

Short communication

## Prevalence and genotyping of human isolates of *Giardia duodenalis* from Albania

F. Berrilli <sup>a,\*</sup>, D. Di Cave <sup>a</sup>, C. D’Orazi <sup>b</sup>, P. Orecchia <sup>a</sup>, L. Xhelilaj <sup>c</sup>, D. Bejko <sup>c</sup>, P. Çaça <sup>c</sup>,  
D. Bebeci <sup>c</sup>, F. Cenko <sup>d</sup>, D. Donia <sup>a</sup>, M. Divizia <sup>a</sup>

<sup>a</sup> Department of Public Health and Cell Biology, Faculty of Medicine, University of Rome “Tor Vergata”, Via Montpellier 1, 00133 Rome, Italy

<sup>b</sup> Azienda Ospedaliera Universitaria Policlinico “Tor Vergata”, Rome, Italy

<sup>c</sup> University Hospital Centre “Mother Theresa”, Paediatric Unit, Tirana, Albania

<sup>d</sup> Albanian Child Survival Project, American Red Cross, Tirana, Albania

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### Abstract

Microscopical and PCR-based techniques were performed in order to investigate the prevalence of infection and the genotypes of *Giardia duodenalis* from 125 stool samples collected from children living in the urban and the rural areas of Tirana (Albania) and hospitalized with acute gastroenteritis. 7 out of 125 samples resulted positive for *Giardia* at the microscopic examination (5.6%). In 50 selected samples including the 7 samples positive for *Giardia* by microscopy, 3 and 15 additional positive samples were detected by immunofluorescence and PCR, respectively. Seasonality appeared as an important parameter to be evaluated in order to better understand the prevalence of infection. Sequence analysis revealed both human Assemblage A and B. This result represents the first data on *G. duodenalis* genotypes in Albania.

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Albania is a small country located in the South Eastern Europe between Greece and Serbia and Montenegro and bordering the Adriatic Sea and Ionian Sea. Although Albania’s economy is constantly growing, the country is still one of the poorest in Europe, with serious socio-economic concerns which reflect on public health and environmental conditions. The capital Tirana is the most overcrowded city, harbouring approximately one sixth of the entire population of the country. The poor infrastructures of the country have contributed to the increase of environmental pollution and the contamination of water resources. These factors may explain the high frequency of outbreaks of acute gastroenteritis reported [1,2]. Causes of diarrhoea include a wide array of bacteria, viruses and protozoa. Recent studies on microbiological and virological environmental pollution identified the quality of drinking water as a major health problem in the suburbs of the Albanian capital [3–5]. Among protozoan parasites, *Giardia duodenalis* is recognised

as one of the most important non-viral infectious agent causing diarrhoeal illness in humans world-wide [6,7]. Therefore, to obtain information about the presence and the genotypes of *Giardia* in humans from Albania, a study on hospitalized children from urban and rural areas around Tirana was carried out.

Over a 1-year period, 125 stool samples were obtained from patients with acute gastroenteritis hospitalized in the Paediatric Unit of Tirana Hospital. All subjects were under 9 years of age, coming from the urban area of Tirana and its rural surrounding. To determine the presence of *Giardia* cysts or trophozoites, faecal samples were concentrated by sedimentation technique and examined as Lugol-stained wet mounts. A sub-sample of 50 isolates, comprising samples assessed positive for *Giardia* by microscopy, was randomly selected from the starting 125 samples, in order to be representative of rural and urban areas and of both sexes. Of the 50 children, 27 were males and 23 females while 25 originated from urban areas and 25 from rural region. The sub-sample isolates were examined by direct immunofluorescent assay MERIFLUOR *Cryptosporidium*/

\* Corresponding author. Tel.: +39 06 72596163; fax: +39 06 72596040.

E-mail address: [berrilli@uniroma2.it](mailto:berrilli@uniroma2.it) (F. Berrilli).

Table 1  
Positive results, prevalence (%) and techniques used

	No. positive/no. examined	%	95% CI
Microscopy	7/125	5.6	1.6–9.6
Immunofluorescence <sup>a</sup>	10/50	20.0	8.9–31.1
PCR <sup>a</sup>	22/50	44.0	30.2–57.7

<sup>a</sup> Fifty selected samples including the 7 samples positive for *Giardia* by microscopy.

*Giardia* Monoclonal Antibody Test (Meridian Diagnostics, Cincinnati, Ohio) and by PCR. Genomic DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen) for PCR and stored at  $-20^{\circ}\text{C}$ . Genotyping was carried out using a nested PCR procedure to amplify a 130-bp region from the 16S-rRNA gene using the primers RH11 and RH4 [8] and the primers GiarR and GiarF [9]. Sterile distilled water was included as negative control in each batch of DNA extraction and PCR reactions. Bands were visualised on ethidium bromide stained 1% agarose gels. PCR products were purified using NucleoSpin<sup>®</sup> Extract (Macherey–Nagel) purification Kit and sequenced. Multiple alignment of sequences was obtained using Clustal X [10]. Prevalence obtained by microscopy, immunofluorescence and by PCR and the relative 95% confidence intervals (CIs) were calculated [11]. The resulting data were statistically compared using the  $\chi^2$ -test for independence.

