SHORT COMMUNICATION

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Cryptosporidium spp. in dogs and cats in Poland

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Abstract

Introduction. *Cryptosporidium* spp. are common protozoan parasites of animals and humans. Due to their zoonotic potential it is important to know their species and prevalence in dogs and cats.

Objective. The aim of the study was to determine the occurrence and molecular characteristics of *Cryptosporidium* spp. in dogs and cats in Poland.

Materials and method. A total of 365 faecal samples (264 dogs and 101 cats) collected from animals living in Poland were analyzed using the Ziehl-Neelsen staining method and genus-specific PCR assay to amplify the *Cryptosporidium* 18S rRNA gene.

Results. *Cryptosporidium* were found in 11 out of the 365 examined stool samples (3%). PCR analysis identified *Cryptosporidium* in 9 out of 264 canine stool samples (3.4%) and 2 out of 101 feline specimens (2%). DNA sequencing confirmed the presence of *C. canis* and *C. parvum* in dogs and *C. felis* in cats.

Conclusion. This is the first molecular characterization of *Cryptosporidium* spp. infection in dogs and cats in Poland.

Key words

Cryptosporidium spp., nested PCR, Protozoa, Ziehl-Neelsen method, zoonosis, dogs, cats

INTRODUCTION

Cryptosporidium is a small (4–6μm), widespread, coccidian parasite affecting the epithelium of the digestive and respiratory tracts in mammals, including humans, reptiles, amphibians and birds [1, 2]. *Cryptosporidium* genus contains over 30 so far recognized species and numerous genotypes [3]. Cats may be infected with *C. felis*, *C. muris, C. ryanae* and *C. parvum* and dogs with *C. canis*, *C. parvum, C. ubiquitum* and *C. andersoni* [4, 5, 6]. Cryptosporidiosis in humans is usually caused by *C. hominis, C. meleagridis, C. parvum*, and also *C. felis* and *C. canis* [7]. Dogs and cats, as intimate companion animals, can be sources of human infections. Prevalence of *Cryptosporidium* spp. ranges from 0.2% – 5.9% in European dogs and from 1.7% – 8.8% in European cats [8, 9, 10, 11]. In Poland, there are only regional data of *Cryptosporidium* prevalence in dogs and cats [12, 13]. Moreover, the genetic diversity of *Cryptosporidium* spp. in dogs in Poland remains unrecognized.

OBJECTIVE

The aim of the study was to determine the occurrence and molecular characteristics of *Cryptosporidium* spp. in dogs and cats in Poland considering their potential to be reservoirs for human cryptosporidiosis.

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MATERIALS AND METHOD

Between October 2016 – June 2019, 365 fresh faecal samples (264 dogs and 101 cats) were obtained from individual, randomly chosen dogs and cats living in different regions of Poland. Samples were collected from 9 of the 16 Polish provinces (Pomerania, Greater Poland, Opole, Silesia, Lesser Poland, Lower Silesia, Łódź, Holy Cross and Subcarpathia). Animals were grouped based on the age (under one year old, between 1–8-years-old and over 8 years old) and faeces condition (formed, diarrheic). Microscopic diagnosis of *Cryptosporidium* was performed and 2 smears were made from each faecal sample to detect oocysts. The smears were stained with the Ziehl-Neelsen method and examined under 1,000x magnification with immersion oil. The oocysts were identified based on their size, morphology and colour. DNA was isolated from each faecal sample using Genomic Mini AX Stool (A&A Biotechnology, Poland), as per the manufacturer's instruction. The DNA samples were stored at -80˚C until further use. To identify *Cryptosporidium* spp. in the stool samples, fragments covering 18S rDNA gene were amplified by nested PCR. First, amplification of the 763 bp region was carried out, and next, for secondary PCR, the 587 bp fragment was amplified using 1 μl of the first PCR product. Primers, mixture composition and PCR conditions have been described by Ryan et al. [14]. The secondary PCR products were examined electrophoretically in 2% agarose gels and visualized after staining with Midori Green Advance DNA stain (Genetics). Microscopic examination, DNA isolation and amplification was performed in the Division of Parasitology at the Wrocław University of Environmental and Life Sciences.

