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ORIGINAL PAPER

Prevalence and associated factors of intestinal parasitisation: a cross-sectional study among outpatients with gastrointestinal symptoms in Catalonia, Spain

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Abstract The aim of this study was to report the prevalence of intestinal parasites in stool specimens from outpatients in Catalonia (Spain), and to evaluate the association of age, seasonality, and gender on general parasitisation and by the most frequent detected species. A total of 13,913 samples from 8,313 patients (1–3 specimens per patient) reporting digestive disorders were examined between 1999 and 2005 as a part of medical examinations. Samples were fixed with MIF solution and microscopically examined as wet mounts. Permanent stain was obtained by the modified Ziehl–Neelsen technique for intestinal coccidia. Nineteen species of intestinal parasites were identified. *Blastocystis hominis* (585 patients) was the predominant species, followed by *Giardia duodenalis* (321), *Dientamoeba fragilis* (131), *Entamoeba coli* (60) and *Cryptosporidium* sp. (59). Prevalence of helminths was low, being *Enterobius vermicularis* as the most frequently reported helminth (49 patients). The overall parasitisation was 1,136/8,313 (13.7%); prevalence in adults was 19.8% with a maximum in spring (14.8%). In the adjusted models, age was the main factor associated with infection: adults, with *B. hominis* and *Entamoeba coli* (odds ratio (OR)=6.0 and OR=8.5, respectively) and children, with *Cryptosporidium* and *Giardia* (OR=2.0 and OR=3.3, respectively). However, seasonality cannot be considered related with infection. The total prevalence was low, taking into account that all the subjects examined presented gastrointestinal symptoms and that species traditionally considered as non-pathogenic were included in the study.

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Introduction

Intestinal infection by protozoa and helminths constitutes one of the most frequent causes of gastrointestinal diseases worldwide. Parasitological studies of large patient populations are scarce in developed countries. In Spain, most of them have been done in public hospitals on specific communities, such as immigrants (López-Vélez et al. 2003; Roca et al. 2002), scholars (Belda et al. 2008), and HIV-positive patients (Moles et al. 1998).

In 1978, the Spanish Constitution established the right of all citizens to health protection and care. The National Spanish Health System is coordinated by the Central Government and the public health services of the autonomous governments. In addition, there is an extensive offer of private health care.

The aim of this study was to describe the prevalence of intestinal parasites in stool specimens from outpatients living in Catalonia (Spain)—mainly Spanish or European citizens—reporting digestive disorders and attending private medical services. In addition, we evaluated the association of age, seasonality, and gender on general parasitisation and by the most frequently detected species. The present work updates data about human infections caused by intestinal protozoa and helminths among all age groups of the general population in Spain.

Material and methods

Subjects This was a retrospective cross-sectional study. A total of 13,913 stool samples from 8,313 patients (1, 2 or 3 specimens per patient) were collected and examined in a clinical parasitological laboratory in Barcelona over 6 years (1999 to 2005). Samples were collected as a part of medical examinations of patients reporting digestive disorders as flatulence, diarrhoea, abdominal cramp, bloating, malabsorption, bloody or odorous stools, irritable bowel or extra-intestinal symptoms. All the patients lived in Catalonia, northeast Spain.

Collection and examination of stool samples Each patient was provided with three clean plastic containers and 5 ml of MIF solution (merthiolate–iodine–formaline) to fix the samples. Specimens were stored at room temperature until analysis. Direct wet smears were taken using 20 μl from the upper sediment. Permanent stained smears were performed by the modified Ziehl–Neelsen technique (phenicated fuchsin-hydrochloric acid 0.5% methylene blue) for intestinal coccidia. For helminth detection, a biphasic formalin–ether concentration was made

Data collection The species detected in each sample were reported in the laboratory database. Patients were considered positive when at least one stool sample was parasitised by at least one protozoan or helminthic species. Patient-related data (date of birth, age, sex, habitual address, and date of sample reception) were collected using a computerised protocol in accordance with Spanish legislation on data protection.

