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Short communication

Zoonotic microsporidia in dogs and cats in Poland

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ABSTRACT

This study investigated the prevalence, genetic diversity, and zoonotic concerns of microsporidia in household dogs and cats in Poland. A total of 126 (82 dogs and 44 cats) fecal specimens were analyzed for the presence of specific DNA of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. using a nested PCR protocol amplifying the internal transcribed spacer region of the rRNA gene. Microsporidia were found in 10 (7.9%) out of the 126 examined stool samples. Of the 82 dogs, 4 (4.9%) and 2 (2.4%) were positive for *E. bieneusi* (genotypes D and PtEbIX) and *Encephalitozoon cuniculi* genotype II, respectively. Of the 44 cats, 4 (9.1%) were positive for *E. bieneusi* (genotypes PtEbIX and eb52). Additionally, one cat (2.3%) was concurrently infected with *E. bieneusi* (PtEbIX) and *E. cuniculi* (genotype II). Considering that all detected microsporidia in dogs and cats have been previously associated with human microsporidiosis, companion animals may be a potential source of microsporidia infections in humans.

1. Introduction

Microsporidia are a diverse group of eukaryotic, spore forming, obligate intracellular pathogens that infect a wide range of invertebrates and vertebrates. Currently, there are more than 1200 species described and most of them cause economically important diseases of insects, fish and fur animals (Cuomo et al., 2012; Didier et al., 2000). Fifteen species of microsporidia are capable of causing infection in humans but not all are clinically relevant. Species of the genus *Encephalitozoon* (*E. cuniculi, E. intestinalis* and *E. hellem*) and *Enterocytozoon bieneusi* are the most frequently detected in patients with impaired immunity (HIV and/or AIDS patients, organ and bone marrow transplant recipients) and also in immunocompetent individuals, mostly travelers and the elderly (Jamshidi et al., 2012; Kicia et al., 2014, 2016). Both *Encephalitozoon* spp. and *E. bieneusi* infect primarily enterocytes, leading to malabsorption, chronic diarrhea, dehydration, weight loss and even death. *E. bieneusi* additionally colonizes the liver, bile ducts and lungs, and species from the genus *Encephalitozoon,* especially *E. cuniculi* and *E. hellem*, are known to cause disseminated infections, and their spores may spread to almost all organs (Didier and Weiss, 2006). Furthermore, *E. bieneusi* and *E. cuniculi* genotypes are commonly reported in dogs and cats, which are considered high-risk hosts for the zoonotic transmission (Mathis et al., 2005). In dogs, *E. cuniculi* causes mainly unspecific neurological symptoms and renal diseases associated with grave prognosis particularly in puppies (Snowden et al., 2009). In cats, encephalitozoonosis was reported mainly in animals with meningoencephalitis and nephritis (Rebel-Bauder et al., 2011). Recent numerous reports have also indicated that feline and canine infections with *E. cuniculi* increasingly often concern ocular disorders *e.g.* cataracts, anterior uveitis and chorioretinal lesions (Benz et al., 2011; Künzel et al., 2014; Nell et al., 2015).

Although the source of microsporidial infections in humans and their transmission routes are not fully recognized, it is evident that animals are involved in the spread of human infections, as spores are excreted in the feces, urine or respiratory secretions of the infected host (Didier et al., 2004; Didier, 2005). In our study, molecular techniques were used to determine the occurrence, genetic diversity, and zoonotic potential of microsporidia in dogs and cats from Poland.

2. Materials and methods

2.1. Study population and sample collection

Fecal samples were obtained from 126 household dogs and cats in

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Poland between June 2013 and December 2016. Among 82 dogs, 46 animals were asymptomatic, 23 diarrheic, 8 suffered from chronic renal failure and 5 from lymphoma. Among 44 cats, 18 were asymptomatic, 9 diarrheic, 13 had chronic renal failure and 4 were diagnosed with lymphoma. The age of dogs ranged from 3 months to 13 years and cats from 5 months to 17 years. One part of each fecal specimen was examined with routine flotation method to evaluate the presence of oocysts and eggs of feline and canine parasites. The animals were screened for the presence of *G. intestinalis* antigens in feces with the use of SNAP *Giardia* (IDEXX Laboratories) immunosorbent assay. The second part of each fecal sample was kept at −20 °C for 1–2 weeks and then used for DNA extraction.

