
U.S.A.	PCR	11.4 (12/105)	STs 1, 3, and 10	[28]
China	**PCR**	**0.6 (2/346)**	**ST1**	**Present study**

–, unavailable data.

an unmatched allele indicates that it is a novel allele within ST1.

T. foetus infection in cats has been reported in many geographic regions, including Europe, North America, and Australia/Oceania, with prevalences ranging from 0 to 81.8% [39]. It has also occasionally been reported in Asia, including South Korea, Japan, and Hong Kong, China [4, 14, 15, 39]. *T. foetus* was the most frequent parasites observed in this study (10.1%), similar to that in Japan (8.8%) but lower than that in some countries from Europe, North America, and Australia/Oceania [4, 39]. It has been divided into a 'cat genotype' and a 'cattle genotype', with the former capable of infecting cats, dogs, and pigs and the latter capable of infecting both cattle and pigs [5, 20]. All isolates in this study belonged to the 'cat genotype', consistent with previous findings in Norway [34].

Investigation of potential risk factors for the four pathogens infection showed that no significant differences were found when age, sex and fecal condition were studied, which is concordance with observations in some earlier studies in Japan, China,U.S.A., and Norway [10, 28, 34, 37]. In contrast, some studies have shown noticeable age-associated differences in the occurrence of these pathogens in cats and in part this variation was associated with a still limited number of animals included in this study [3, 10, 17, 26]. Moreover, a relatively low prevalence of four pathogens was also observed in cats in the present study and maybe related partly to a better hygiene and management of domestic cats and the detection methodologies including the fecal specimen process used [3, 28]. Considering that the prevalence of these parasites was associated with many risk factors, further studies are needed to find out the major factor driving prevalence rates of these pathogens in cats [3].

In summary, the molecular prevalence of *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *T. foetus* were determined in cats in Eastern China. The presence of zoonotic species, assemblages, and subtypes poses a threat to public health. The findings reinforce the need to clarify the significance of cats in the epidemiology of enteric pathogens in humans and animals.

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REFERENCES

1. Abe, N., Kimata, I. and Iseki, M. 2003. Identification of genotypes of *Giardia intestinalis* isolates from dogs in Japan by direct sequencing of the PCR amplified glutamate dehydrogenase gene. *J. Vet. Med. Sci.* **65**: 29–33. [Medline] [CrossRef]
2. Alfellani, M. A., Stensvold, C. R., Vidal-Lapiedra, A., Onuoha, E. S., Fagbenro-Beyioku, A. F. and Clark, C. G. 2013. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Trop.* **126**: 11–18. [Medline] [CrossRef]
3. Bouzid, M., Halai, K., Jeffreys, D. and Hunter, P. R. 2015. The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Vet. Parasitol.* **207**: 181–202. [Medline] [CrossRef]
4. Doi, J., Hirota, J., Morita, A., Fukushima, K., Kamijyo, H., Ohta, H., Yamasaki, M., Takahashi, T., Katakura, K. and Oku, Y. 2012. Intestinal *Tritrichomonas suis* (=*T. foetus*) infection in Japanese cats. *J. Vet. Med. Sci.* **74**: 413–417. [Medline] [CrossRef]
5. Doi, J., Abe, N. and Oku, Y. 2013. Molecular survey of *Tritrichomonas suis* (=*T. foetus*) 'cat' and 'cattle' genotypes in pigs in Japan. *J. Vet. Med. Sci.* **75**: 475–479. [Medline] [CrossRef]
6. Duboucher, C., Caby, S., Dufernez, F., Chabé, M., Gantois, N., Delgado-Viscogliosi, P., Billy, C., Barré, E., Torabi, E., Capron, M., Pierce, R. J., Dei-Cas, E. and Viscogliosi, E. 2006. Molecular identification of *Tritrichomonas foetus*-like organisms as coinfecting agents of human Pneumocystis pneumonia. *J. Clin. Microbiol.* **44**: 1165–1168. [Medline] [CrossRef]
7. Duda, A., Stenzel, D. J. and Boreham, P. F. 1998. Detection of *Blastocystis* sp. in domestic dogs and cats. *Vet. Parasitol.* **76**: 9–17. [Medline] [CrossRef]
8. Fayer, R. 2010. Taxonomy and species delimitation in *Cryptosporidium*. *Exp. Parasitol.* **124**: 90–97. [Medline] [CrossRef]
9. Feng, Y. and Xiao, L. 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin. Microbiol. Rev.* **24**: 110–140. [Medline] [CrossRef]
10. Ito, Y., Itoh, N., Kimura, Y. and Kanai, K. 2016. Molecular detection and characterization of *Cryptosporidium* spp. among breeding cattery cats in Japan. *Parasitol. Res.* **115**: 2121–2123. [Medline] [CrossRef]
11. Khademvatan, S., Abdizadeh, R., Rahim, F., Hashemitabar, M., Ghasemi, M. and Tavalla, M. 2014. Stray cats gastrointestinal parasites and its association with public health in ahvaz city, South Western of iran. *Jundishapur J. Microbiol.* **7**: e11079. [Medline] [CrossRef]