PCR products sequencing was performed by Genomed (Poland) in both directions. Identity of the obtained sequences was checked by a blast search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Copromicroscopic analyses were

performed using the flotation technique with a sodium nitrate solution (density 1.2 g/mL) to evaluate the presence or absence of cysts, oocysts and helminth eggs. Confidence intervals (CI) at the level of 95% ($p = 0.05$) was calculated according to the Wilson method. 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 *Cryptosporidium* infection rates among the investigated groups. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Cryptosporidium spp. were identified by nested PCR in 9 out of 264 canine stool samples (3.4%) and 2 out of 101 feline specimens (2%). The oocysts of *Cryptosporidium* spp. were detected microscopically in 7 (2.7%) canine and 1 (1%) feline stool samples. This frequency was significantly lower than that described for Poland by Gundłach (27.4% in dogs and 33.3% in cats) and Bajer (12.5% in sled dogs) [12, 13]. The results obtained may reflect an improvement in the living conditions of companion animals in Poland, but may be also due to the use of different diagnostic methods. The commercial *Cryptosporidium* coproantigen ELISA used by Gundłach might yield false positive results [15]. Frequency of *Cryptosporidium* spp. in dogs from Poland presented in the current study equaled that in other European countries, such as Spain (4.1% – 5.5%), Greece (5.9%), France (2.6%), United Kingdom (4.6%), and the Czech Republic (0.6–3.2%) [6, 9, 11, 16, 17]. A significantly lower prevalence in Europe was reported for dogs in Italy (0.2%) [8]. The incidence of *Cryptosporidium* spp. in cats showed in the presented study (2.3%) was comparable to that in Austria (1.7%) [10]. A higher frequency in cats was reported for Spain (8.8%) and Greece (6.8%) [9, 11]. In the current study, no statistically significant differences were observed related to animal age. *Cryptosporidium* spp. frequency was similar in diarrheic and non-diarrheic dogs, but in cats the parasite was only in diarrheic ones. The occurrence of other gastrointestinal parasites may influence faecal consistency. Three out of 9 *Cryptosporidium*-positive dogs were infected with other parasites (*Giardia duodenalis*, *Ancylostomatidae* and *Cystoisospora* spp.). One of 2 *Cryptosporidium*-positive cats that suffered from diarrhea were concurrently infected with *Toxocara cati* (Tab. 1). Mixed infections with *Cryptosporidium* and other protozoan parasites (*G. duodenalis* and *Cystoisospora canis* especially) do not seem to be rare and have been previously described [18]. Sequences obtained by genotyping were compared with sequences deposited in GenBank base and 3 cases of *C. canis* and 2 cases of *C. parvum* in dogs were detected (Accession Nos. of reference sequences with the highest similarity to obtained sequences: MK886593.1, KY711523.1 and JX886768.1). In cats, 1 out of 2 positive secondary PCR products was sequenced and *C. felis* identified (KM977642.1). The sequences showed high similarity (≥99%) to sequences deposited in the GenBank. *C. canis,* previously restricted only to dogs, is currently considered as a potentially zoonotic species occurring in human patients [19, 20]. *C. parvum* is considered to be a zoonotic species, rarely found in dogs. [3, 6, 21, 22]. *C. felis,* as one of the most frequently found species in cats, was also occasionally detected in humans, suggesting the possibility of transmission between humans and cats [4, 7, 23, 24]. Similar to other studies performed worldwide, the nested PCR method used in the current study proved to be more sensitive in the detection of *Cryptosporidium,* compared with the traditional Ziehl-Neelsen staining [25, 26] (Tab. 1).

CONCLUSION

This is the first molecular characterization of *Cryptosporidium* spp. infection in dogs and cats in Poland. The research showed a low frequency of *Cryptosporidium* in these animals. The presence of *C. canis*, *C. parvum* and *C. felis* in canine and feline population in Poland suggests that companion animals could be potential reservoirs for human infections of these pathogens.

Table 1. Occurrence of *Cryptosporidium* spp. in dogs and cats related to clinical symptoms

Animal species (n)	Clinical symptoms (n)	No. of positive animals	Infection frequencies (CI*)	Age of animals	Methods of detection		Other parasites (Flotation method)
					Cryptosporidium spp. (PCR)**	Ziehl-Neelsen	
Dog (264)	Asymptomatic (117)	3	2.6% (0.9 – 7.3)	2 months	*C. canis* MK886593.1	positive	-
				6 months	*C. parvum* JX886768.1	positive	*Cystoisospora* spp.
				9 months	*C. spp.*	positive	-
	Diarrhea (147)	6	4.1% (1.9– 8.6)	2 months	*C. canis* MK886593.1	negative	*Giardia duodenalis*
				5 months	*C. spp*	positive	*Uncinaria stenocephala*
				6 months	*C. canis* MK886593.1	positive	-
				8 years	*C. spp*	positive	-
				10 years	*C. parvum* KY711523.1	positive	-
				Unknown	*C. spp.*	negative	-
Cat (101)	Asymptomatic (57)	0	0.0% (0-6.3)	-	-	-	-
	Diarrhea (44)	2	4.6% (1.3-15.1)	9 months	*C. felis* KM977642.1	positive	-
				11 months	*C. spp.*	negative	*Toxocara cati*

* CI – 95% confidence interval according to the Wilson method.
** Accession No. of reference sequences deposited in GenBank (reference sequences chosen according to high similarity to obtained sequences).

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Conflict of interest
The authors declare that they have no conflict of interest.

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