

Intestinal parasites isolated in a large teaching hospital, Italy, 1 May 2006 to 31 December 2008

L Masucci (lmasucci@rm.unicatt.it)¹, R Graffeo¹, S Bani¹, F Bugli¹, S Boccia^{2,3}, N Nicolotti², B Fiori¹, G Fadda¹, T Spanu¹

1. Institute of Microbiology, Università Cattolica del Sacro Cuore, Policlinico "A. Gemelli", Rome, Italy

2. Institute of Hygiene and Preventive Medicine, Università Cattolica del Sacro Cuore, Policlinico "A. Gemelli", Rome, Italy

3. IRCCS San Raffaele Pisana, Rome, Italy

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Intestinal parasites account for the majority of parasitic diseases, particularly in endemic areas. Most are transmitted via contaminated food. Because of increased immigration and travel, enteric parasitoses are now distributed worldwide. Between May 2006 and December 2008, we examined stool specimens from 5,351 patients (4,695 Italians, 656 non-Italians) for ova and parasites using microscopy, culture techniques, and molecular methods. Stools from 594 patients (11.1%) were contaminated and for all patients samples combined, a total of 700 intestinal parasites were counted. Ninety of the 594 infected patients had more than one parasite in their stools. Parasites causing intestinal disease occurred in 8.8% of patients. The prevalence was over twice as high among non-Italians (26.8% vs 8.9% in Italians, $p < 0.001$) and higher in males (13.0% vs 9.5% in females, $p = 0.003$). Most isolates were pathogenic protozoa, including in decreasing order of frequency: *Blastocystis hominis*, *Giardia intestinalis*, *Entamoeba histolytica*, and *Cyclospora cayentanensis*. The latter two species tended to be more common in Italians, although not at significant level (3.6% (15/418) vs 1.7% (3/176) in non-Italians, OR: 2.15; 95%CI: 0.60–11.70, $p = 0.22$). Helminthes were found in 28 patients, mainly non-Italians (5.7% (10/176) vs 4.3% (18/418), OR: 1.34; 95%CI: 0.54–3.13, $p = 0.47$). *Ascaris lumbricoides* and *Hymenolepis nana* were the most common. *Strongyloides stercoralis*, *Enterobius vermicularis*, *Taenia* spp. and *Trichuris trichiura* were also found. Intestinal parasites are a serious problem in developing countries, but should not be underestimated in industrialised countries.

Introduction

Enteric parasites are the most common cause of parasitic diseases, and they cause significant morbidity and mortality, particularly in endemic areas [1]. Patients with intact or compromised immunity are affected with similar frequencies [2]. Children and young adults are the most affected group, particularly in regions with limited resources and those in which observation of hygienic measures is lax [3]. Intestinal symptoms are frequent and include abdominal pain and acute or chronic

diarrhoea and/or constipation, but systemic manifestations (fatigue, anaemia, weight loss, rash) are by no means uncommon. Most intestinal parasites are transmitted by the faecal-oral route as a result of the ingestion of water, vegetables, and/or soil contaminated with ova, cysts, or oocysts; in other cases (i.e. *Ancylostoma duodenale*) transmission occurs via the skin through direct penetration by larvae living in the soil.

The diseases caused by intestinal parasites, once considered rare phenomena confined to the tropics, are now being diagnosed with increased frequency in Europe and other industrialised countries [1]. This trend can be attributed to various factors, including globalisation of the food supply, the increased consumption of fresh foods, increased travel to developing countries, and more intensive immigration originating from these areas. Contact with other cultures has led to increased consumption of raw or undercooked foods, a potential source of parasites that could be eliminated by proper food processing [4]. Seafood is a classic example, but the rapid transport of fresh fruits and produce from developing countries has made such products more available to European consumers, and thus increased their contact with intestinal parasites. In at-risk groups, sexual practices may also represent a contributor to the transmission of parasites such as *Entamoeba histolytica*, *Hymenolepis nana* and/or *Enterobius vermicularis* [5].