Patients were grouped in three age categories: children (<12 years old), teenagers and young adults (13–25 years old) and adults (>25 years old). The mean age of population sample was 19.41 (SD=23.05); 60.0% of the outpatients were children; 5.2% were teenagers and young adults, and 34.5% were adults. Most of the patients lived in the city of Barcelona, while the rest were distributed around Catalonia. Males and females accounted for 41.3% and 47.4% of the patients, respectively. During the study period, 1,100–1,500 samples were analysed every year, approximately 300 per month. Months of sample reception were grouped by season (winter, spring, summer and autumn). Autumn and winter were the coolest seasons, whereas summer was the hottest. The wettest periods were normally during autumn and spring.

Statistical analysis Statistical analysis was performed using SPSS 15.0 statistical software. A descriptive analysis was conducted to establish the distribution of parasitisation and the species detected by gender, age group and season. Associations between qualitative variables were assessed using the chi-square test. A multivariate analysis by logistic regression was performed to determine the effect of these factors on the odds of presenting both general parasitic infection and most common species. All p values <0.05 were considered significant.

Results

Descriptive analysis

The overall prevalence of protozoan and helminth infections was 13.7% (1,136/8,313 patients). Nineteen species of intestinal parasites were detected, 13 of which were considered pathogenic species (*Giardia duodenalis*, *Cryptosporidium* sp., *Dientamoeba fragilis*, *Entamoeba histolytica/Entamoeba dispar*, *Cyclospora cayetanensis*, *Fasciola hepatica*, *Schistosoma mansoni*, *Trichuris trichiura*, *Enterobius vermicularis*, *Hymenolepis nana*, *Ascaris lumbricoides* and *Ancylostoma duodenale*), five non-pathogenic protozoa (*Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii, Entamoeba hartmanni* and *Chilomastix mesnilii*), and the *incerta sedis Blastocystis hominis*, in which its pathogenic potential is under debate. The prevalence of parasitisation was 10.7% in children, 15.7% in teenagers and young adults, and 19.8% in adults (p<0.001). Males and females recorded parasitisation of 14.0% and 14.1%, respectively (p=0.892). Infections were more prevalent in spring, while the lowest prevalence was registered in winter (p=0.211; Table 1).

The prevalence of parasitisation by protozoan species was 13.0%, including *B. hominis*, and 0.8% by helminth species. *B. hominis* was the most frequently detected parasite in single and multiple infections, followed by *G. duodenalis* and *D. fragilis* (Table 2). Single infections accounted for 90.4% of the cases (1,027 subjects) and multiple infections 9.6% (109 subjects; 98 patients presented two species, ten patients with three species and one patient showed four species). The most common combinations were *B. hominis* and *D. fragilis* (29 patients; p<0.001), and *B. hominis* and *E. coli* (27 patients; p<0.001). *E. vermicularis* was the most common helminth infection in outpatients (0.6%); of these, 17 patients were children and 19 were adults.

In terms of seasonality, *B. hominis* infections presented the highest peak (30.8%; p<0.001) during spring. In contrast, *G. duodenalis* and *Cryptosporidium* sp. were more frequently detected in autumn (38.0%; p<0.001 and 44.1%; p=0.033, respectively) and *E. coli* was especially frequent in winter (33.3%; p=0.045; Tables 3 and 4).

Multivariate analysis

In the multivariate analysis, performed by logistic regression and adjusted for age groups, gender and season

Table 1 Intestinal parasitisation and distribution by sex, age groups and season among 8,313 outpatients inhabiting Catalonia

	Patients			Univariate analysis	Multivariate analysis
Factor Total	Negative (%) 7,177 (86.3)	Positive (%) 1136 (13.7)	Sig.[a]	OR (95% CI)	OR[b] (95% CI)
Sex					
Male	2,956 (86.0)	481 (14.0)			
Female	3,386 (85.9)	556 (14.1)	0.892	1.01 (0.89–1.15)	0.91 (0.79–1.05)
Age groups					
<12 years old	3756 (89.3)	449 (10.7)		Ref.	Ref.
13–25 years old	306 (84.3)	57 (15.7)		1.56 (1.16–2.10)	1.56 (1.15–2.12)
>25 years old	1927 (80.2)	477(19.8)	<0.001	2.07 (1.80–2.38)	2.06 (1.78–2.39)
Season					
Winter	1767 (87.5)	252 (12.5)		Ref.	Ref.
Spring	1652 (85.2)	286 (14.8)		1.21 (1.01–1.46)	1.17 (0.95–1.44)
Summer	1659 (86.5)	259 (13.5)		1.10 (0.91–1.32)	1.06 (0.86–1.31)
Autumn	2099 (86.1)	339 (13.9)	0.211	1.13 (0.59–1.35)	1.23 (1.01–1.50)

