Parasitol Res (2007) 101:S153–S156 DOI 10.1007/s00436-007-0623-7

Observations on *Giardia* Infection in Dogs from Veterinary Clinics in Germany

D. Barutzki[1] (✉), R. C. A. Thompson[2], C. Wielinga[2], U. Parka[2], R. Schaper[3]

[1] Veterinary Laboratory Freiburg, Freiburg i. Br., Germany
[2] Centre for Biomolecular Control of Disease, School of Veterinary and Biomedical Sciences, Murdoch University, Australia
[3] Bayer HealthCare AG, Animal Health, Leverkusen, Germany

✉ E-mail: barutzki@uni-freiburg.de

Abstract

Recent studies have shown that dogs in Germany harbour different genotypic assemblages, predominantly C and D, but also the assemblage A, which has been confirmed as a pathogenic human genotype. To determine the prevalence of assemblage A, a first study was conducted in dogs which showed clinical signs of diarrhoea or other symptoms indicating a possible infection with *Giardia*. A total of 92 dogs were identified positive for *Giardia* by a coproantigen test, of which 65 samples were positive for cysts of *Giardia* using the MIFC technique. These samples were genotyped, 50 *Giardia* isolates were identified as assemblage D, 33 as assemblage C and eight as a mixture of both. One dog harboured a mixture of assemblage D and A.

It was concluded that the predominant dog-specific assemblages C and D in this first study might be more commonly associated with GI disorders than the zoonotic genotype A. Alternatively the coproantigen test used in this study might select assemblages C + D in dogs. Therefore, in a second study randomly selected dogs presented at local veterinary clinics were examined for cysts of *Giardia* using the MIFC technique. A total of 58 samples of *Giardia*-positive dogs were genotyped: 57% belonged to assemblage D, 36% to assemblage C and 7% could be identified as the zoonotic genotype assemblage A. Comparing the 65 MIFC-positive samples from the first study with the 58 samples of the second study a significantly higher prevalence of assemblage A ($P = 0.0467$) was found in randomly selected dogs.

Introduction

Flagellates of the genus *Giardia* are ubiquitous in their distribution and found in the intestinal tract of humans and animals of several species throughout the world. *Giardia* spp. are common parasites of dogs in Germany. In recent years, studies have shown that dogs harbour different assemblages such as the dog-specific assemblages D and C, but also the assemblage A, which has been confirmed as zoonotic (Barutzki *et al.* 2006, Leonhard *et al.* 2006). Dogs have a potentially important role as carrier of zoonotic assemblages due to the close contact to susceptible humans. To determine the prevalence of assemblage A, a first study (Barutzki *et al.* 2006) was conducted in dogs which showed clinical signs of diarrhoea or other symptoms indicating a possible infection with *Giardia*. A total of 92 dogs were identified positive for *Giardia* by a coproantigen test (ProSpecT® Giardia Microplate Assay), of which 65 samples were positive for cysts of *Giardia* using the MIFC technique (Fig. 1). For genotyping, a nested PCR-based procedure for the direct characterisation of *Giardia* from faeces based on a ~1100 bp region of SSU rDNA was used. All 92 samples were genotyped, 50 *Giardia* isolates were identified as assemblage D, 33 as assemblage C and 8 as a mixture of both. One dog harboured a mixture of assemblage D and A. It was concluded that the predominant dog-specific assemblages C and D in this first study might be more commonly associated with GI disorders than the zoonotic genotype A. Alternatively the coproantigen test used in this study might select assemblages C + D in dogs.

Therefore, in a second study reported here, randomly selected dogs presented at local veterinary clinics have been examined for cysts of *Giardia* using the MIFC technique. Positive samples have been genotyped to investigate a potential relation between clinical signs of GI disorders and the genotype of *Giardia* infecting dogs.