Physicians in non-endemic areas are often poorly prepared to deal with these 'exotic' diseases. Microscopic examination of stool for ova and parasites is commonly ordered to investigate gastrointestinal complaints, eosinophilia, or liver abscesses or as part of preventive assessments of travellers, immigrants, or patients scheduled for transplantation procedures. However, the physicians who order these tests often have a limited knowledge of what they actually entail and how the results should be interpreted. Paradoxically, laboratory and physician surveys suggest that physicians often test for parasites when the likelihood of infection is low and fail to use essential tests when suspicion is high [6].

There is an abundance of epidemiological data on the diffusion and prevalence of intestinal parasitic diseases in developing areas [2,7,8], but in industrialised countries these infections are rarely reported [9]. The microbiology laboratory of our hospital is receiving an increased number of requests for the analysis of stool specimens for ova and parasites. This trend prompted us to evaluate the distribution of intestinal parasites isolated by our laboratory over a 30-month period.

Patients and methods

Patient population and study design

This retrospective study was conducted at the Catholic University Hospital, a 1,600-bed academic medical centre located in Rome, Italy, that admits approximately 60,000 patients per year. We systematically searched the computerised database of the medical centre's central microbiology laboratory to identify all stool specimens submitted for parasitology investigation from 1 May 2006 through 31 December 2008.

Laboratory methods

All commercial products and devices described below were used in accordance with manufacturers' instructions unless otherwise stated.

Samples were stored in fresh normal saline smears. Formalin ethyl acetate concentration was performed with a Midi PARASEP kit (DiaSys Europe Ltd – Wokingham, UK) with Lugol's iodine. Smears were examined with a 40x phase-contrast objective.

Permanent smears were prepared from unpreserved stool and examined with a 100x oil-immersion objective after specific staining, which included trichrome stain (Scientific Device Laboratory, Inc.; Des Plaines, Illinois) and/or Weigert's iron hematoxylin for the detection of protozoa; modified Ziehl-Neelsen acid-fast stain for *Cryptosporidium*, *Cyclospora*, and *Isoospora* species; and modified trichrome (chromotrope) stain for Microsporidia.

We also used DNA-based methods to detect *E. histolytica*, *E. dispar*, *Cryptosporidium* spp., *Microsporidia* spp. and *Cyclospora*. In brief, we extracted genomic DNA from samples using the EZ1 DNA Tissue Kit (Qiagen, Valencia, CA - USA). DNA extracts were stored at -20°C prior to PCR analysis, which was done as previously described [10-15]. PCR for Microsporidia was performed only in patients who were immunocompromised or immunosuppressed.

Stool specimens were cultured to detect the presence of certain parasites. For isolation of *E. histolytica*, we used a culture medium based on BOECK & DRBOHLAV formulation (DiaSys Entamoeba kit, DiaSys Europe Ltd; Wokingham, UK). Cultures were incubated at 35–37°C for four days, and each day a drop of culture medium was examined under a 40x phase-contrast objective. Cell cultures were used to detect human-infecting

Microsporidia [16]. For detection of *Strongyloides stercoralis* larvae, we streaked stool samples onto nutrient agar plates (1.5% agar, 0.5% meat extract, 1.0% peptone, 0.5% NaCl) and incubated them for at least two days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, creating visible tracks [17]. When requested by clinicians, IgG antibodies to *Strongyloides stercoralis* were searched using a commercial ELISA (Bordier Affinity Products SA, Crissier, Switzerland).

Laboratory quality control

United Kingdom National External Quality Assessment Service (UKNEQAS – Department of Clinical Parasitology – Hospital for Tropical Diseases- London) provides specimens for faecal parasitology quality control since 2003. The quality management system of the laboratory of Microbiology is certified by Det Norske Veritas (DNV Italia S.r.l.) to conform to the quality management standard ISO 9001:2008.

