Research Note

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Stray cats are more frequently infected with zoonotic protists than pet cats

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Abstract: Faecal samples were collected from cats kept as pets (n = 120) and stray cats (n = 135) in Central Europe (Czech Republic, Poland and Slovakia) and screened for the presence of *Cryptosporidium* spp., *Giardia intestinalis* (Kunstler, 1882), *Encephalitozoon* spp. and *Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisce et Modigliani, 1985 by PCR analysis of the small-subunit of rRNA (*Cryptosporidium* spp. and *G. intestinalis*) and ITS (microsporidia) genes. Sequence analysis of targeted genes revealed the presence of *C. felis* Iseki, 1979, *G. intestinalis* assemblage F, *E. cuniculi* Levaditi, Nicolau et Schoen, 1923 genotype II, and *E. bieneusi* genotype D. There was no correlation between the occurrence of detected parasites and sex, presence of diarrhoea or drug treatment (drug containing pyrantel and praziquantel). Compared to pet cats (7%), stray cats (30%) were statistically more frequently infected with protist parasites and overall may present a greater risk to human health.

Keywords: *Cryptosporidium*, *Giardia*, *Encephalitozoon*, *Enterocytozoon bieneusi*, antiparasitics, PCR

Since their domestication in the Fertile Crescent at the dawn of human civilisation (Driscoll et al. 2009), cats have risen to become the most popular pets, with a global population estimated at 600 million (McLamb 2013). Cats can host several parasite species that are infectious for humans including nematodes of the genera *Toxocara* Stiles, 1905 and *Ancylostoma* Dubini, 1843, the cestodes *Dipylidium* Leuckart, 1883 and *Echinococcus* Rudolphi, 1801 and protists of the genera *Toxoplasma gondii* (Nicolle et Manceaux, 1908), *Cryptosporidium* Tyzzer, 1910, *Giardia* Kunstler, 1882, *Encephalitozoon* Levaditi, Nicolau et Schoen, 1923, and *Enterocytozoon* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisce et Modigliani, 1985 (Baneth et al. 2016).

The present study focuses on eukaryotes of the genera *Cryptosporidium*, *Giardia*, *Encephalitozoon* and *Enterocytozoon*, which have been less well studied in cats, and are associated with disease in immunocompetent and, more frequently, immunocompromised individuals (Thellier and Breton 2008, Ortega and Kváč 2013).

Out of more than 30 species and 100 genotypes of the genus *Cryptosporidium*, five (*C. felis* Iseki, 1979, *C. parvum*

Tyzzer, 1912, *C. muris* Tyzzer, 1907, *C. ryanae* Fayer, Santín et Trout, 2008, and *Cryptosporidium* rat genotype III) have been reported in cats (Sargent et al. 1998, Santín et al. 2006, Yang et al. 2015). Although most cases of human zoonotic cryptosporidiosis are caused by *C. parvum* (see Nichols et al. 2014), *C. felis* is more prevalent in cats and may be the most likely infectious risk for cat owners (Lucio-Forster et al. 2010, Beser et al. 2015).

Giardia intestinalis (Kunstler, 1882) also has global distribution and is the most common intestinal protist pathogen in humans and animals. *Giardia intestinalis* consists of seven assemblages with different host specificities (Monis et al. 2003). Among them, assemblages A and B have a broad host range, including humans and cats (i.e. zoonotic genotypes), whereas assemblage F primarily infects cats (Heyworth 2016).

Most species of the genus *Encephalitozoon* and genotypes of *E. bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisce et Modigliani, 1985 infect a broad range of hosts, including pets, livestock, wildlife and humans (Dengjel et al. 2001). Although infections in immunocompetent hosts are usually

Table 1. Number of samples positive to *Cryptosporidium felis* Iseki, 1979, *Giardia intestinalis* (Kunstler, 1882), *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen, 1923 and *Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse et Modigliani, 1985 by PCR in pet and stray cats in the Czech Republic, Poland and Slovakia.

Country	Type of cats	No. of cats	<i>Cryptosporidium felis</i>	<i>Giardia intestinalis</i> assemblage F	<i>Encephalitozoon cuniculi</i> genotype II	<i>Enterocytozoon bieneusi</i> genotype D
Czech Republic	pet	55	1	2	0	0
	stray	63	5 ¹	5	3 ¹	3
Slovakia	pet	34	0	3	1	0
	stray	39	3	3	2 ²	5 ²
Poland	pet	31	0	1	0	0
	stray	33	2	2	3 ^{3,4}	4 ^{3,4}

¹⁻⁴ upper indices indicate an animal with coinfection.

inapparent, they can cause disseminated infection manifesting serious health problems (Mathis et al. 2005).

