SHORT COMMUNICATION

Genotyping of *Giardia duodenalis* Among Children and Dogs in a Closed Socially Deprived Community From Italy

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Impacts

- To add data on the presence of *Giardia* in a well-defined context with homogeneous environmental condition as suggested by the most recent directives from the International Scientific Community.
- To identify *Giardia* genotypes circulating among people and dogs within the marginalized close community.
- To obtain by sensitive molecular tools more reliable information on the risk that stray dogs infected by giardiasis may represent for human health.

Keywords:
Giardia; children; dogs; zoonoses; Rom community; Italy

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Summary

Molecular characterization of *Giardia duodenalis* cysts from humans and animals living in well-defined contexts is useful to study the circulation of isolates and represents a tool to evaluate zoonotic infection risk. The presence of giardiasis in children living in a disadvantaged and socially deprived small Rom community, as well in dogs roaming freely in the same context was carried out by microscopic analysis and beta-giardin gene amplification. Five out of 14 children were found positive at microscopic examination for *G. duodenalis* and six positive at PCR, while eight out of 14 dogs tested both microscopically and molecularly positive for *G. duodenalis*. Moreover, most of the children and dogs were symptomatic. Molecular characterization of *Giardia* positive samples from children and dogs showed 99.5% identity with *Giardia* Assemblage A1. The dog-specific genotypes C and D were not found. The findings of this survey provide the first European evidence to support the possible role of dogs in zoonotic transmission involving children and stray dogs in a closed context with very low standards of hygiene (i.e. Rom community), and these results show the need to monitor the health of marginal populations to safeguard ethnic minority groups.

Introduction

Giardia is one of the most important protozoan transmitted by the faecal-oral route and connected to faecal contamination of the environment (Kirkpatrick and Green, 1985). A wide range of animals is infected by *Giardia duodenalis*, including humans and pet, livestock and wild animal species; it causes a self-limiting disease in immuno-competent subjects and severe diarrhoea in immunocompromised subjects (Thompson and Monis, 2004; Savioli et al., 2006).

Giardia duodenalis is a species complex with seven Assemblages (A–G); Assemblage A – and in particular the sub-Assemblage A1 – is zoonotic, infecting humans and a wide range of animals (Thompson, 2004; Cacciò et al., 2005). Among the different DNA targets characterized for *Giardia*, the beta-giardin gene is conserved and considered reliable for the discrimination and identification of *Giardia* Assemblages and the main sub-Assemblages (Smith et al., 2006; Wielinga and Thompson, 2007).

Over the past decade, there has been an increase in reports of *Giardia* infection in social groups living in

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overcrowded situations with poor hygiene and health conditions, and giardiasis has recently been classified among the 'Neglected Diseases Initiatives' (Savioli et al., 2006).

Therefore, the scientific community is interested in *Giardia* infection in humans and animals because of the implications for public health. Molecular technologies have contributed to solving taxonomic and epidemiological issues regarding *Giardia* spp. circulating within and among different species of animals (Xiao and Fayer, 2008). Recent research has shown a strict genetic relationship between *G. duodenalis* genotype A isolated from children and dogs in some communities in Mexico (Eligio-García et al., 2005) and in Brazil (Volotao et al., 2007).

In Europe, although a number of isolates from different host species have been typed and zoonotic Assemblages (Assemblages A and AI) have been identified in humans and dogs (Giangaspero et al., 2007), data are lacking on the frequency of *Giardia* transmission from animals to humans. To acquire better understanding of zoonotic transmission of *Giardia*, it is useful to study its ecological features and transmission patterns in well-defined contexts and animal populations in homogeneous environmental conditions (Savioli et al., 2006; Smith et al., 2007). In developed countries, Rom communities may represent a suitable model for studying epidemiology of human giardiasis because they are organized as a closed social and cultural system, sharing the same limited environment which is often characterized by poor hygienic conditions. Rom populations originate from Eastern Europe and are the largest and poorest minority in Europe, living as nomads and also in permanent settlements. In Italy, the Rom population is estimated at 90 000–110 000 individuals, living in family groups concentrated in the biggest towns (Kosa and Adany, 2007).