*Giardia* cysts were found in 7 out of the 125 samples examined by microscopy (5.6% CI 1.6–9.6). Immunofluorescence and PCR of 50 selected samples, including the 7 samples positive for *Giardia* by microscopy, not only confirmed all microscopically positive isolates, but also detected 3 and 15 additional positive samples, respectively. All immunofluorescence-positive samples were also positive by PCR (Table 1). The prevalence obtained using nested PCR by sex, season (spring/summer vs. autumn/winter) and locality are reported in Table 2. Eleven of the 22 PCR positive isolates originated from urban areas, the other 11 samples from rural regions. A correlation between seasonality and occurrence of *Giardia* was found ( $P=0.004$ ). The prevalence of giardiasis appeared higher in warmest months (51.0% CI 34.9–67.1) rather than in coldest (23.0% CI 0.12–45.9). Sequence analysis revealed 10 isolates corresponding to Assemblage A (20.0%) and 12 isolates to Assemblage B (24.0%). No mixed assemblages were detected. The distribution of both assemblages by sex, season and locality is shown in Table 2 and no significant correlation was observed.

In general, the prevalence of infection is higher in young people and appear to vary depending on the geographic origin of the studied population, ranging from 1.2% to 32.0% [12]. Prevalence observed by molecular analysis in the current study (44.0%) are higher than those observed in previous researches based only on microscopical examinations [13]. However, considering that the fifty samples selected contain all of microscopy positives, the infection rates observed by immunofluorescence and PCR may overestimate the prevalence of *Giardia* infection. Nevertheless, more sensitive techniques such as PCR may indeed detect *Giardia* infection with low parasitic load or evidence the parasite when it is undetectable in stool sample [14,15]. Values of prevalence observed from rural and urban areas are identical, suggesting a comparable risk of infection in both the environment. Most likely, drinking water and contact with animals could be increasingly recognised as the major vehicles for the transmission of the cysts in Albania. Several studies confirm the large circulation of waterborne enteric pathogens in the country because of the large population movement from rural to urban areas and the absence of any wastewater treatment plant [16,17]. Seasonality appears to be as a factor associated to the morbidity. The seasonal patterns with highest infection rates in the summer have also been reported in other studies [17,18]. Since it is well known that *Giardia* cysts are able to survive for prolonged periods in the environment also at low temperature, the higher risk during warmer season could be related to an increase of human outdoor activities.

Concerning molecular investigation, the genetic analysis of *Giardia* isolates evidenced the presence of both human Assemblages A and B. The greatest zoonotic risk is from Assemblage A *Giardia* genotype and to a lesser measure from Assemblage B genotype which appears to be predominantly human-specific [18]. Recently it has been reported a likely association between assemblage A and subgenotype A2 infections and an increased odds ratios for diarrhoea whereas higher parasite DNA loads and a higher overall prevalence were observed for assemblage B infections which was statistically related with asymptomatic *Giardia* infection [19]. Since our results showed no significant correlation in the distribution of both assemblages according to the sex, seasonality and locality of the sample, diverse transmission cycles which include both humans and domestic animals are possibly involved. The understanding of the circulation of

Table 2  
*Giardia* prevalence (%) by PCR and genotypes by sex, season and locality

Sample (no. positive/no. examined)	Prevalence (%) 95% CI	Genotype				
		Assemblage A		Assemblage B		
		Tot	% 95% CI	Tot	% 95% CI	
Sex	Male (13/27)	48.0 (29.0–66.8)	6	22.0 (6.4–37.6)	7	26.0 (9.4–42.5)
	Female (9/23)	39.0 (19.0–58.9)	4	17.0 (1.6–32.3)	5	22.0 (5.1–38.9)
Season	Spring/summer (19/37)	51.0 (34.9–67.0)	8	22.0 (8.6–35.3)	11	30.0 (15.2–44.7)
	Autumn/winter (3/13)	23.0 (0.12–45.9)	2	15.0 (–4.4–34.4)	1	8.0 (–6.7–22.7)
Locality	Rural (11/25)	44.0 (24.5–63.4)	6	24.0 (7.2–40.7)	5	20.0 (4.3–35.6)
	Urban (11/25)	44.0 (24.5–63.4)	4	16.0 (1.6–30.3)	7	28.0 (10.4–45.6)

*Giardia* genotypes in Albania results particularly relevant, as a consequence of the social isolation of Albania until the beginning of the 1990s and its role as a starting point for massive migration fluxes toward European and non-European countries. The results obtained may reflect the living conditions, lifestyle and environmental situation of the population. As a result, the level of giardiasis could be decreased significantly by implementing relatively simple strategies, such as better wastewater treatment and hygiene education.

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