Cats have a tendency towards independence and frequently spend periods outdoors, where they can come into contact with infected animals and contaminated food and water. We hypothesised that stray cats, which spend longer periods outside, are more frequently infected with zoonotic protists than pet cats and may represent a human health risk during the rehoming process. We undertook the present study to describe the presence of *Cryptosporidium* spp., *G. intestinalis*, and microsporidia of the genera *Encephalitozoon* and *Enterocytozoon* in pet and stray cats. Associations with antiparasitic treatment, sex of the animal and the presence of diarrhoea were also examined.

A total of 255 faecal samples were collected from pet (n = 120) and stray (n = 135) cats. Pet cats were defined as those living with people in their homes and under periodic veterinary control. Stray cats were defined as those found roaming freely without human supervision around human settlements and subsequently kept in shelters in the Czech Republic, Poland or Slovak Republic. Faecal samples from pet and stray cats were collected by cat owners and shelter employees, respectively. Each sample was processed as a mixture of three samples collected within three days with a one-day pause between collections. All examined animals were older than one year. Each cat was sampled once and a faecal sample was individually placed in a plastic dish without fixation, stored in the dark at 4 °C before examination for parasites. Consistency of faeces (presence of diarrhoea) and the known treatment history of the animal were noted at the time of sampling.

Genomic DNA was extracted from 200 mg of each faecal sample. A nested PCR approach was used to amplify a region of the internal transcribed spacer (ITS) of *E. bieneusi* (see Buckholt et al. 2002) or *Encephalitozoon* spp. (see Didier et al. 1995, Katzwinkel-Wladarsch et al. 1996) and the small ribosomal subunit rRNA gene (18S rRNA) of *Cryptosporidium* spp. (see Alves et al. 2003) and *Giardia* spp. (see Read et al. 2002). DNA of *C. suis* Ryan, Monis, Enemark, Sulaiman, Samarasinghe, Read, Buddle, Robertson, Zhou, Thompson et Xiao, 2004, *G. intestinalis* assemblage E, *E. cuniculi* genotype III, and *E. bieneusi* genotype CZ3, respectively, was used as positive control.

Purified secondary PCR products (GenElute Gel Extraction Kit, Sigma, St. Louis, MO, USA) were sequenced twice in both directions with secondary primers using

a BigDye Terminator v3.1 cycle sequencing kit in an ABI Prism 3130 genetic analyser (Applied Biosystems, Carlsbad, CA, USA). The nucleotide sequences of each gene obtained in this study were aligned with previously published sequences saved in GenBank using the MAFFT version 7 online server (<http://mafft.cbrc.jp/alignment/server/>). Phylogenetic trees with bootstrap support were inferred by maximum likelihood using the MEGA6 software.

Out of 255 cats sampled in Central Europe, 11, 16, 9 and 12 were positive for DNA of species of *Cryptosporidium*, *Giardia*, *Encephalitozoon*, and *E. bieneusi*, respectively.

A Maximum Likelihood tree constructed from partial sequences of 18S rDNA of species of *Cryptosporidium* and *Giardia* showed the presence of *C. felis* (identical to GenBank Accession no. AF112575) and *G. intestinalis* assemblage F (identical to AF199444), respectively. Sequence analyses of the ITS of microsporidia revealed the presence of *E. cuniculi* genotype II (identical to GQ422153) and *E. bieneusi* genotype D (identical to KF383393; phylogenies not shown). *Cryptosporidium felis* was found in one pet and 10 stray cats. *Giardia intestinalis* was found in six pet and ten stray cats. *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen, 1923 was detected in one pet and eight stray cats. *Enterocytozoon bieneusi* was not found in pet cats but was detected in 12 stray cats (Table 1).

Most positive animals (40 out of 44) were infected with just one of the parasite species examined, three were co-infected with *E. bieneusi* and *E. cuniculi*, and one was co-infected with *C. felis* and *E. cuniculi*. Multiple species infections were detected only in stray cats (Table 1). Statistical analyses on the entire dataset revealed significantly higher prevalence of each parasite in stray cats than in pet cats (Table 2). There was no association between sex of the animal, presence of diarrhoea or drug treatment (drug containing pyrantel and praziquintel) and the occurrence of *C. felis*, *G. intestinalis*, *E. cuniculi* or *E. bieneusi* (p-value = 0.0637–0.7118; Table 2).

In the present study, PCR and sequence analysis were used to detect species and genotypes of *Cryptosporidium*, *Giardia*, *Encephalitozoon*, and *E. bieneusi* in cats from Central Europe. All *Cryptosporidium*-positive cats were shedding *C. felis*, which is consistent with the reported dominance of this species in domestic cats, whereas other species and genotypes are reported less frequently (Santín et al. 2006, Palmer et al. 2008, Xu et al. 2016). There are many reports of cryptosporidiosis of cats, with a prevalence

Table 2. Factors examined for association with shedding of (oo)cysts of *Cryptosporidium felis* Iseki, 1979 and *Giardia intestinalis* (Kunstler, 1882) and spores of *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen, 1923 and *Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse et Modigliani, 1985 in cats.