12. Koehler, A. V., Korhonen, P. K., Hall, R. S., Young, N. D., Wang, T., Haydon, S. R. and Gasser, R. B. 2017. Use of a bioinformatic-assisted primer design strategy to establish a new nested PCR-based method for *Cryptosporidium*. *Parasit. Vectors* **10**: 509. [Medline] [CrossRef]
13. Koompapong, K., Mori, H., Thammasonthijarern, N., Prasertbun, R., Pintong, A. R., Popruk, S., Rojekittikhun, W., Chaisiri, K., Sukthana, Y. and Mahittikorn, A. 2014. Molecular identification of *Cryptosporidium* spp. in seagulls, pigeons, dogs, and cats in Thailand. *Parasite* **21**: 52. [Medline] [CrossRef]
14. Köster, L. S., Chow, C. and Yao, C. 2015. Trichomonosis in cats with diarrhoea in Hong Kong, China, between 2009 and 2014. *JFMS Open Rep* **1**: 2055116915623561. [Medline]
15. Lim, S., Park, S. I., Ahn, K. S., Oh, D. S., Ryu, J. S. and Shin, S. S. 2010. First report of feline intestinal trichomoniasis caused by *Tritrichomonas foetus* in Korea. *Korean J. Parasitol.* **48**: 247–251. [Medline] [CrossRef]
16. Li, N., Xiao, L., Wang, L., Zhao, S., Zhao, X., Duan, L., Guo, M., Liu, L. and Feng, Y. 2012. Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Negl. Trop. Dis.* **6**: e1809. [Medline] [CrossRef]
17. Li, W., Li, Y., Song, M., Lu, Y., Yang, J., Tao, W., Jiang, Y., Wan, Q., Zhang, S. and Xiao, L. 2015. Prevalence and genetic characteristics of *Cryptosporidium, Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet. Parasitol.* **208**: 125–134. [Medline] [CrossRef]
18. López, J., Abarca, K., Paredes, P. and Inzunza, E. 2006. [Intestinal parasites in dogs and cats with gastrointestinal symptoms in Santiago, Chile]. *Rev. Med. Chil.* **134**: 193–200 (in Spanish). [Medline]
19. Mohsen, A. and Hossein, H. 2009. Gastrointestinal parasites of stray cats in Kashan, Iran. *Trop. Biomed.* **26**: 16–22. [Medline]
20. Mueller, K., Morin-Adeline, V., Gilchrist, K., Brown, G. and Šlapeta, J. 2015. High prevalence of *Tritrichomonas foetus* 'bovine genotype' in faecal samples from domestic pigs at a farm where bovine trichomonosis has not been reported for over 30 years. *Vet. Parasitol.* **212**: 105–110. [Medline] [CrossRef]
21. Nagel, R., Cuttell, L., Stensvold, C. R., Mills, P. C., Bielefeldt-Ohmann, H. and Traub, R. J. 2012. *Blastocystis* subtypes in symptomatic and asymptomatic family members and pets and response to therapy. *Intern. Med. J.* **42**: 1187–1195. [Medline] [CrossRef]
22. Osman, M., El Safadi, D., Cian, A., Benamrouz, S., Nourrisson, C., Poirier, P., Pereira, B., Razakandrainibe, R., Pinon, A., Lambert, C., Wawrzyniak, I., Dabboussi, F., Delbac, F., Favennec, L., Hamze, M., Viscogliosi, E. and Certad, G. 2016. Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium, Giardia, Blastocystis* and *Dientamoeba* among schoolchildren in Tripoli, Lebanon. *PLoS Negl. Trop. Dis.* **10**: e0004496. [Medline] [CrossRef]
23. Oliveira-Arbex, A. P., David, É. B. and Guimarães, S. 2018. *Blastocystis* genetic diversity among children of low-income daycare center in Southeastern Brazil. *Infect. Genet. Evol.* **57**: 59–63. [Medline] [CrossRef]
24. Palmer, C. S., Thompson, R. C. A., Traub, R. J., Rees, R. and Robertson, I. D. 2008. National study of the gastrointestinal parasites of dogs and cats in Australia. *Vet. Parasitol.* **151**: 181–190. [Medline] [CrossRef]
25. Profizi, C., Cian, A., Meloni, D., Hugonnard, M., Lambert, V., Groud, K., Gagnon, A. C., Viscogliosi, E. and Zenner, L. 2013. Prevalence of *Tritrichomonas foetus* infections in French catteries. *Vet. Parasitol.* **196**: 50–55. [Medline] [CrossRef]