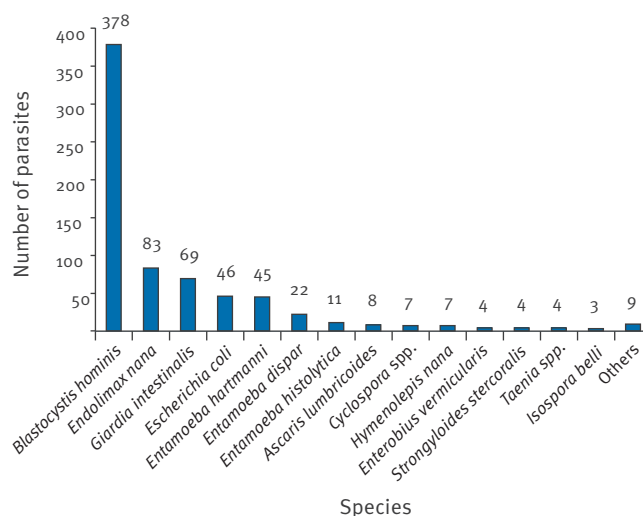
Data analysis

For each case considered, the patient's demographic characteristics were obtained from the hospital and laboratory databases, and these data were used to calculate age-, sex-, and nationality-specific incidence rates for each type of pathogenic parasites.

We also reviewed the results of stool examinations for ova and parasites to determine the incremental value of examining more than one specimen. For categorical variables, results are expressed as absolute numbers or percentages. Statistical significance was determined with the chi-square test. Odds ratios (ORs) and 95%

FIGURE

Intestinal parasite counts detected in stool samples from 594 patients, by species, Rome, Italy, 1 May 2006–31 December 2008 (n=700)



'Others' include *Cryptosporidium* spp. (n=2), *Encephalitozoon intestinalis* (n=2), *Dientamoeba fragilis* (n=1), *Trichuris trichiuria* (n=1), *Trichomonas hominis* (n=1), *Chilomastix mesnili* (n=1), and *Iodamoeba bütschlii* (n=1).

Taenia spp. include *Taenia saginata* and *T. solium*.

confidence intervals (CIs) were calculated to evaluate the strength of the associations that emerged. Two-tailed tests were used to determine statistical significance; a p value of <0.05 was considered significant.

Results

From May 2006 to December 2008, 9,456 faecal samples from 5,351 patients were examined for ova and parasites. Most patients (87.7%, n=4,695) were of Italian nationality; the remaining (12.3%, n=656) were born in Africa, Asia, Central and South-America. Ages ranged from 0 to 99 years and slightly more than half of the patients (54.43%, n=2,913) were female. Inpatients accounted for 60% (n=3,216) of the patients in the study.

Overall, 11.1% (594/5,351) of patients had stools contaminated with parasites, and for all combined patients' samples, a total of 700 intestinal parasites were counted. Ninety patients (90/594) had more than one parasite count in their stools. Enteric parasites were more commonly detected in non-Italian patients than in Italians (26.8% (176/656) vs 8.9% (418/4,695); OR: 3.01; 95% CI: 2.47–3.68, $p<0.001$), and in males than in females (13.0% (263/2,135) vs 9.5% (276/2,913); OR: 1.30; 95% CI: 1.08–1.56, $p=0.003$). Figure summarises the findings from the parasitological survey. Protozoa predominated and were identified in 96.8% (575/594) of the subjects, while helminths were present in 4.7% (28/594).

Table 1 provides specific quantitative data regarding the association between number of stool samples examined and the successful recovery of parasites. The majority of series (60.5% (3,237/5,351)) consisted of only a single faecal specimen. Intestinal parasites were detected in 7.3% (392/5,351) of patients when either a single specimen (when only one was submitted) or the first of a series of multiple specimens was examined. Examination of a second specimen, when submitted, increased the percentage of positivity by another 1.9% of patients (100/5,351). Examination of three faecal samples allowed the detection of parasites in another 1% of patients (53/5,351) who had not had the first or second

specimen test positive for parasites. Examination of more than three specimens, when obtained, uncovered the remaining 0.5% of patients (29/5,351) not diagnosed in the initial specimen of a series.

When only parasites known to cause intestinal disease were considered, an overall prevalence of parasitic infections of 8.8% (469/5,351) was found. The prevalence of pathogenic parasites was lower 7.8% (30/387) in children aged less than five years, increased to 14% among patients aged 5–14 years, and for patients aged more than 14 years the prevalence decreased with age (Table 2).