2.2. DNA extraction and PCR assays

Total DNA was extracted from 200 mg of feces by bead disruption using a Pulsing Vortex Mixer (Labnet International) followed by isolation using the Genomic Mini AX Stool Spin kit as per the manufacturer's instructions (A & A Biotechnology, Poland). To identify the *Encephalitozoon* spp. and *E. bieneusi* in the samples to the species/genotype level, a nested PCR protocol was used to amplify partial sequence of 16S ribosomal DNA (16S rDNA), complete sequence of internal transcribed spacer (ITS), and partial sequence of 5.8S ribosomal DNA (5.8.S rDNA) gene (Buckholt et al., 2002; Didier et al., 1995; Katzwinkel-Wladarsch et al., 1996). PCR amplicons were sequenced directly in both directions with ABI 3130 sequence analyzer (Applied Biosystems, Foster City, CA, USA). Alignments were manually edited using ChromasPro 1.7.4 (Technelysium, Pty, Ltd.), including trimming at both ends, aligned with previously published sequences using the MAFFT version 7 online server. The identity of the obtained sequences was examined by a BLAST search (www.ncbi.nlm.nih.gov/blast).

2.3. Statistical analysis

The confidence intervals (CI) at level 95% ($P = 0.05$) were calculated according to the adjusted Wald method (Sauro and Lewis, 2005). The Chi-square test ($\chi 2$) with Yates correction implemented in the STATISTICA ver. 12.0 software package was used to compare the differences in microsporidia infection rates among groups of animals and subgroups within animals. Differences were considered significant when $P < 0.05$.

3. Results and discussion

In this study, molecular techniques were used to report the occurrence and species and genotype determination of microsporidia in dogs and cats in Poland. Microsporidia were found in 10 (7.9%) out of 126 examined fecal samples. Phylogenetic analysis (trees not shown) revealed the presence of three different genotypes of *E. bieneusi* and *E. cuniculi* belonging to genotype II (identical with GenBank accession no. KJ469978). Of the 82 dogs examined, four (4.9%) were positive for *E. bieneusi* identical to genotype PtEbIX (n = 2) and genotype D (n = 2) (GenBank accession nos. AF101200 or AF101200), and two (2.4%) for *E. cuniculi* (Table 1). Of the 44 cats examined, three were positive for *E. bieneusi* genotype PtEbIX and one for genotype eb52 (AF059610). Additionally, one cat was concurrently infected with *E. cuniculi* II and *E. bieneusi* genotype PtEbIX (Table 1). *Enterocytozoon bieneusi*, initially found mainly in HIV-infected patients, is a species with multiple genotypes, diverse pathogenicity, and a broad range of hosts (Matos et al., 2012). Based on the ITS sequence analysis, there are at least 204 reported genotypes, both animal host-adapted and human-pathogenic, divided into eight groups. Only group I consists of genotypes with zoonotic potential, in the rest host-specific genotypes are grouped (Karim et al., 2014a,b). In the case of *E. cuniculi*, four genotypes are known: I, II, III, and IV also named "rabbit strain", "mouse strain", "dog strain", and "human", respectively, as they differ in their biology and epidemiology (Didier et al., 1998; Hinney et al., 2016). In our study, three different genotypes of *E. bieneusi* were found. One of them was zoonotic that belonged to group 1a (genotype D) and two remaining (genotype eb52 and PtEbIX) seemed to be feline and canine specific. While *E. bieneusi* genotype D has been so far reported *e.g.* in dogs, cats, humans, pigs, cattle, beaver, falcons, fox, macaque, muskrat or raccoon (Li et al., 2012; Santín et al., 2006), genotype eb52 was previously found only in cats (Abe et al., 2009). Similarly, PtEbIX genotype, known as the most divergent of *E. bieneusi* genotypes (Lobo et al., 2006; Mori et al., 2013), and detected in two dogs and three cats in this study, was previously found only in dogs and cats (Abe et al., 2009; Karim et al., 2014a; Mori et al., 2013; Santín and Fayer, 2011).