Materials and methods

Faecal samples

Trial 1: Faecal samples were obtained from privately owned dogs presented at local veterinary practitioners for medical problems, predominantly GI disorders. These samples have been investigated for *Giardia* using a coproantigen test (ProSpecT® *Giardia* Microplate Assay) and the MIFC (Merthiolate Iodine Formaldehyde Concentration) technique. Those found positive in the MIFC test were included in this comparative analysis.

Trial 2: Randomly selected dogs presented at local veterinary clinics were examined for cysts of *Giardia* using the MIFC technique.

PCR method in trials 1 + 2

DNA was extracted from washed (DMSO) preserved samples using a stool extraction kit (Qiagen) with initial freeze/thaw treatment of cysts.

PCR amplification and sequencing of the 18S rDNA

To determine the respective genotypes, a nested PCR was employed for amplification at the 18S rDNA locus. For the primary reaction, primers RH11 and RH4 (Hopkins *et al.* 1997) and for the secondary reaction primers GiarF and GiarR (Read *et al.* 2002) were used. Amplification conditions for these primers varied slightly from the published methods (increased reaction volume and a touch down annealing PCR programme).

Products were isolated from agarose gel using a DNA purification kit (UltraClean GelSpin, Mo Bio). Sequencing reactions were performed by using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Products of the 18S rDNA PCR were sequenced in the reverse direction only (GiarR). Sequencing profiles were analysed using SeqEd v1.0.3 (Applied Biosystems). Isolates were grouped into their genetic assemblages based on their poly-

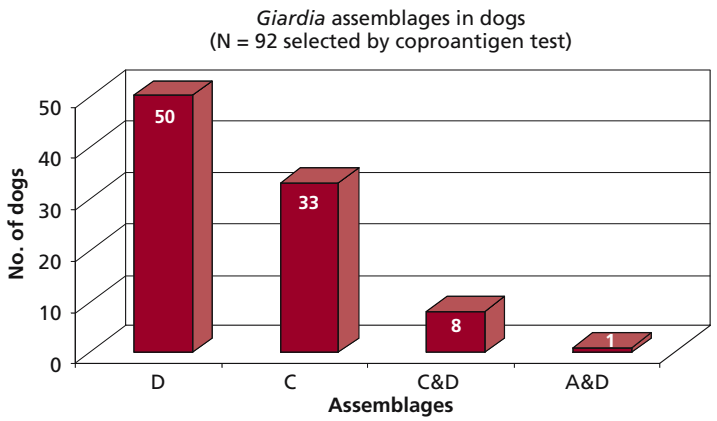

Fig. 1 Distribution of *Giardia* assemblages in dogs with clinical signs of gastrointestinal disorders

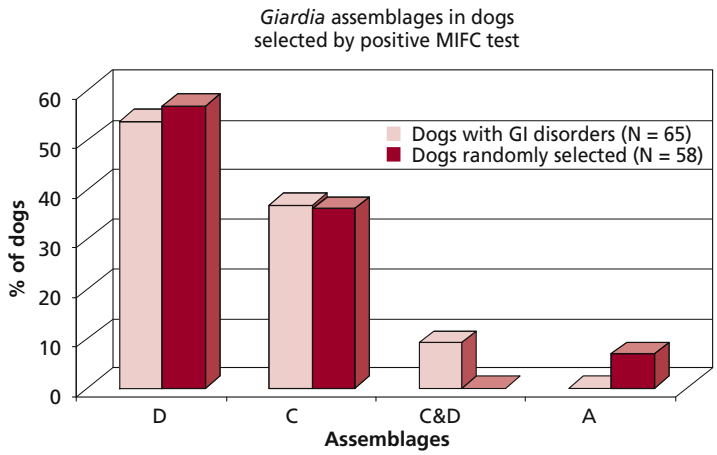

Fig. 2 Distribution of *Giardia* assemblages in randomly selected dogs

morphisms within the 130 base pair sequence (Hopkins *et al.* 1997, Monis *et al.* 1999).