Factor	No. of animals	<i>Cryptosporidium felis</i>			<i>Giardia intestinalis</i>			<i>Encephalitozoon cuniculi</i>			<i>Enterocytozoon bieneusi</i>		
		No. of positive	χ^2	P-value	No. of positive	χ^2	P-value	No. of positive	χ^2	P-value	No. of positive	χ^2	P-value
Housing													
pet cat	120	1	5.15	0.0232	6	0.28	0.5943	1	3.45	0.0629	0	9.29	0.0020
stray cat	135	10			10			8			12		
Sex													
male	143	5	1.72	0.6779	6	2.39	0.1219	6	0.33	0.5681	7	0.02	0.8912
female	112	6			10			3			5		
Faecal consistency													
diarrhoea	23	2	0.29	0.5847	4	3.43	0.0637	1	0.14	0.7118	1	0.18	0.6664
solid specimen	232	9			12			8			11		
Treatment													
yes	216	8	0.49	0.4838	11	2.16	0.1408	8	0.01	0.9072	9	0.29	0.5849
no	39	3			5			1			3		

ranging from 1 to 25% (e.g. Rambozzi et al. 2007, Yang et al. 2015), but few studies have compared the occurrence of cryptosporidia in pet and stray cats. Similarly to Yang et al. (2015) and Xu et al. (2016) we found to be more frequently present in stray cats (8%) than pet cats (1%). The prevalence of *G. intestinalis* in cats (6%) was similar to that in Columbia, Italy and the United States (3–11%; Hill et al. 2000, Spain et al. 2001, Santín et al. 2006), but lower than prevalence found in Australia (80%; McGlade et al. 2003). In accordance with Paoletti et al. (2011), we found a higher occurrence of this parasite in stray cats than pet cats.

Similarly, *E. cuniculi* and *E. bieneusi* were more prevalent in stray cats than in pet cats. The prevalence of microsporidia was low in stray and pet cats, which corresponds to previous findings (Santín et al. 2006, Xu et al. 2016). *Encephalitozoon cuniculi* was the only species of *Encephalitozoon* found in cats in the present study and it is the dominant species reported in other studies on cats (Hsu et al. 2011, Piekarska et al. 2017), with only one report of another species, *E. intestinalis*, in a cat from Brazil (Velasquez et al. 2012). Although *Encephalitozoon* spp. can cause severe generalised encephalitozoonosis or cataracts and uveitis in cats, these zoonotic agents are probably relatively rare, as evidenced by low seroprevalence (2%) in healthy cats (Buyukmihci et al. 1977, Hsu et al. 2011, Rebel-Bauder et al. 2011). Whereas 13 genotypes of *E. bieneusi* have been reported in cats (Karim et al. 2014), we detected only genotype D, which is one of the most frequently reported genotypes (Santín et al. 2006, Xu et al. 2016). In contrast to previous surveys (Dengjel et al. 2001, Xu et al. 2016), we did not find *E. bieneusi* in pet cats.

Similar to other studies (Santín et al. 2006, de Lucio et al. 2017), only cat-specific *G. intestinalis* assemblage F was found in the present study, suggesting that cats do not represent a risk in the transmission of *G. intestinalis* to humans. The zoonotic potential of feline cryptosporidiosis is considered to be medium and the zoonotic transmission of *C. felis* between cat and humans in a household has been reported (Beser et al. 2015). About 100 cases of *C. felis* have been reported in humans to date, with the majority

reported in immunocompromised individuals (Caccio and Putignani 2014). Both *E. cuniculi* genotype II and *E. bieneusi* genotype D have a broad host specificity and have been reported in humans. Although the sources of microsporidia infecting humans are not well known, there is evidence of zoonotic transmission (Dengjel et al. 2001) and cats should be considered a potential source (Mathis et al. 2005).

Although species of *Cryptosporidium*, *Giardia*, *Encephalitozoon* and *Enterocytozoon* occur frequently in populations of domestic, captive and wild animals, coinfections with parasites of two or more of these genera had a low prevalence in the present study, which is consistent with previous findings (Mynařová et al. 2016, Peng et al. 2016). This may be explained by animals shedding parasites intermittently, which may be missed by the sampling approach employed (Sak et al. 2010).

Treatment of cats with drugs containing pyrantel and praziquantel had no effect on the prevalence of protist infections, which is unsurprising considering that these drugs were designed to treat helminth infections. Previous studies have shown that 3–5 doses of Drontal, which contains pyrantel, praziquantel and febantel, cleared infection with *G. intestinalis* in dogs (Barr et al. 1998, Montoya et al. 2008); however, Payne et al. (2002) found that dogs treated with Drontal for three consecutive days did not clear giardiasis. Similarly to the present study, Pavlásek (2005) reported that praziquantel and pyrantel were not effective at treating *Cryptosporidium* infection in cats.

Due to the lack of clinical signs, the parasites detected in the present study are unlikely to be suspected during routine health examinations by veterinarians. Therefore, people who come into contact with cats, particularly stray cats, may be unaware of the potential health risk.

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