This study aimed to evaluate the presence of *Giardia* in children living in a disadvantaged and socially deprived small Rom community, as well in dogs in the same context, and also to use molecular techniques to identify the isolates.

Material and Methods

Samples were collected from a Rom community in a camp (~800 m^2) on the outskirts of the city of Foggia (Southern Italy), consisting of a total of 119 individuals (63 males and 56 females) making up 25 families. The Rom people live in huts or caravans close together with two separate cinderblock buildings housing communal toilets, one for men and one for women. Outside doors are not always closed, enabling free access by birds and dogs. Faeces contaminate floors. Living standards are extremely low (Fig. 1), with dogs roaming freely around the camp (i.e. both in homes and in the toilets) (Fig. 2), eating garbage or leftovers. The huts and/or caravans stand in a double row along a main street. The surface of asphalt and stones has numerous puddles of waste water and/or rainwater.

Fig. 1. Rom camp showing the very low standard of hygiene.

Over a 2-month period from June to July 2007, faecal samples were simultaneously collected from 14 children aged 2–13 years (i.e. able to walk around freely) from 14 families living in the camp (one child per family), and from all 14 free-roaming dogs in the same enclave. We obtained permission to take a single specimen from only one child in each of the families and we preferred to ask samples from the children referring diarrhoea or some gastrointestinal distress.

Children's parents were supplied with sterile containers and medical history as well as gender, and age data was collected for the children. Faecal samples were also individually collected directly from the rectum of the 14 different stray dogs freely roaming during the sampling

Fig. 2. Stray dogs roaming freely around the Rom camp.

time, and age, sex and breed data were recorded. The consistency (diarrheic or not diarrheic) of all faecal samples was recorded. All fresh faecal samples were concentrated with a sucrose solution (Roberts-Thompson et al., 1976) and examined twice. Each faecal sample was also processed by extracting genomic DNA using QIAamp DNA Stool Mini Kit (Qiagen, Milano, Italy). A partial sequence (about 171 bp) encoding for beta-giardin was amplified by PCR (Cacciò et al., 2002). Samples with DNA extracted from *G. duodenalis* (American Type Culture Collection, ATCC 30957) and samples without DNA were included in PCR reactions as positive and negative internal controls. After purification, positive samples were sequenced and all the sequences were aligned by ClustalX and compared with those available in GenBank for *G. duodenalis* under the following accession number: X85958 (AI), AY072723 (AII), AY072725 (B), AY545646 (C), AY545647 (D).

Results and Discussion

The age, gender, faecal consistency and results at microscopic examination and PCR of children and dogs are reported in Tables 1 and 2 respectively. Five faecal samples from the 14 children were positive by both molecular and microscopic testing for *G. duodenalis*, while one was positive only at PCR. Of the six diarrheic samples, three were positive for *G. duodenalis* by both microscopy and PCR. No other parasites except *Giardia* were identified in children. Eight of the 14 dogs examined were positive for *G. duodenalis* both by microscopic and molecular testing, while one animal was positive only by PCR. Three of the above positive animals had diarrheic faeces. An overall percentage of *Giardia* infection of 42.8 and 64.2% was registered in children and dogs respectively. Eggs of *Toxocara canis* were found in faeces from 6 dogs. Sequences of positive children and dog samples displayed 99.5% identity with *G. duodenalis* Assemblage A, sub-Assemblage A1 (accession number: X85958), showing that they belong to the same zoonotic genotype (Cacciò et al., 2002). The dog-specific genotypes C and D were not found. Due to limited number of samples, no statistical evaluation on the correlation with the age, gender of the children and dogs and the results was counted.

The study demonstrates giardiasis infection in children and dogs in a small socially isolated settlement characterized by poor sanitary conditions and the presence of stray free-ranging dogs. Rom habits and behaviour may contribute to such infection, because children are often barefoot, moving from one housing unit to the other, playing in an environment contaminated by garbage, refuse, faeces (animal and human) and puddles of dirty water.

