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Prevalence of intestinal parasitoses detected in Padua teaching hospital, Italy, March 2011 - February 2013

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SUMMARY

The aim of this study was to evaluate the distribution of parasitic intestinal infections in patients attending Padua teaching hospital during a two-year period. Between 1st March 2011 and 28th February 2013, we examined stool specimens from 7341 patients (6127 Italians, 1214 non-Italians) for ova and parasites using microscopy, rapid enzyme immunoassays, culture techniques and molecular methods. Stools of 1080 patients (14.71%) were positive for parasites; a total of 1349 intestinal parasites were counted. Protozoa were detected in 1028/1080 patients (95.19%), while helminths were present in 80/1080 patients (7.41%). The protozoa most commonly detected were *Blastocystis* spp., *Dientamoeba fragilis* and *Giardia duodenalis*. *Enterobius vermicularis* was the helminth most frequently encountered. Of the 1080 infected patients, 227 (21.02%) had more than one parasite in their stool. To conclude, in Italy intestinal parasitoses must be unquestionably considered in differential diagnoses of gastrointestinal diseases. For this purpose, sound knowledge of epidemiology is essential.

Keywords: *Dientamoeba fragilis*, epidemiology, helminths, intestinal parasitoses, Italy.

INTRODUCTION

Diarrhoeal disease remains one of the major causes of morbidity and mortality worldwide, despite on-going improvement in our basic understanding of its epidemiology, pathogenesis and treatment [1]. Enteric parasites are the most frequent cause of parasitic illness [2].
Parasitic infections, caused by helminths and protozoan parasites, are among the most common infections in humans in developing countries. In developed countries, such as Italy, protozoan parasites more frequently cause gastrointestinal infections compared to helminths [3, 4]. Enteric parasites are mostly transmitted by the faecal-oral route due to ingestion of water, food and vegetables, soil contaminated with ova, cysts or oocysts. In some cases, transmission occurs via the skin through direct penetration by larvae living in the soil [5].
Intestinal parasites are now being diagnosed with increased frequency in Europe and other industrialised countries [3, 4, 6, 7]. The prevalence of these infections in non-endemic areas is still underestimated. This fact is due to the characteristics of the infection such as mild or non-specific symptoms, long period of incubation and, in other cases, to inadequacy of laboratory methods. Furthermore, in non-endemic areas, physicians have often a limited knowledge about those diseases and test for parasitoses are frequently requested when the chance of infection is low. In industrialised countries, intestinal parasitoses are usually not notified and consequently few epidemiological data on the distribution are available [3]. The increase of the frequency of parasitoses in developed countries can be attributed to various factors such as globalisation of the food supplies, increased consumption of fresh food, increased travels to the

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developing countries, more intensive immigration from the latter areas and the phenomenon of international child adoption. Contact with other cultures has led to increased consumption of raw or undercooked food as potential source of parasites [3, 4, 7]. Conversely the rapid transport of fresh fruits and products from developing countries enhanced contact with intestinal parasites [4].
The aim of this study was to assess the distribution of parasitic intestinal infections in our area over the period 1st March 2011-28th February 2013.

PATIENTS AND METHODS

Patient population and study design
This retrospective study was performed at Padua teaching hospital, an academic medical centre with approximately 60000 admissions per year [8]. We systematically searched the computerised and hardcopy archive of the Microbiology and Virology Laboratory to identify all stool specimens submitted for parasitology investigation from 1st March 2011 through 28th February 2013.

Laboratory methods
All commercial products illustrated below were used in accordance with manufacturers' instructions.
Samples for routine parasitological research were collected and transported in stool transport vials containing sodium acetate formalin (Para-Pak® ULTRA SAF) as liquid fixative. Our operating protocol provides the direct distribution to the patients of the stool transport vials. This precaution allows the fixation of the material immediately after the evacuation. The patients were also provided with a record card for medical history and symptoms collection.
Each patient was required to collect three stool samples to be carried out on alternate days, in so far for diagnostic purposes a single sample is not sufficient due the fact that the shedding of parasites is not continuous. Data suggest that a single stool specimen submitted for microscopic examination will detect from 58 to 72% of protozoa present. Optimal recovery and microscopic identification of protozoa from patients with intestinal infections is dependent on proper collection and preservation of faecal specimen [9].
Stool samples were subjected to macroscopic examination for the presence of mucus, blood or worms.
Ova and parasite test (O&P) contemplates fixed samples enrichment by gravity filtration and direct wet examination by microscopy (100x and 400x) to demonstrate the presence of worm eggs, larvae, protozoan trophozoites or cysts. Even under ideal conditions with prompt preservation of stool this test needs to be performed by a skilled technologist.
Simultaneously, for each patient, a pool obtained from all the three samples was tested with a rapid enzyme immunoassay (ImmunoSTAT!® Crypto/Giardia) for the qualitative detection of *Cryptosporidium parvum* and *Giardia lamblia* (syn *Giardia intestinalis* and *Giardia duodenalis)* antigens.
In doubtful cases and/or for further investigations we also performed:
– permanent specific stains, from unpreserved stool (Giemsa staining, modified Ziehl-Neelsen acid fast staining and modified trichrome staining);
– PCR research, from unpreserved stool, for *Entamoeba histolytica/dispar* and *Dientamoeba fragilis* [10].