Statistical analysis

First, the two trials were tested on differences in distribution relating to their genotyping with a two-sided Mann-Whitney U test.

In a second step, the two trials were analysed on differences in the characteristic value of genotyping "A or any combination with A" and "C, D and CD" (Fisher's exact test).

Results and discussion

Giardia duodenalis is an enteric protozoon, which can produce gastrointestinal disorders such as diarrhoea and malabsorption in various animal species and humans. The transmission of the parasite can be direct from human to human, animal to animal as well as from animal to humans or from humans to animals, and through environmental contamination. Until today, the role of dogs in the transmission of *Giardia* to humans is unclear. In Germany, analysis of coproscopical examinations for the years 1997 to 2000 revealed a prevalence of 16. 5% for *Giardia* in dogs (Barutzki *et al.* 2003). Of particu-

lar importance are the assemblages A and B, which are accepted to be zoonotic genotypes of which the first occurs also in dogs (Traub *et al.* 2004, Leonhard *et al.* 2006). The prevalence of assemblage A in dogs varies considerably depending on the geographic location. In addition, this variability might be influenced by the population selected. In Germany, dogs presented at veterinary clinics from private owners showed a prevalence of assemblage A of 70%, and those selected from dog shelters showed 43% (Leonhard *et al.* 2006). In contrast to these findings, in our first study assemblage A was found in only one dog out of 92. In this study the examined dogs were selected based on the existence of clinical symptoms of GI disorders. In addition, the results could be influenced by using the coproantigen test which might select for dog-specific assemblages C and D. Therefore, in contrast to the first study the method was changed. Only randomly selected dogs presented at local veterinary clinics and shedding *Giardia* cysts confirmed by the MIFC technique were included in the analysis. Until now 58 samples of these *Giardia*-positive dogs have been genotyped: 57% belong to assemblage D and 36% to assemblage C which are regarded as dog-specific. Another 7% could be identified as the zoonotic genotype A. (Fig. 2). The two trials showed no significant difference when tested on differences in distribution relating to their genotyping. Compared with *Giardia*-positive dogs showing GI disorders (from trial 1) the prevalence of assemblage A was significantly higher (P = 0.0467) in randomly selected dogs.

Conclusions

- A total of 7% of randomly selected *Giardia*-positive dogs proved to be infected with the zoonotic *Giardia* genotype assemblage A.
- Randomly selected *Giardia*-positive dogs harbour a significantly higher percentage of assemblage A compared to positive dogs with GI symptoms.
- Dogs without clinical signs of GI symptoms should be periodically checked for *Giardia* and treated, if positive, due to the risk of zoonotic infection.

Acknowledgements

The study reported herein was performed in compliance with current, applicable, local laws and regulations.

References

Barutzki D, Schaper R (2003). Prevalence of *Giardia* spp. in dogs in Germany. In: *Giardia* the Cosmopolitan Parasite (ed) Olsen, BE, Olsen, ME, Wallis, PM, pp. 91–95.

Barutzki D, Thompson RCA, Wielinga C, Traub RJ, Schaper R (2006). *Giardia* spp. in dogs: Genotyping of 100 samples from Germany. (Poster) German Society of Parasitology Annual Meeting 2006, p. 131.

Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RCA (1997). Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J Parasitol 83:44–51.

Monis PT, Andrews RH, Mayrhofer G and Ey PL (1999). Molecular systematics of the parasitic protozoan *Giardia intestinalis*. Mol Biol Evol, 16:1135–1144.

Leonhard S, Pfister K, Beelitz P, Wielinga C, Traub RJ, Thompson RCA (2006). The molecular characterization of *Giardia* from dogs in southern Germany. (Poster) German Society of Parasitology Annual Meeting 2006, p. 163.

Read C, Walters J, Robertson ID, Thompson RCA (2002). Correlation between genotype of *Giardia duodenalis* and diarrhoea. Int J Parasitol 32:229–231.

Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N, Thompson RCA (2004). Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128:253–262.