The distribution of intestinal parasites stratified by nationality and sex is shown in Tables 3 and 4. The prevalence of parasitic infections was about 2.6 times higher among patients in the non-Italian group (19.3% (127/656) vs 7.3% (342/4,695) in the Italian group; OR: 2.66; 95% CI: 2.12–3.32; $p<0.001$) and 1.3 times higher in males than in females (9.9% (241/2,438) vs 7.8% (228/2,913); OR: 1.26; 95% CI: 1.04–1.53; $p=0.02$). Protozoa accounted for the vast majority of the infections, being detected in 97.6% (458/462) of patients harbouring pathogenic parasites. Among those, *Blastocystis hominis* was the most common parasite, detected in 7.1% (378/5,351) of the patients, particularly among non-Italians (14.1% (93/656) vs 6.1% (285/4,695) in Italians; OR: 2.56; 95% CI: 1.97–3.30, $p<0.001$). Although not at significant level, more males also appeared to be infected than females (7.5% (184/2,438) vs 6.6% (194/2,913); OR: 1.14; 95% CI: 0.92–1.42, $p=0.21$). The level of infection by *B. hominis* was lower among children less than five years of age (2.3% (8/387) vs 7.5% (370/4,964); OR: 0.28; 95% CI: 0.13–0.58, $p<0.001$) and equal among the individuals in all the other age groups. *Giardia intestinalis* was the second most common species. The overall prevalence of infected patients was 1.3% (69/5,351). *Giardia intestinalis* was detected more frequently in non-Italians than in Italians (5.0% (33/656) vs 0.8% (36/4,695); OR: 6.86; 95% CI: 4.11–11.39; $p<0.01$), in males than in females (1.6% (38/2,438) vs 0.7% (21/2,913); OR: 2.18; 95% CI: 1.24–3.92; $p<0.001$). Prevalence of giardiasis

TABLE 1

Detection of patients infected by intestinal parasites in function of number of stool samples per patient examined, Rome, Italy, 1 May 2006–31 December 2008

Number of patients	Number of samples examined per patient	Patients testing negative for intestinal parasites n (%)	Patients testing positive for intestinal parasites n (%) in function of number of samples examined						
			1	2	3	4	5	≥6	
3,237	1	3,020 (93.3)	217 (6.7)						
841	2	730 (86.9)	74 (8.8)	37 (4.3)					
949	3	811 (85.5)	69 (7.2)	32 (3.4)	37 (3.9)				
146	4	122 (83.6)	8 (5.48)	9 (6.2)	4 (2.7)	3 (2.0)			
49	5	35 (71.4)	3 (6.1)	8 (16.3)	2 (4.1)	0 (0.0)	1 (2.0)		
129	≥6	58 (45.0)	21 (16.3)	14 (10.8)	10 (7.7)	12 (9.3)	3 (2.3)	11 (19.0)	
5,351	total	4,776 (89.2)	392 (7.3)	100 (1.9)	53 (1.0)	15 (0.3)	4 (0.05)	11 (0.2)	

TABLE 2

Patients infected with intestinal parasite pathogens, by age group, Rome, Italy, 1 May 2006–31 December 2008 (n=469)

Pathogenic parasites	Number of patients positive for intestinal parasite pathogens					
	Patient age in years	<5	5–14	15–44	45–65	>65
	Number of patients	(n=387)	(n=410)	(n=1,865)	(n=1,259)	(n=1,430)
<i>Ascaris lumbricoides</i> ^a	8		1	3	3	1
<i>Blastocystis hominis</i> ^b	378	8	35	142	89	104
<i>Cryptosporidium</i> spp.	2			1		1
<i>Cyclospora cayetanensis</i>	7	1	1		4	1
<i>Dientamoeba fragilis</i>	1			1		
<i>Entamoeba histolytica</i> ^b	11		3	4	3	1
<i>Enterobius vermicularis</i> ^a	4	1	1	1	1	
<i>Giardia intestinalis</i> ^b	69	17	11	23	10	8
<i>Hymenolepis nana</i> ^a	7	2	4		1	
<i>Isospora belli</i>	3	1	1	1		
<i>Microsporidia</i>	2			1	1	
<i>Strongyloides stercoralis</i> ^a	4		1	1		2
<i>Taenia</i> spp. ^a	4			2	2	
<i>Trichuris trichiura</i> ^a	1					1
Total	501	30 (7.7%)	58 (14.1%)	180 (9.6%)	114 (9.0%)	119 (8.3%)
p value for each group distribution				<0.001		

Total is higher than 469, because a patient could be infected with more than one parasite species.