In selected cases or when requested by clinicians we carried out:
– agar plate culture for the recovery of *Strongyloides stercoralis* larvae;
– scotch tape test for the detection of pinworm ova.

Laboratory quality control
United Kingdom National External Quality Assessment Service (UKNEQAS - Department of Clinical Parasitology - Hospital for Tropical Diseases - London) provided specimens for faecal parasitology external quality assessment.

Data analysis
We obtained, for each case considered, the patient's demographic characteristics from the record cards and the laboratory database, and we used this data to evaluate age, sex and nationality-specific incidence rates for each type of pathogenic parasites.
Statistical significance was calculated with the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined to evaluate the strength of the associations that emerged.

Two-tailed tests were used to calculate statistical significance; a p-value of <0.05 was considered significant.

RESULTS

From March 2011 to February 2013, faecal samples from 7082 patients were submitted to our service for O&P and for the detection of *C. parvum* and *G. lamblia* antigens. Among them, 240 patients were not evaluated because of unsuitable samples: excessive or poor material, inappropriate containers, lack of SAF. So, we analysed 20526 samples from 6842 patients. Among these patients, 97/6842 also performed scotch tape tests in addition to O&P.
These unselected samples came to our attention for several reasons: gastro-intestinal symptoms and extra-intestinal disorders, such as hypereosinophilia, itching, allergies of unknown origin. A considerable number of samples were analysed as an unspecified screening.
In addition to O&P, samples from 499 further patients were evaluated performing the following tests led by medical history and clinical signs:
– 412 scotch tape tests;
– 31 agar culture for *S. stercoralis*;
– 56 specific detection test for *Cryptosporidium* spp. (rapid enzyme immunoassay and permanent stain).

Overall we evaluated specimens from 7341 patients. Most patients (83.46%, n=6127) were of Italian nationality; the remaining (16.54%, n=1214) were born in Europe, Africa, Asia and Americas. Ages ranged from 0 to 101 years; 58.19% (n=4272) patients were female and 41.81% (n=3069) were male. Outpatient accounted for 82.85% (n=6082) of the patient in the study (Table 1).
One thousand eighty of 7341 (14.71%) patients had stools contaminated with parasites, and for all combined patients' samples, a total of 1349 intestinal parasites (1268 protozoa and 81 helminths) were counted.
Protozoa accounted for most infections, being detected in 1028/1080 patients (95.19%), while helminths were present in 80/1080 patients (7.41%).
The prevalence of parasitic diseases was about 1.4 times higher among patients in the non-Ital-

Table 1 - Summary of patients present in the study. O&P+Ag: Ova and parasites test + rapid enzyme immunoassay for detection of *C. parvum* and *G. lamblia* antigens, STT: scotch tape test, ACS: agar culture for *S. stercoralis*, Cry: specific test for *Cryptosporidium sp.*

	Total patients	*Patients not evaluable*	*Patients evaluated*	*Non-Italians*[a]	*Italians*[a]	*Male*[a]	*Female*[a]	*Inpatients*[a]	*Outpatients*[a]
O&P+Ag	7082	240	6842[b]	1116 (16.31%)	5726 (83.69%)	2833 (41.41%)	4009 (58.59%)	1182 (17.28%)	5660 (82.72%)
STT	476	64	412	74 (17.96%)	338 (82.04%)	194 (47.09%)	218 (52.91%)	48 (11.65%)	364 (88.35%)
ACS	47	16	31	6 (19.35%)	25 (80.65%)	15 (48.39%)	16 (51.61%)	10 (32.26%)	21 (67.74%)
Cry	59	3	56	18 (32.14%)	38 (67.86%)	27 (48.21%)	29 (51.79%)	19 (33.93%)	37 (66.07%)
Total	7664	323	7341	1214 (16.54%)	6127 (83.46%)	3069 (41.81%)	4272 (58.19%)	1259 (17.15%)	6082 (82.85%)

[a]: in brackets the percent proportions of each group of patients calculated on the total of the patients evaluated.
[b]: 97/6842 also performed scotch tape tests in addition to O&P.