Univariate and multivariate analyses

[a] Chi-square test

[b] Odds ratio for occurrence of intestinal parasitisation, adjusted for sex, age group and season

Table 2 Intestinal parasite species and prevalences detected

	Outpatients		
	Infected patients	% Overall outpatients[a] (n=8,313)	% Outpatients with positive samples[b] (n=1,136)
Protozoa			
Blastocystis hominis	585	7.0	51.5
Giardia duodenalis	321	3.9	28.3
Dientamoeba fragilis	131	1.6	11.5
Entamoeba coli	60	0.7	8.2
Cryptosporidium sp.	59	0.7	5.2
Endolimax nana	12	0.1	1.1
Entamoeba histolytica/dispar	9	0.1	0.8
Iodamoeba bütschlii	4	<0.1	0.4
Entamoeba hartmanni	2	<0.1	0.2
Cyclospora cayetanensis	1	<0.1	0.1
Chilomastix mesnilii	1	<0.1	0.1
Helminth			
Enterobius vermicularis	49	0.6	4.3
Taenia saginata	7	0.1	0.6
Trichuris trichiura	3	<0.1	0.3
Fasciola hepatica	1	<0.1	0.1
Hymenolepis nana	1	<0.1	0.1
Ascaris lumbricoides	1	<0.1	0.1
Ancylostoma duodenale	1	<0.1	0.1
Schistosoma mansoni	1	<0.1	0.1

[a] Occurrence of each species among the overall number of outpatients

[b] Occurrence of each species among outpatients with positive samples

Table 3 Associated factors with *Blastocystis hominis*, *Entamoeba coli*, and *Dientamoeba fragilis* infections

	Blastocystis hominis (*n*=585)					*Entamoeba coli* (*n*=60)					*Dientamoeba fragilis* (*n*=131)				
	n	(%)	Sig.[a]	OR[b] (95%CI)	Sig.	*n*	(%)	Sig.[c]	OR[d](95%CI)	Sig.	*n*	(%)	Sig.[e]	OR[f](95%CI)	Sig.
Sex															
Male	254	46.1		Ref.		29	52.7		Ref.		52	41.9		Ref.	
Female	297	53.9	0.814	0.79 (0.65–0.96)	0.015	26	47.3	0.359	0.53 (0.30–0.95)	0.032	72	58.1	0.296	1.12 (0.77–1.62)	0.562
Age groups															
< 12 years old	116	22.7		Ref.		8	16.3		Ref.		72	57.6		Ref.	
13–25 years old	43	8.4		4.63 (3.17–6.76)		4	8.2		6.06 (1.76–20.88)		2	1.6		0.30 (0.07–1.22)	
>25 years old	352	68.9	<0.001	6.01 (4.77–7.56)	<0.001	37	75.5	<0.001	8.51 (3.76–19.26)	<0.001	51	40.8	0.090	1.12 (0.77–1.64)	0.176
Season															
Winter	135	23.1		Ref.		20	33.3		Ref.		31	23.7		Ref.	
Spring	180	30.8		1.25 (0.96–1.62)		17	28.3		0.79 (0.40–1.58)		33	25.2		1.25 (0.74–1.12)	
Summer	118	20.2		0.80 (0.60–1.07)		15	25.0		0.47 (0.21–1.06)		30	22.9		1.15 (0.67–1.97)	
Autumn	152	25.9	<0.001	1.11 (0.85–1.45)	0.014	8	13.3	0.045	0.30 (0.12–0.77)	0.046	37	28.2	0.964	1.01 (0.64–1.81)	0.861

[a] Presence and absence of *Blastocystis hominis* is compared by chi-square test

[b] Odds ratio for occurrence of parasitisation by *Blastocystis hominis*, adjusted for sex, age group and season

[c] Presence and absence of *Entamoeba coli* is compared by chi-square test

[d] Odds ratio for occurrence of parasitisation by *Entamoeba coli*, adjusted for sex, age group and season

[e] Presence and absence of *Dientamoeba fragilis* is compared by chi-square test

[f] Odds ratio for occurrence of parasitisation by *Dientamoeba fragilis*, adjusted for sex, age group and season

Table 4 Associated factors with *Giardia duodenalis* and *Cryptosporidium* sp. infections