^a Helminth.^b Pathogenic protozoan.**TABLE 3**

Patients having a single intestinal parasite species, by nationality or sex, Rome, Italy, 1 May 2006–31 December 2008 (n=389)

	Total	Number of positive patients that were:			
		Italians (n=4,695)	Non-Italians (n=656)	Female (n=2,913)	Male (n=2,438)
<i>Ascaris lumbricoides</i>	7	4	3	5	2
<i>Blastocystis hominis</i>	305	250	55	163	142
<i>Cryptosporidium</i> spp	1	0	1	1	0
<i>Cyclospora cayetanensis</i>	2	2	0	1	1
<i>Dientamoeba fragilis</i>	1	0	1	0	1
<i>Encephalitozoon intestinalis</i>	2	2	0	0	2
<i>Entamoeba histolytica</i>	6	6	0	3	3
<i>Enterobius vermicularis</i>	3	3	0	2	1
<i>Giardia intestinalis</i>	52	30	22	15	37
<i>Hymenolepis nana</i>	1	1	0	0	1
<i>Isospora belli</i>	2	0	2	1	1
<i>Strongyloides stercoralis</i>	2	2	0	1	1
<i>Taenia</i> spp.	4	3	1	3	1
<i>Trichuris trichiura</i>	1	0	1	1	0
Total	389	303 (6.4%)	86 (13.1%)	196 (6.7%)	193 (7.9%)
p value			<0.001		0.11

Taenia spp. include *Taenia saginata* or *solium*.

was highest in children under five years of age (4.4% (17/387); OR: 6.81; 95% CI: 3.27–14.14; $p < 0.001$) and in people aged 5–14 years (2.7% (11/410); OR: 4.09; 95% CI: 1.73–9.21, $p < 0.001$), and lowest in people aged 45 years or more (0.7% (18/2,689); OR: 1, reference group). *E. histolytica* was the third most common species; it was isolated from 11 patients, all but one, Italians. It was detected more commonly in males. The fourth common species was *Cyclospora cayetanensis*, which was detected in seven patients, five were Italians, and three of these denied travel to endemic areas within

the previous 12 months. *Encephalitozoon intestinalis* and *Cryptosporidium parvum* were detected in faecal samples of four HIV-infected patients. As regard to helminthes, nematodes such as *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Enterobius vermicularis* and *Trichiuris trichiuria* were identified in 17 patients, of whom 10 were Italians, while the plathelminthes *Hymenolepis nana* and *Taenia* species were responsible for 11 infections which occurred in seven Italians with no history of travel, and four non-Italians, three of whom were adopted children from endemic areas.

TABLE 4

Patients with mixed infections, by combinations of pathogenic parasites, by patient nationality or sex, Rome, Italy, 1 May 2006–31 December 2008 (n=80)