Table 2 - Origin, age and sex of the patients included in the study.

		Total patients N = 7341	*Patients with intestinal parasitoses*[a] *N = 1080*	*% of patients with intestinal parasitoses on the respective group*	*OR (95% CI)*	*p*
Origin	Non-Italians	1214 (16.54%)	241 (22.31%)	19.85%	1.56 (1.33-1.83)	< 0.001
	Italians	6127 (83.46%)	839 (77.69%)	13.69%		
Age	≤14 years	1827 (24.89%)	276 (25.56%)	15.11%	1.04 (0.90-1.21)	0.584
	>14 years	5514 (75.11%)	804 (74.44%)	14.58%		
Sex	Male	3069 (41.81%)	479 (44.35%)	15.61%	1.13 (0.99-1.29)	0.066
	Female	4272 (58.19%)	601 (55.65%)	14.07%		

[a]: in brackets the percent proportions of each group of patients calculated on the total of the patients with parasitoses are indicated

ian group (non-Italians 19.85% vs Italians 13.69%, OR: 1.56, CI: 1.33-1.83, p<0.001). No significant differences between age groups (age ≤14 years 15.11% *sv* >14 years 14.58%, OR: 1.04, CI: 0.90-1.21, p=0.584) and sexes (male 15.61% vs female 14.07%, OR: 1.13, CI: 0.99-1.29, p=0.066) were observed (Table 2).

In Tables 3 and 4 we report the frequency of the infections by protozoa and by helminths in our studied population, considering the parasitoses once at a time (both single and mixed infections).

The most commonly detected intestinal protozoan was *Blastocystis* spp. (n=674, 9.18%) mainly identified in foreigner patients (non-Italians 11.29% *vs* Italians 8.76%, OR: 1.32, CI: 1.09-1.62, p=0.005). The occurrence of the infections was lower in children than in adults (age ≤14 years 5.20% *vs* >14 years 10.50%, OR: 0.47, CI: 0.37-0.58, p<0.001).

The second most common species was *D. fragilis* identified in 424 patients (5.78%), more frequently detected in non-Italians and children (non-Italians 11.29% *vs* Italians 8.76%, OR: 1.32, CI: 1.09-1.62, p=0.005; age ≤14 years 9.52% *vs* >14 years 4.53%, OR: 2.22, CI: 1.81-2.71, p<0.001).

G. duodenalis was the third most prevalent species detected in 69 patients (0.94%). In this case, we report that the level of infection is higher in males than in females (male 1.50% *vs* female 0.54%, OR: 2.81, CI: 1.70-4.65, p<0.001). The fourth common species was *Entamoeba coli* (n=44, 0.60%), then *Endolimax nana* (n=34) and *Chilomastix mesnili* (n=11).

As far as helminths are concerned, *Enterobius vermicularis* was the most widespread: 70 patients, particularly in children (age ≤14 years 3.28% *vs* >14 years 0.18%, OR: 18.69 CI: 9.55-36.58, p<0.001). We found 5 *Taenia* spp., 2 *Hymenolepis nana*, 2 *Ascaris lumbricoides*, 1 *Trichuris trichiura* and 1 *S. stercoralis*.

Table 3 - Protozoa found in stools samples with mentions to demographic data.

