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Research paper

Giardia in a selected population of dogs and cats in Germany – diagnostics, coinfections and assemblages

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ABSTRACT

Infections with the zoonotic endoparasite *Giardia duodenalis* are widely spread among dogs and cats worldwide. Since the question whether the infection might be transmitted from domestic animals to their owners is still an important topic, a reliable detection of patent *Giardia* infections and the determination of the associated *Giardia* assemblages is of major concern. The objectives of the present study were to determine the prevalence of *Giardia* infections in dogs and cats living in Germany using different diagnostic tests and to identify the *Giardia* assemblages of infected animals. Furthermore, a possible correlation of coinfections with other endoparasites was analysed. All samples were investigated by enzyme-linked immunosorbent assay (ELISA), merthiolate-iodine-formalin concentration technique (MIFC) and zinc chloride flotation. ELISA-positive samples were additionally screened with a direct immunofluorescence assay (IFA). Faecal DNA was extracted from all *Giardia* cyst-positive samples and used for multilocus sequence typing with nested PCRs targeting the following gene loci: SSU rRNA (SSU), glutamate dehydrogenase (gdh) and triosephosphate isomerase (tpi). Samples from dogs and cats tested positive for *Giardia* coproantigen (ELISA) in 30.6% and 17.9%, respectively. The MIFC technique revealed *Giardia* cysts in 33.9% of canine and in 34.6% of feline ELISA-positive samples, while using IFA, cysts were present in 90.4% of canine and in 76.9% of feline ELISA-positive samples. Coinfections with other endoparasites besides *Giardia* were found in both dogs and cats, yet a statistically significant correlation could solely be drawn for the canine samples. The success rate of the different PCR protocols varied between 23.1% (tpi) and 91.3% (SSU) for dogs and between 25.0% (gdh) and 90.0% (SSU) for cats. Dog-specific *Giardia* assemblages C and D were detected in 42 and 55 canine isolates, respectively. The cat-specific *Giardia* assemblage F was detected in 14 feline isolates. Two canine and two feline samples harboured the zoonotic assemblage A. According to the results of the study, *Giardia* is a common endoparasite in dogs and cats from Germany. The exclusive application of MIFC is insufficient for a reliable identification of patent *Giardia* infections since the IFA revealed a higher sensitivity for the detection of *Giardia* cysts in feline and canine faecal samples. Even though the majority of investigated animals harboured the species-specific *Giardia* assemblages C, D and F, a zoonotic potential arising from assemblage A could not be excluded.

1. Introduction

The reliable detection of patent *Giardia* infections in dogs and cats as close companions of humans is of major importance due to the zoonotic potential of this worldwide occurring endoparasite. Especially young animals with or without the presence of diarrhoea tend to be infected with *Giardia* spreading cysts into the environment, although adult animals might also be affected (Barutzki and Schaper, 2011). Even though a variety of tests for the diagnosis of *Giardia* is available, the steady recognition of the endoparasite, the correct interpretation of results and the subsequent treatment might be challenging. For the direct detection of *Giardia* cysts, methods like merthiolate-iodine-formalin concentration technique (MIFC), sodium acetate formaldehyde (SAF) technique, zinc sulphate ($ZnSO_4$) faecal flotation and immunofluorescence assay (IFA) are used (Barutzki and Schaper, 2003, 2013; Geurden et al., 2008; Rishniw et al., 2010). The indirect confirmation of *Giardia* via coproantigen is usually performed with different enzyme-linked immunosorbent assays (ELISA) and immunochromatographic assays (SNAP) (Carlin et al., 2006; Costa et al., 2016; Zimmerman and Needham, 1995). Recent surveys have confirmed the presence of

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Giardia in symptomatic as well as in asymptomatic dogs and cats from Germany with prevalences ranging from 2.2–30.0% in canine and 0.7–24.6% in feline samples (Barutzki and Schaper, 2003, 2011; Becker et al., 2012; Cirak and Bauer, 2004; Epe et al., 2004; Epe et al., 2010). The frequent detection of *Giardia* in dogs or cats poses the question whether the infection might also be transmitted to the animal owner. To date, *Giardia* is divided into two potentially zoonotic assemblages A and B as well as six species-specific assemblages C–H and corresponding subassemblages (Lasek-Nesselquist et al., 2010; Thompson, 2004; Thompson and Monis, 2012). Nevertheless, the determination of *Giardia* assemblages in companion animals is rarely part of the routine diagnostics so far. Both zoonotic assemblages A and B as well as canine-specific assemblages C and D have been determined in different canine populations worldwide (Claerebout et al., 2009; Covacin et al., 2011; Dado et al., 2012; Gil et al., 2017; Leonhard et al., 2007; McDowall et al., 2011; Sommer et al., 2015; Upjohn et al., 2010; Xu et al., 2016). In cats, the zoonotic assemblage A and the feline-specific assemblage F usually predominate (Kostopoulou et al., 2017; Paoletti et al., 2011; Piekarska et al., 2016; Santin et al., 2006). Although several studies have been performed to investigate the current assemblages in dogs and cats in various countries, further research is needed in order to gain reliable data on possible distribution patterns of *Giardia* between animals and humans (García-Cervantes et al., 2017; Thompson, 2004). In comparison with other countries, limited genotyping data is available for *Giardia* infections in dogs and cats from Germany. In the present study, *Giardia* assemblages and corresponding gene variations in all animals shedding *Giardia* cysts were identified. Furthermore, the prevalence for *Giardia* infections and endoparasitic coinfections in dogs and cats living in Germany was determined by different diagnostic methods.