These findings provide evidence to support the potential role of dogs in zoonotic *Giardia* transmission involving children and domestic/stray dogs in the Rom community examined here. The index case is unknown. It is not clear if infections were initiated by dogs or children, nor is it clear how the parasites spread. But it is apparent that the same genotype is present in all the infected children and dogs. *Giardia* infection caused by the zoonotic Assemblage A has recently been recorded in small Asiatic communities (Traub et al., 2004; Inpankaew

Table 1. Faecal samples collected from children

Identification no.	Gender	Age	Faecal consistency	Results of faecal examination		Genotyping β-giardin
				Microscopy	PCR	
1	M	2 years	d.	+	+	A1
2	M	2.5 years	d.	–	–	
3	F	7 years	n.d.	–	–	
4	M	8 months	d.	–	–	
5	M	8 years	n.d.	–	–	
6	F	4 years	d.	+	+	A1
7	F	10 years	d.	+	+	A1
8	M	8 months	n.d.	+	+	A1
9	F	10 years	n.d.	+	+	A1
10	M	12 years	d.	–	–	
11	F	8 years	n.d.	–	–	
12	F	7 years	n.d.	–	–	
13	M	6 years	n.d.	–	–	
14	F	10 years	n.d.	–	+	A1

Gender (M, males; F, females), faecal consistency (reported as diarrheic, -d. or not diarrheic, n.d.) and results at microscopic examination and PCR (expressed as positive, + or negative, –).

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Table 2. Faecal samples collected from dogs

Identification number	Gender	Age	Breed	Faecal consistency	Results of faecal examination		Geno typing β-giardin
					Microscopy	PCR	
1	F	5 months	Cross-breed	n.d.	+	+	A1
2	F	6 years	Cross-breed	d.	–	–	
3	M	2 years	Cross-breed	n.d.	–	+	A1
4	F	1 year	Cross-breed	d.	+	+	A1
5	M	5 years	Cross-breed	n.d.	–	–	
6	M	3 years	Cross-breed	n.d.	+	+	A1
7	M	8 months	Shepherd breed	d.	+	+	A1
8	F	1 year	Cross-breed	n.d.	+	+	A1
9	M	4 years	Dobermann	d.	–	–	
10	F	3 years	Cross-breed	n.d.	+	+	A1
11	F	4 years	Cross-breed	n.d.	–	–	
12	F	1 year	Cross-breed	d.	+	+	A1
13	F	2 years	Cross-breed	d.	–	–	
14	M	6 months	Cross-breed	n.d.	+	+	A1

Gender (M, males; F, females), faecal consistency (reported as diarrheic, -d. or not diarrheic, n.d.) and results at microscopic examination and PCR (expressed as positive, + or negative, –).

et al., 2007), with up to 8% of humans and 20% of dogs infected. Genotype A in both children and dogs have been also recorded in Mexicans (Eligio-García et al., 2005) and more recently in a Brazilian (Volotao et al., 2007) community, and in both situations the infection was attributed to the socio-cultural factors and sanitary conditions of the studied populations.

In Italy, the prevalence of giardiasis ranges from 0.9 to 4.7% in children and from 3.6 to 74% with the highest prevalence in dogs from kennels (Giangaspero et al., 2007). In this study, the high percentage of positivity registered in both children and dogs (42.8 and 64.2% respectively) is not surprising considering the peculiar environment studied which can be easily considered as a 'focus of transmission' of giardiasis, also considering that the dog-specific genotypes C and D were not found.

Although research on the occurrence of different genotypes of *Giardia* has created awareness about the public health risk of infection from domestic animals (i.e. dogs/cats), data are lacking on the occurrence of zoonotic *Giardia*. This work contributes information on the epidemiology of giardiasis in a small community in Southern Europe where no other data have been available, and indicates the need for a larger investigation and to identify the sources of the infection with *Giardia* in this community and shed light on the dynamic of the infection within this and other socially isolated and economically deprived communities with poor sanitation and health care. Considering the presence of more than one zoonotic parasite (i.e. *Giardia* and *Toxocara*), our findings also show the need to monitor and safeguard the health of marginal populations to prevent the potential spread of infection to other communities.

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