		Origin				*Age*				*Sex*			
Parasite	*No. of parasites*[a]	*Non-Italians*[b]	*Italians*[b]	*OR (95% CI)*	*p*	*≤14 years*[b]	*>14 years*[b]	*OR (95% CI)*	*p*	*Male*[b]	*Female*[b]	*OR (95% CI)*	*p*
Protozoa	**1268**	**298**	**970**	**-**	**-**	**307**	**961**	**-**	**-**	**569**	**699**	**-**	**-**
Blastocystis spp.	674 (9.18%)	137 (11.29%)	537 (8.76%)	1.32 (1.09-1.62)	0.005	95 (5.20%)	579 (10.50%)	0.47 (0.37-0.58)	<0.001	296 (9.64%)	378 (8.80%)	1.10 (0.94-1.29)	0.243
Dientamoeba fragilis	424 (5.78%)	110 (9.06%)	314 (5.12%)	1.84 (1.47-2.31)	<0.001	174 (9.52%)	250 (4.53%)	2.22 (1.81-2.71)	<0.001	183 (5.96%)	241 (5.64%)	1.06 (0.87-1.29)	0.560
Giardia duodenalis	69 (0.94%)	12 (0.99%)	57 (0.93%)	1.06 (0.57-1.99)	0.841	10 (0.54%)	59 (1.07%)	0.51 (0.26-1.00)	0.045	46 (1.50%)	23 (0.54%)	2.81 (1.70-4.65)	<0.001
Entamoeba coli	44 (0.60%)	20 (1.65%)	24 (0.39%)	4.26 (2.35-7.74)	<0.001	18 (0.99%)	26 (0.47%)	2.10 (1.15-3.84)	0.014	24 (0.78%)	20 (0.47%)	1.68 (0.92-3.04)	0.086
Endolimax nana	34 (0.46%)	12 (0.99%)	22 (0.36%)	2.77 (1.37-5.61)	0.003	6 (0.32%)	28 (0.51)	0.65 (0.27-1.56)	0.327	14 (0.45%)	20 (0.47%)	0.97 (0.49-1.93)	0.920
Chilomastix mesnili	11 (0.15%)	2 (0.16%)	9 (0.14%)	1.12 (0.24-5.20)	-	1 (0.05%)	10 (0.18%)	0.30 (0.04-2.36)	-	1 (0.03%)	10 (0.23%)	0.14 (0.02-1.09)	11 (0.15%)
Other protozoa[c]	12 (0.16%)	5 (0.41%)	7 (0.11)	-	-	3 (0.16%)	9 (0.16%)	-	-	5 (0.16%)	7 (0.16%)	-	-

[a]: in brackets the percentages on 7341 total patients are reported, calculated as the occurrence of the infections by protozoa in the population studied, not considering their involvement either in single or in mixed infections but considering the parasitoses (and subsequently the respective parasites involved) once at a time.

[b]: in brackets the percent proportions of each group of patients calculated on the respective total are indicated.

[c]: Other protozoa detected: *Entamoeba dispar* (3), *Entamoeba hartmanni* (2), *Entamobea histolytica* (2), *Cryptosporidium* spp. (2), *Iodamoeba bütschlii* (2), *Retortamonas intestinalis* (1).

As we summarised in Tables 5 and 6, 853 infections were caused by only one agent. Moreover 21.02% of positive patients (227/1080) had mixed infections. The most frequent association was *Blastocystis* spp./*D. fragilis* observed in 118 cases, corresponding to 10.93% out of the total, then *D. fragilis*/*E. vermicularis* (16 cases, 1.48%) and *Blastocystis* spp./*G. duodenalis* (11 cases, 1.02%).

Table 4 - Helminths found in stools samples with mentions to demographic data.

		Origin				*Age*				*Sex*			
Parasite	*No. of parasites*[a]	*Non-Italians*[b]	*Italians*[b]	*OR (95% CI)*	*p*	*≤14 years*[b]	*>14 years*[b]	*OR (95% CI)*	*p*	*Male*[b]	*Female*[b]	*OR (95% CI)*	*p*
Helminths	**81**	**21**	**60**	**-**	**-**	**63**	**18**	**-**	**-**	**41**	**40**	**-**	**-**
Enterobius vermicularis	70 (0.95%)	17 (1.40%)	53 (0.87%)	1.63 (0.94-2.82)	0.080	60 (3.28%)	10 (0.18%)	18.69 (9.55-36.58)	<0.001	37 (1.21)	33 (0.77%)	1.57 (0.98-2.51)	0.060
Taenia spp.	5 (0.07%)	0	5 (0.08%)	-	-	0	5 (0.09%)	-	-	2 (0.07%)	3 (0.07%)	0.93 (0.16-5.56)	-
Hymenolepis nana	2 (0.03%)	2 (0,16%)	0	-	-	2 (0.11%)	0	-	-	2 (0.07%)	0	-	-
Ascaris lumbricoides	2 (0.03%)	1 (0.08%)	1 (0.02%)	5.05 (0.32-80.80)	-	1 (0.05%)	1 (0.02%)	3.02 (0.19-48.30)	-	0	2 (0.05%)	-	-
Trichuris trichiura	1 (0.01%)	0	1 (0.02%)	-	-	0	1 (0.02%)	-		0	1 (0.02%)	-	-
Strongyloides stercoralis	1 (0.01%)	1 (0.08%)	0	-	-	0	1 (0.02%)	-	-	0	1 (0.02%)	-	-