2. Material and methods

2.1. Sample origin

From 2015–2016, a total of 376 canine and 145 feline faecal samples were collected from animals living in different parts of Germany. Animals included into the study had to fulfil the following criteria: a maximum age of two years, or if older than two years the presence of gastrointestinal (GI) symptoms or a diagnosed immunodeficiency at the time of sample collection. One part of the samples was specifically recruited for the present study while the other part of samples was obtained in the framework of the routine diagnostics at the Department for Experimental Parasitology, Ludwig-Maximilians-University, Munich.

The majority of animals derived from private households, the rest of the samples was obtained from animals originating from animal shelters and breeding or laboratory facilities. A portion of animals (38.6% of dogs and 63.4% of cats) was presented to veterinarians for several reasons including gastrointestinal disorders, vaccinations or routine examinations. Dogs and cats of various breeds and both sexes were investigated. Most samples (430/521) were collected over three consecutive days in order to increase the chance to detect intermittently shed *Giardia* cysts. Veterinarians or owners sending the samples marked on a questionnaire information on the origin, age, sex and the presence of gastrointestinal symptoms at the time of collection. All samples were processed directly after their arrival at the diagnostic laboratory in Munich.

2.2. Screening for Giardia coproantigen with ELISA

In order to detect *Giardia* coproantigen, all faecal samples were investigated with the ELISA ProSpecT™ Giardia Microplate assay (Remel, Lenexa, USA) following the manufacturer's instructions.

2.3. Screening for Giardia cysts with merthiolate-iodine-formalin concentration (MIFC) technique and direct immunofluorescence assay (IFA)

For the direct detection of *Giardia* cysts, all faecal samples were screened with MIFC technique as described previously (Pfister et al., 2013). All ELISA-positive samples were additionally investigated using the IFA Merifluor® Cryptosporidium/Giardia (Meridian Bioscience, Luckenwalde, Germany). The number of *Giardia* cysts in a single slide was described as rare (+, < 50 cysts/slide), numerous (++, 50–500 cysts/slide) and plentiful (+++, > 500 cysts/slide) in both the MIFC technique and the IFA.

2.4. Screening for endoparasitic coinfections with $ZnCl_2$-NaCl flotation

For the detection of coinfections with endoparasites, a saturated $ZnCl_2$-NaCl flotation (specific gravity 1.28) was performed with all faecal samples (Thienpont et al., 1979). Slides were investigated under a light microscope using a 100 × magnification.

2.5. DNA extraction

DNA from all samples containing *Giardia* cysts was extracted straight after the initial investigation using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommended protocol. In total, 104 canine and 20 feline samples were used for DNA-extraction and subsequent genotyping. DNA concentration and purity were measured with the Nanodrop™ ND 1000-Spectrometer (Peqlab, Erlangen, Germany).

2.6. Nested and semi nested PCR protocols

Nested PCR protocols were performed for the amplification of fragments of the SSU rRNA gene (Hopkins et al., 1997; Read et al., 2002), the glutamate dehydrogenase (gdh) gene (Cacciò et al., 2008) and the triosephosphate isomerase (tpi) gene (Sulaiman et al., 2003). In order to distinguish between *Giardia* assemblages A and F, all ambiguous samples at the SSU locus were additionally screened with a semi nested protocol (Pallant et al., 2015). Moreover, an extra protocol specifically targeting *Giardia* assemblages C and D was performed for the second amplification of the nested tpi-PCR with all samples (Lebbad et al., 2008). Detailed information on the master mix components, primers and cycling conditions is available in the supplementary material.

2.7. Visualisation of PCR products

PCR products were analysed on 2% Top Vision Agarose gels (Fermentas, St. Leon-Rot, Germany) produced with TAE buffer 50 × (Qiagen, Hilden, Germany) and TBE buffer 10 × (Fermentas, St. Leon-Rot, Germany). The agarose was dyed with GelRed™ nucleic acid stain, 10,000 × in water (Biotium, Hayward, USA) and a Gene Ruler 100 bp Plus DNA ladder (Fermentas, St. Leon-Rot, Germany) was added to every agarose gel. A gel documentation system was used for visualising gel images under UV light (Peqlab, Erlangen, Germany).

2.8. Sequencing and species identification

PCR-positive products were purified using the QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Forward and reverse sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany). Obtained reverse sequences were reversed, complemented and aligned to the forward sequences using online tools (Reverse Complement: http://www.bioinformatics.org/sms/rev_comp.html, Clustal Omega: http://www.ebi.ac.uk/Tools/msa/clustalo/). The obtained sequences were compared with sequences from the GenBank (BLAST: http://blast.ncbi.nlm.