Pathogenic parasites	Patients				
	Total	Italians (n=4,695)	Non-Italians (n=656)	Female (n=2,913)	Male (n=2,438)
<i>Blastocystis hominis</i> / <i>Ascaris lumbricoides</i>	1	0	1	1	0
<i>B. hominis</i> / <i>Cryptosporidium parvum</i>	1	1	0	0	1
<i>B. hominis</i> / <i>Cyclospora cayetanensis</i>	4	2	2	2	2
<i>B. hominis</i> / <i>Endolimax nana</i>	23	15	8	10	13
<i>B. hominis</i> / <i>E. nana</i> / <i>Escherichia coli</i>	1	0	1	1	0
<i>B. hominis</i> / <i>E. nana</i> / <i>E. coli</i> / <i>Entamoeba hartmanni</i>	1	0	1	1	0
<i>B. hominis</i> / <i>E. nana</i> / <i>E. hartmanni</i>	2	0	2	0	2
<i>B. hominis</i> / <i>E. nana</i> / <i>Entamoeba histolytica</i>	1	0	1	0	1
<i>B. hominis</i> / <i>E. nana</i> / <i>Giardia intestinalis</i>	2	1	1	2	0
<i>B. hominis</i> / <i>E. nana</i> / <i>Hymenolepis nana</i>	1	1	0	0	1
<i>B. hominis</i> / <i>E. nana</i> / <i>E. coli</i>	1	0	1	1	0
<i>B. hominis</i> / <i>E. coli</i>	11	3	8	5	6
<i>B. hominis</i> / <i>E. coli</i> / <i>G. intestinalis</i>	2	0	2	1	1
<i>B. hominis</i> / <i>Entamoeba dispar</i>	2	2	0	0	2
<i>B. hominis</i> / <i>E. dispar</i> / <i>G. intestinalis</i>	1	1	0	0	1
<i>B. hominis</i> / <i>E. hartmanni</i>	6	3	3	4	2
<i>B. hominis</i> / <i>E. histolytica</i>	2	2	0	0	2
<i>B. hominis</i> / <i>E. histolytica</i> / <i>G. intestinalis</i>	1	1	0	1	0
<i>B. hominis</i> / <i>Enterobius vermicularis</i>	1	1	0	0	1
<i>B. hominis</i> / <i>G. intestinalis</i>	1	0	1	0	1
<i>B. hominis</i> / <i>G. intestinalis</i>	4	2	2	1	3
<i>B. hominis</i> / <i>H. nana</i> / <i>G. intestinalis</i> / <i>Strongyloides stercoralis</i>	1	0	1	0	1
<i>B. hominis</i> / <i>H. nana</i>	1	0	1	1	0
<i>B. hominis</i> / <i>H. nana</i> / <i>G. intestinalis</i>	1	0	1	0	1
<i>B. hominis</i> / <i>S. stercoralis</i>	1	0	1	0	1
<i>C. cayetanensis</i> / <i>E. hartmanni</i>	1	1	0	0	1
<i>E. histolytica</i> / <i>E. nana</i>	1	1	0	0	1
<i>G. intestinalis</i> / <i>E. coli</i>	1	0	1	0	1
<i>G. intestinalis</i> / <i>H. nana</i>	1	1	0	0	1
<i>G. intestinalis</i> / <i>Iodamoeba Butschlii</i>	1	0	1	0	1
<i>G. intestinalis</i> / <i>Isospora belli</i>	1	0	1	1	0
<i>H. nana</i> / <i>Chilomastix mesnili</i> / <i>E. hartmanni</i>	1	1	0	0	1
Total	80	39 (0.8%)	41 (6.2%)	32 (1.1%)	48 (2.0%)
p value		0.31		0.51	

Discussion

An estimated 3.5 billion people—roughly half of the world's population—have intestinal parasitoses, which cause almost 450 million deaths every year. The problem is particularly serious in endemic areas with poor sanitation, but parasitic infections are also becoming more common in non-endemic areas as a result of intensified immigration and travel [1].

Very little is known about the prevalence of intestinal protozoa and helminthes in Italy [9]. The present study shows that, over a 30-month period, 8.8% of the patients referred to a large teaching hospital in Rome for ova and parasite analysis of stool samples harbour at least one species of pathogenic parasite.

The infection rate was lowest in children under five years of age and peaked in the 5–14 year-old group, declining progressively thereafter. The frequency of parasitic infections was found to be associated with patient, sex and nationality. The prevalence was substantially higher (almost 20% (127/656)) among the non-Italian patients of our study, and even higher rates have been reported in the immigrant population living in southern Italy (61.9%) [18]. On the whole, intestinal parasites were more common in males, although certain species, such as *A. lumbricoides* and *Taenia* spp., were detected more often in females.

Overall, *Blastocystis hominis* was the most common parasite encountered in this study. It was detected in 7.1% (378/5,351) of the patients we examined (14.1% (93/656) of the non-Italians and 7.5% (184/2,438) of the males). *Blastocystis* is an enteric protozoan that parasitises humans and many animals. It has a worldwide distribution and is often the most commonly isolated organism in parasitological surveys [8–19]. Accumulating evidence (epidemiological, in vivo, and in vitro) strongly suggests that *Blastocystis* is a pathogen [20]. Many genotypes exist in nature, and recent observations indicate numerous zoonotic genotypes also infect humans. Tan et al. have suggested that conflicting observations on the pathogenesis of *Blastocystis* stem from genetic diversity within the species, which includes both pathogenic and non-pathogenic genotypes [19]. The controversial pathogenicity of *Blastocystis* has been attributed to subtype variations in virulence, and while more recent studies seem to support this view, other factors also appear to contribute to the clinical outcome of the infection [19].