[a]: in brackets the percentages on 7341 total patients are reported, calculated as the occurrence of the infections by helminths in the population studied, not considering their involvement either in single or in mixed infections but considering the parasitoses (and subsequently the respective parasites involved) once at a time. [b]: in brackets the percent proportions of each group of patients calculated on the respective total are indicated.

Table 5 - Patients having a single intestinal parasite species, by origin, age and sex.

		Origin		*Age*		*Sex*	
	Total	*Non-Italians (N=1214)*	*Italians (N=6127)*	*≤14 years (N=1827)*	*>14 years (N=5514)*	*Male (N=3069)*	*Female (N=4272)*
Blastocystis spp.	487	84	403	46	441	208	279
Dientamoeba fragilis	237	62	175	109	128	96	141
Enterobius vermicularis	45	12	33	38	7	24	21
Giardia duodenalis	45	7	38	5	40	28	17
Entamoeba coli	14	6	8	3	11	6	8
Endolimax nana	10	5	5	2	8	3	7
Chilomastix mesnili	4	1	3	0	4	0	4
Taenia spp.	4	0	4	0	4	1	3
Entamoeba dispar	3	1	2	0	3	3	0
Cryptosporidium spp.	2	0	2	1	1	0	2
Ascaris lumbricoides	1	1	0	1	0	0	1
Strongyloides stercoralis	1	1	0	0	1	0	1
Total	**853**	**180 (14.83%)**	**673 (10.98 %)**	**205 (11.22%)**	**648 (11.75%)**	**369 (12.02%)**	**484 (11.33%)**
		OR: 1.41 (CI: 1.18-1.68) p<0.001		**OR: 0.95 (CI: 0.80-1.12) p=0.538**		**OR: 1.07 (CI: 0.93-1.24) p=0.360**	

Clinical data were obtained using the record card directly provided to the user or through interviews with patients and clinicians. The record card reports: personal data, clinical signs and symptoms, travel history, risk behaviours, laboratory tests and comorbidities.

As for the nationality, out of the total of 241 immigrant patients with intestinal parasitoses 105 (43.56%) came from Eastern Europe, 59 (24.48%) from Northern Africa, 36 (14.94%) from Sub-Saharan Africa, 18 (7.47%) from South America, 11 from Asia, six from Middle East, four from Western Europe, two from Central America. Moreover, we report 13 positive patients belonging to the group of international child adoption.

As shown in Table 7 the more frequently observed symptoms in patients positive for single infection were: skin itching/rash (168, 19.70%), then non-specific gastrointestinal disorders (151, 17.70%, as nausea, vomiting, abdominal bloating and meteorism) and abdominal pain (76, 8.91%). The remaining patients had not declared the presence of clinical symptoms as they carried out the examination as an unspecified screening.

Table 6 - Patients with mixed infections, by combination of pathogenic parasites, by origin, age and sex.

		Origin		*Age*		*Sex*	
	Total	*Non-Italian (N=1214)*	*Italian (N=6127)*	*≤14 years (N=1827)*	*>14 years (N=5514)*	*Male (N=3069)*	*Female (N=4272)*
Blastocystis spp./*D. fragilis*	118	27	91	32	86	49	69
D. fragilis/E. vermicularis	16	2	14	15	1	8	8
Blastocystis spp./*G. duodenalis*	11	1	10	1	10	9	2
Blastocystis spp./*E. coli*	10	3	7	1	9	7	3
Blastocystis spp./*D. fragilis/E. coli*	9	7	2	3	6	5	4
Blastocystis spp./*D. fragilis/ E. nana*	8	2	6	1	7	4	4
D. fragilis/G. duodenalis	6	0	6	2	4	5	1
Blastocystis spp./*E. nana*	5	3	2	0	5	1	4
D. fragilis/E. nana	5	1	4	1	4	4	1
D. fragilis/E. coli	5	3	2	3	2	3	2
Blastocystis spp./*D. fragilis/ E. vermicularis*	4	1	3	4	0	3	1
Blastocystis spp./*D. fragilis/ G. duodenalis*	4	2	2	0	4	3	1
Blastocystis spp./ *E. vermicularis*	3	1	2	1	2	2	1
Other mixed infections[a]	23	8	15	7	16	7	16
Total	**227**	**61**	**166**	**71**	**156**	**110**	**117**
		OR: 1.90 (CI: 1.41-2.56) p<0.001		OR: 1.39 (CI: 1.04-1.85) p=0.024		OR: 1.32 (CI: 1.01-1.72) p=0.039	