	Giardia duodenalis (*n*=321)					*Cryptosporidium* sp. (*n*=59)				
	n	(%)	Sig.[a]	OR[b] (95%CI)	Sig.	*n*	(%)	Sig.[c]	OR[d] (95%CI)	Sig.
Sex										
Male	142	51.8		Ref.		30	52.6		Ref.	
Female	132	48.2	0.076	0.97 (0.74–1.26)	0.805	27	47.4	0.358	0.88 (0.50–1.55)	0.661
Age groups										
< 12 years old	206	76.0		1.98 (1.46–2.70)		44	84.6		3.28 (1.53–7.07)	
13–25 years old	7	2.6		0.82 (0.37–1.82)		0	0			
>25 years old	58	21.4	<0.001	Ref.	<0.001	8	15.4	0.001	Ref.	0.010
Season										
Winter	70	21.8		Ref.		14	23.7		Ref.	
Spring	54	16.8		0.89 (0.59–1.36)		13	22.3		0.82 (0.34–1.95)	
Summer	75	23.4		1.27 (0.86–1.87)		6	10.2		0.58 (0.22–1.54)	
Autumn	122	38.0	0.001	1.39 (0.98–1.99)	0.073	26	44.1	0.033	1.52 (0.75–3.08)	0.126

[a] Presence and absence of *Giardia duodenalis* is compared by chi-square test

[b] Odds ratio for occurrence of parasitisation by *Giardia duodenalis*, adjusted for sex, age group and season

[c] Presence and absence of *Cryptosporidium* sp. is compared by chi-square test

[d] Odds ratio for occurrence of parasitisation by *Cryptosporidium* sp., adjusted for sex, age group and season

(Table 1), age was the only predictor factor associated with parasitisation for any species. The highest association effect was observed in adults (odds ratio (OR)=2.06; 95% confidence interval (CI)=1.78–2.39). Gender and seasonality had no effect on intestinal parasite infection in adjusted or non-adjusted models.

The logistic regression analysis was reproduced for the most frequent species (Tables 3 and 4). For *B. hominis* and *E. coli* infections, adults showed an OR=6.01 (95%CI=4.77–7.56) and an OR=8.51 (95%CI=3.76–19.26), respectively, when the children category was taken as a reference group. Moreover, gender was statistically associated with these two parasite species, females showing an OR=0.79 (95%CI=0.65–0.96) and an OR=0.53 (95%CI=0.30–0.95), respectively. Regarding *G. duodenalis* and *Cryptosporidium* sp., age was also statistically associated with infection by these species. When adults category was taken as a reference group, children presented an OR=1.98 (95% CI=1.46–2.70) and an OR=3.28 (95%CI=1.53–7.07) for *G. duodenalis* and *Cryptosporidium* sp., respectively. No factors were associated with *D. fragilis* infection.

Discussion

Parasitological research is rarely conducted on large patient cohorts in developed countries. The prevalence of intestinal parasitisation found in the present study (13.7%) contrasts strongly with that reported in the general population of the USA (32%) (Amin 2002), Turkey (37.2%) (Celiksöz et al. 2005), and Albania (66.6%) (Spinelli et al. 2006), and among children in Turkey (31.0%; Ostan et al. 2007). To date, our study is the first to examine the prevalence of intestinal parasites in a large cohort of outpatients (8,313) in Spain. However, several studies analysing intestinal parasitisation among children and immigrant communities reported a higher prevalence than the present one, such as those found in Córdoba (27.1%; Pérez Armengol et al. 1997), Valencia (26.7%; Belda et al. 2008) and Madrid (22.9%; López-Vélez et al. 2003).

Among the 19 species reported, the most frequent were *B. hominis*, *G. duodenalis*, *D. fragilis*, *E. coli* and *Cryptosporidium* sp. These findings are consistent with those reported by Amin (2002) in the USA and Östan (2007) in Turkey. Several authors are beginning to show awareness of the pathogenic potential of organisms such as *B. hominis, E. hartmanni* and *E. coli* (Stark et al. 2007; Garcia 2001; Wahlgren 1991). So, taking into account the high prevalence detected in the present study, their sanitary role should be reconsidered in further studies.