To date, it was shown that dogs and cats could be involved in the spread of zoonotic microsporidia species (Karim et al., 2014a; Künzel et al., 2014, Xu et al., 2016). In Europe, natural infection with *E. bieneusi* was detected in dogs in Portugal (11.7%), Spain (10.8%) and Switzerland (8.3%) (Mathis et al., 2005; Santín and Fayer, 2011). In Iran, the zoonotic species of microsporidia were found in 31% of dogs (*e.g. E. cuniculi* (18/100), *E. bieneusi* (8/100) and *E. intestinalis* (5/100)), and in 7.5% of cats (*E. bieneusi* (3/40)) (Jamshidi et al., 2012). In China, the reported rates of *E. bieneusi* infection ranged from 6.0 to 15.5% in dogs (including stray and pet dogs) (Karim et al., 2014a). Low prevalence of *E. bieneusi* in cats was consistent with other reports from Columbia, China, Germany, and Iran, where its occurrence in the range of 6–17% was described (Jamshidi et al., 2012; Santín et al., 2008; Xu et al., 2016). Similar to other studies performed worldwide (Sasaki et al., 2011; Hsu et al., 2011), low prevalence of *E. cuniculi* within the investigated population of dogs and cats was observed in Poland. The results of our study revealed a prevalence of microsporidia comparable to the other countries.

E. bieneusi was detected only in diarrheic dogs and cats with chronic renal failure or diarrhea whereas asymptomatic animals were negative. However, three of five microsporidia positive animals that suffered from diarrhea were concurrently infected with *Cystoisospora* spp. or *Giardia intestinalis*. Thus, diarrhea could be primarily caused by other intestinal parasites than microsporidia (Epe et al., 2010; Mitchell et al., 2007). On the other hand, infections caused by *E. cuniculi* were found in asymptomatic dogs and one cat with chronic renal failure (Table 1). Although infection with microsporidia was more frequently recorded in animals with a clinical symptom than in asymptomatic animals, there was no significant difference among groups ($\chi 2 = 0.010–0.681$, $P = 0.4091–0.9204$; Table 1). Also, the difference in microsporidia occurrence between dogs and cats was not statistically significant ($\chi 2 = 0.000$, $P = 0.9956$). Though the prevalence of microsporidia in feline and canine fecal samples seems to be low, it is known that mainly healthy hosts could shed spores intermittently, and therefore real prevalence is probably much higher than that observed during a one shot sampling study; with the increasing number of samples tested the probability of microsporidia detection increases (Kotková et al., 2013). This study and earlier reports also demonstrated that clinically healthy dogs and cats might harbor pathogenic strains of microsporidia and act only as shedders (Jamshidi et al., 2012). Infections with *E. cuniculi* in cats are observed both in symptomatic animals with characteristic ocular signs of cataract and anterior uveitis and in subclinically infected cats (Benz et al., 2011). In the course of generalized encephalitozoonosis in cats muscle cramps, superficial keratitis, depression, shock and mortality were observed with no changes in laboratory parameters (Didier et al., 1998). In other clinical cases, cerebellar hypoplasia and chronic kidney disease were reported (Hsu et al., 2011).

Considering the presence of zoonotic microsporidia genotypes in pets, the role of dogs and cats as microsporidia reservoir and a potential risk factor of transmission for immunocompromised patients cannot be excluded (Galván-Díaz et al., 2014; Künzel et al., 2014; Santín et al., 2008; Velásquez et al., 2012). There was one case of *E. cuniculi* and *E. bieneusi* co-infection in human and dog confirmed (Weitzel et al., 2001). In another case, the spores of *E. cuniculi* in fecal samples of a patient