[a]: Other mixed infections detected: *Blastocystis* spp./*C. mesnili/D. fragilis* (2), *C. mesnili/D. fragilis* (2), *D. fragilis/E. nana/E. coli* (2), *A. lumbricoides/T. trichiura* (1), *Blastocystis* spp./*C. mesnili* (1), *Blastocystis* spp./*C. mesnili/Taenia* spp. (1), *Blastocystis* spp./*D. fragilis/E. nana/E. coli* (1), *Blastocystis* spp./*D. fragilis/E. nana/E. vermicularis* (1), *Blastocystis* spp./*D. fragilis/E. hartmanni* (1), *Blastocystis* spp./*D. fragilis/E. vermicularis/G. duodenalis* (1), *Blastocystis* spp./*D. fragilis/G. duodenalis/H. nana* (1), *Blastocystis* spp./*E. nana/I. bütschlii* (1), *Blastocystis* spp./*E. nana/R. intestinalis* (1), *Blastocystis* spp./*E.hartmanni* (1), *Blastocystis* spp./*E. histolytica* (1), *Blastocystis* spp./*H. nana* (1), *Blastocystis* spp./*I. bütschlii* (1), *C. mesnili/E. coli* (1), *D. fragilis/E. coli/G. duodenalis* (1), *E. coli/E. histolytica* (1).

Table 7 - Patients having a single intestinal parasite species, by clinical signs and symptoms.

		Clinical signs and symptoms						
	Total	*Diarrhoea*	*Abdominal pain*	*Gastrointestinal disorders*	*Pruritus ani*	*Skin itching/ rash*	*Eosinophilia*	*Bruxism*
Blastocystis spp.	487	41	34	88	10	106	5	0
Dientamoeba fragilis	237	15	33	44	16	45	5	2
Enterobius vermicularis	45	0	2	2	20	8	0	2
Giardia duodenalis	45	8	3	9	1	4	1	0
Entamoeba coli	14	0	2	3	1	2	0	0
Endolimax nana	10	1	2	0	0	0	0	0
Chilomastix mesnili	4	0	0	1	0	3	0	0
Taenia spp.	4	0	0	2	0	0	0	0
Entamoeba dispar	3	0	0	0	0	0	0	0
Cryptosporidium spp.	2	0	0	0	0	0	0	0
Ascaris lumbricoides	1	0	0	1	0	0	0	0
Strongyloides stercoralis	1	0	0	1	0	0	0	0
Total	**853**	**65 (7.62%)**	**76 (8.91%)**	**151 (17.70%)**	**48 (5.63%)**	**168 (19.70%)**	**11 (1.28%)**	**4 (0.46%)**

As for patients with mixed infection we report the data about the most frequent combination (*Blastocystis* spp./*D. fragilis*): 30 patients declared skin itching/rash, 24 non-specific gastrointestinal disorders, 16 diarrohea, 15 abdominal pain, six anal itching, one eosinophilia.

DISCUSSION

Intestinal parasitoses are cause of almost 450 million deaths every year worldwide and their importance is now demonstrated also in areas considered non-endemic (2).
From these considerations, it became mandatory to clarify the current situation in our area to demonstrate the importance of investing resources and personnel in this discipline of medicine considered neglected.
Our study, spanning over a period of two years, considered all the samples received in our laboratory for O&P examination and further parasitological investigation, whether demanded by clinical suspicion or performed for non-specific screening.
The overall prevalence emerged in our survey was 14.71% that is pretty high considering that we are in a so-called non-endemic area.
In our study 241 positive patients were foreigners, coming mainly from Eastern Europe and Africa, highlighting the fact that immigration from endemic countries certainly is a mentionable issue.
Moreover, the phenomenon of international adoption should not be underestimated: we found that 13 children, mostly adopted from Africa and Eastern Europe, were suffering from parasitoses, seven of them with mixed infection (up to four parasites simultaneously). These data suggest the importance of promptly monitoring such children.
Immigration and international adoptions cannot be the only risk factor to consider in our reality, we must contemplate that a faecal-oral route exists also in our area.
From the analysis performed on our patients we found that protozoa are the parasites most frequently diagnosed; among these, the most commonly detected was *Blastocystis* spp., a worldwide distributed protozoon, with a prevalence of 9.18%; despite its still controversial role, several studies presently show the pathogenicity of this protozoan. As a matter of fact, it has been demonstrated the existence in nature of differ-