The second most common species in our study population was *Giardia intestinalis*. This common, ubiquitous flagellated protozoan has a significant impact on public health. Giardiasis is characterised by high prevalence, and it frequently causes major outbreaks and substantial effects on the growth and cognitive functions of infected children [21]. *Giardia* generally causes a self-limited illness characterised by diarrhoea, abdominal cramps, bloating, weight loss, and malabsorption. However, asymptomatic infections

are frequent, especially in developing countries [22]. Infection rates are generally lower in industrialised countries, and figures ranging from 0.4% to 6.2% have been reported in Italy [23]. The overall prevalence observed in the present study was 1.3% (69/5351), but the rate in children less than 15 years of age was three times higher (4.8% (38/797)). Rates were also higher in the non-Italian subgroup (5% (33/656) vs 0.8% (36/4,695) in Italians), and 78.6% (22/28) of infected patients aged less than 15 years were non-Italians. High infection rates have been reported for certain relatively poor regions in developed countries, and Giangaspero et al. reported a rate of 42.9% in a small, socially deprived Roma community in Italy [24]. A lower prevalence (4.5%) was found, however, in immigrants living in southern Italy [18].

The third most common species, although only detected in 11 patients, was *Entamoeba histolytica*. *Entamoeba* spp. can cause harmless colonisation of the intestine or severe infections characterised by invasion of the colon wall and damage to other host tissues, such as the liver, lung, and brain (amoebiasis). In most cases, a clinical diagnosis of amoebiasis can be confirmed microbiologically, generally by light microscopic visualisation of parasites in a wet smear or stained specimens. This procedure is simple and inexpensive, but it has several limitations. The most important is that it cannot distinguish between the cysts and trophozoites of *E. histolytica* (the disease-causing species), *E. dispar* (which is non-pathogenic), and *E. moshkovskii*, the amphizoic amoeba that occasionally infects humans. In addition, multiple samples often have to be examined, and the diagnosis can be even more difficult when there are cysts of different species of *Entamoeba*, *Iodamoeba*, or *Endolimax* [25]. Differentiating between these three species has become increasingly important (for diagnosis and for epidemiological studies) since sporadic cases of human infection with *E. moshkovskii* were reported [26]. Recent findings indicate that this species is highly prevalent in young children in Bangladesh, where it is often associated with *E. histolytica* and *E. dispar* [27]. In our study, *E. histolytica* and/or *E. dispar* were detected by microscopy in 33 patients. However, when these cases were analysed with molecular tools, the presence of *E. histolytica* was confirmed in only 11 patients (10 of whom were Italians): the other 22 (18 Italians) were infected with *E. dispar*, *Isospora belli*, *E. intestinalis*, and *Cryptosporidium parvum* were detected only in immunocompromised patients, all but one of who were HIV-infected. Helminthes were found in only 0.5% (27/5,351) of our patients.

Our findings confirm that certain intestinal parasites, such as *E. histolytica*, *C. cayetanensis*, *S. stercoralis*, and *H. nana*, are not restricted to endemic areas in the tropics. However, our study has certain limitations that must be acknowledged. Our analysis was retrospective, and it was performed at a single hospital, so the results are not necessarily applicable to other settings. However, this shortcoming is to some extent

outweighed by the large size of the sample population and the fairly large variety of the parasites identified. In addition, the relationship between parasitic infection rates and nationality has been consistently demonstrated in other studies [18-24].

In conclusion, knowledge of the organisms responsible for intestinal parasite infections in non-endemic areas is an essential step toward effective patient care and the implementation of appropriate control measures. Our findings demonstrate that intestinal parasitoses must be considered in the differential diagnosis of gastrointestinal diseases even in industrialised areas. Examination of multiple stool specimens per patient is still the recommended approach for excluding parasitic infection [28]. We agree that multiple stool analyses for ova and parasites can be clinically useful in populations with increased risk of infection, but it also is important to identify populations at low risk for infection, such as that in our study. Stool examinations are costly and labour-intensive, and routine requests should be avoided unless they are supported by epidemiological (e.g. international travel, unsafe drinking water) and clinical features (e.g. diarrhoea for more than seven days, especially if the patient is immunocompromised). In this context, close collaboration between physicians and clinical microbiologists should produce significant positive effects.

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