26. Ramírez, J. D., Sánchez, A., Hernández, C., Flórez, C., Bernal, M. C., Giraldo, J. C., Reyes, P., López, M. C., García, L., Cooper, P. J., Vicuña, Y., Mongi, F. and Casero, R. D. 2016. Geographic distribution of human *Blastocystis* subtypes in South America. *Infect. Genet. Evol.* **41**: 32–35. [Medline] [CrossRef]
27. Ramírez, J. D., Flórez, C., Olivera, M., Bernal, M. C. and Giraldo, J. C. 2017. *Blastocystis* subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. *PLoS One* **12**: e0172586. [Medline] [CrossRef]
28. Ruaux, C. G. and Stang, B. V. 2014. Prevalence of *blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *PLoS One* **9**: e107496. [Medline] [CrossRef]
29. Ryan, U., Fayer, R. and Xiao, L. 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* **141**: 1667–1685. [Medline] [CrossRef]
30. Sánchez, A., Munoz, M., Gómez, N., Tabares, J., Segura, L., Salazar, Á., Restrepo, C., Ruíz, M., Reyes, P., Qian, Y., Xiao, L., López, M. C. and Ramírez, J. D. 2017. Molecular epidemiology of *Giardia, Blastocystis* and *Cryptosporidium* among indigenous children from the Colombian Amazon Basin. *Front. Microbiol.* **8**: 248. [Medline] [CrossRef]
31. Sargent, K. D., Morgan, U. M., Elliot, A. and Thompson, R. C. A. 1998. Morphological and genetic characterisation of *Cryptosporidium* oocysts from domestic cats. *Vet. Parasitol.* **77**: 221–227. [Medline] [CrossRef]
32. Scanlan, P. D., Stensvold, C. R., Rajilić-Stojanović, M., Heilig, H. G., De Vos, W. M., O'Toole, P. W. and Cotter, P. D. 2014. The microbial eukaryote *Blastocystis* is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiol. Ecol.* **90**: 326–330. [Medline] [CrossRef]
33. Scicluna, S. M., Tawari, B. and Clark, C. G. 2006. DNA barcoding of *blastocystis*. *Protist* **157**: 77–85. [Medline] [CrossRef]
34. Tysnes, K., Gjerde, B., Nødtvedt, A. and Skancke, E. 2011. A cross-sectional study of *Tritrichomonas foetus* infection among healthy cats at shows in Norway. *Acta Vet. Scand.* **53**: 39. [Medline] [CrossRef]
35. Wang, L., Xiao, L., Duan, L., Ye, J., Guo, Y., Guo, M., Liu, L. and Feng, Y. 2013. Concurrent infections of *Giardia duodenalis, Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. *PLoS Negl. Trop. Dis.* **7**: e2437. [Medline] [CrossRef]
36. Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A. A., Montali, R. J., Fayer, R. and Lal, A. A. 1999. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. *Appl. Environ. Microbiol.* **65**: 1578–1583. [Medline]
37. Xu, H., Jin, Y., Wu, W., Li, P., Wang, L., Li, N., Feng, Y. and Xiao, L. 2016. Genotypes of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Giardia duodenalis* in dogs and cats in Shanghai, China. *Parasit. Vectors* **9**: 121. [Medline] [CrossRef]
38. Yang, R., Ying, J. L., Monis, P. and Ryan, U. 2015. Molecular characterisation of *Cryptosporidium* and *Giardia* in cats (*Felis catus*) in Western Australia. *Exp. Parasitol.* **155**: 13–18. [Medline] [CrossRef]
39. Yao, C. and Köster, L. S. 2015. *Tritrichomonas foetus* infection, a cause of chronic diarrhea in the domestic cat. *Vet. Res. (Faisalabad)* **46**: 35. [Medline] [CrossRef]
40. Zheng, G., Hu, W., Liu, Y., Luo, Q., Tan, L. and Li, G. 2015. Occurrence and molecular identification of *Giardia duodenalis* from stray cats in Guangzhou, southern China. *Korean J. Parasitol.* **53**: 119–124. [Medline] [CrossRef]