ent genotypes with variations of virulence that could explain the variability of symptoms and the clinical outcome [11-13]. Moreover, other authors suggested that the finding of greater than five parasites per high power field (400x) or, less commonly, oil immersion (1000x) objective is associated with the presentation of significant gastrointestinal symptoms [11]. In our series, we found significant symptomatology in 259/487 (53.18%) single *Blastocystis* spp. infection. It remains unclear whether this organism should be considered as pathogenic.

The second most prevalent species encountered in our research was *D. fragilis*, a flagellated protozoon whose complete life cycle has not yet been determined; to date the cyst stage has not been identified and the trophozoite is the only stage found in stools of infected individuals [14]. Its role is still debated and even if considered a neglected parasite, it is not rare and an increasing number of studies consider this faecal agent responsible for enteritis and extra-intestinal disorders [15-17]. In our survey the prevalence was substantially high (5.78%, prevalence that reach 6.20% considering only patients that submitted samples just for O&P only), finding that aligns with what is reported in a recent Italian study [18]. Even if the rate was higher in foreigner patients, the frequency in Italian patients is significant (5.12%). These data help to confirm the endemic character of this protozoan whose spread is apparently increasing despite the hygiene standards traditionally practiced in our community. Moreover, we underline that its detection was associated to allergic phenomena such as skin itching and rash in 45/237 (18.99%) patients and to diarrhoea and other gastrointestinal disorders in 92/237 (38.82%) of cases, considering single infection only. A significant finding, yet to be explored, is constituted by the high number of healthy carriers, individuals who, while harbouring *D. fragilis*, have not declared the presence of clinical symptoms; this factor certainly contributes to the high prevalence and apparent high contagiousness [15].

Lastly, we mention the frequent finding of *D. fragilis* within families and close contacts; in particular, in our research we have identified 19 families involved within which at least two subjects were affected, considering it important to establish a screening of all contacts in order to prevent reinfection.

We consider it important to emphasise that identification of *D. fragilis* by microscopic examination is not always easy: it is therefore essential to ensure an appropriate collection and storage of samples (at least three samples on alternate days and usage of container with fixative) and to resort to expert microscope operator; in doubtful cases, we used to require an additional sample to be stained or to perform a real-time PCR.

In our reality *G. duodenalis* was the third most commonly encountered with a prevalence of 0.49% that is consistent with data about infection in Italy reported on specialised studies [19, 20]. The rate was higher in adults than in children and in males than in females. The symptoms most frequently associated were diarrhoea and other gastrointestinal disorders in 20/45 (44.44%) of cases, contemplating single infection only.

Concerning helminths, the most frequent parasite identified was *E. vermicularis* that was found in 70 patients, 60 of whom were children. It is likely that this finding is underestimated because, in presence of suspected symptoms, the scotch tape test, in addition to O&P, is not always requested.

In our survey, we report only one case of strongyloidiasis relating to an African woman with enteritis in HIV infection, despite this helminthiasis was considered endemic in Northern Italy [21]. The diagnosis of infection by *S. stercoralis* might be underestimated because stool examination is relatively insensitive [22]. We consider the opportunity of the introduction of serological tests supporting stool investigation in certain clinical situations, such as immunocompromised patients.

In conclusion, in our country, although considered a non-endemic area, intestinal parasitoses must be unquestionably contemplated in differential diagnosis of gastrointestinal diseases; for this purpose the knowledge of the epidemiology is essential.

We wind up by underlying the importance of examination of multiple stool specimens per patient for a better diagnosis and we always recommend carrying out these investigations whenever clinical suspicion is well-founded. To this purpose a close collaboration between the parasitologist and the attending physician is always desirable.

Conflict of interest

The authors have no conflicts of interest to declare.

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