In the present study, few samples were positive for helminths (0.8%), the pinworm *E. vermicularis* being the most frequently reported helminth among paediatric samples. However, their prevalence could be underestimated because it was not systematically detected by means of the Graham Test. Other parasitological studies carried out in different countries as Albania, Thailand and Republic of Korea showed a higher prevalence of helminths (Spinelli et al. 2006; Kim et al. 2009; Kitvatanachai et al. 2008). In our sanitary context, several authors have described in the

recent years an increase of imported helminthiasis due the immigration and international travels (Pardo et al. 2007). Although the prevalence of helminthiasis is high in immigrants, the risk of transmission to the Spanish population is low, a situation that is reflected in the present study.

Our results indicate that age was the only factor associated with parasitisation, both in bivariate and multivariate analysis, adults being the category showing most parasitisation. These results are consistent with those reported by Spinelli et al. (2006) on healthy subjects in Albania and by Mahfouz et al. (1997) in Egypt who detected an almost threefold increased risk of parasitisation in subjects over 2 years old. Regarding seasonality, the lowest prevalence was recorded in winter and the highest in spring. This observation was also made by Amin (2002) in the USA.

Regarding *B. hominis* infection, adults showed a higher association of infection by this parasite than children, reaching the highest peaks during spring. However, in the USA, Amin (2002) detected the highest prevalence between August and October. *E. coli* showed a similar association pattern with age as *B. hominis*, but a major transmission during the cold season. In the present study, *D. fragilis* infection was homogenously distributed considering gender, age groups and season; this finding is consistent with a previous study carried out in Australia (Stark et al. 2005). In contrast, Schuster and Jackson (2009) reported higher prevalence (around 15%) among patients with chronic gastrointestinal disorders consulting complementary medicine practitioners in the British Isles.

We assessed associations between age and seasonality and the presence of two potential waterborne parasites, *G. duodenalis* and *Cryptosporidium* sp. These organisms presented a number of similar and contrasting features. A contrasting feature between these parasites was that the prevalence of *Giardia* infection was almost five times higher than *Cryptosporidium* sp. infection, as has been previously reported by some authors (Cohen et al. 2008; Laupland and Church 2005). There were two main common features among these parasites. First, they were both more frequent in children than in adults, age being the only independently associated factor in the adjusted models for both infections. These findings are consistent with results found in prior studies (Laupland and Church 2005; Makri et al. 2004; Pollock et al. 2005). Second, gender was not an independent factor associated with parasitisation by *G. duodenalis* and *Cryptosporidium* sp. This finding contrasts with that reported by Laupland and Church (2005) in Canada. Some authors have suggested that the incidence of *Giardia* and *Cryptosporidium* varies spatially and temporally (Cohen et al. 2008). In the present study, the highest peaks of G. *duodenalis* and C*ryptosporidium* sp. infections were observed during autumn (between September to December), coinciding with wet weather and moderate temperatures, in contrast to findings by Moles et al. (1998) in Zaragoza (Spain) who detected a peak of parasitisation by *Cryptosporidium* sp. in winter. However, the effect of seasonality was not an independent factor for these infections when the logistic models were adjusted for other factors. Therefore, seasonality cannot be considered related with *Cryptosporidium* and *Giardia* parasitisation in the sanitary context in Catalonia.

The present study is novel in Spain. It provides data on the occurrence of intestinal parasites and describes the influence of several demographic and seasonal factors of acquiring parasitological infections. However, this study has some limitations. The first is that the population studied comprised outpatients, mainly Spanish and European citizens, attending a private medical service. Attendance of such services might contribute to a selection bias, which should be taken into account when extrapolating these findings to the general Spanish population. Second, this was a laboratory-based study and thus, detailed information on patient clinical variables, treatments and outcomes was unknown. Finally, differences in the seasonality of intestinal parasite prevalence between geographical locations may depend on a variety of factors and therefore comparisons with other studies should be interpreted with caution.

In conclusion, we present results on intestinal parasitisation among 8,313 outpatients in Spain attended between 1999 and 2005. *G. duodenalis* and *Crypstosporidium* sp. were more prevalent in children, while *E. coli* and *B. hominis* were most common in adults. Seasonality was not independently associated with parasitisation in our sanitary context. The total prevalence was low given that all the subjects examined presented gastrointestinal symptomatology and that the study included species traditionally considered as non-pathogenic. This finding reinforces the need to re-examine the status of these parasites, in which its pathogenic potential is under debate.

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