The current spectrum and prevalence of intestinal parasitosis in Campania (region of southern Italy) and their relationship with migration from endemic countries

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SUMMARY

Background: In Italy, the current clinical–epidemiological features of intestinal parasitosis and the impact of recent massive migration flows from endemic areas on their distribution are not very well known.
Methods: An analysis was carried out involving 1766 patients (720 natives and 1046 immigrants) observed during the period 2009–2010 (the 'current group') and 771 native patients observed during the period 1996–1997 (the 'historical group'), a time at which immigration in the area was minimal. Patients were analyzed for intestinal parasitosis at four healthcare centres in Campania.
Results: A wide variety of intestinal parasites was detected in the study subjects. Immigrants had a significantly higher prevalence of parasitosis and multiple simultaneous infections than natives in both groups. In both study groups of natives, the detection of at least one parasite was significantly associated with a history of travel to endemic areas. Among immigrants, we found an inverse correlation between the frequency of parasite detection and the amount of time spent in Italy. No circulation of parasites was found among contacts of parasitized patients.
Conclusions: Intestinal parasites are still a cause of intestinal infection in Campania. Although immigrants have a significantly higher prevalence of parasitosis than natives, this does not increase the risk of infection for that population. This is likely due to the lack of suitable biological conditions in our area.

1. Introduction

Intestinal parasites represent a major public health problem in developing countries. In 2004, the World Health Organization (WHO) estimated that 3.5 billion people, mostly in tropical and sub-tropical areas of the world, were infected with intestinal parasites, and that 450 million people, mainly children, had evidence of related disease.[1]

Most parasites are ubiquitous, and until a few decades ago, Italy was also considered to be an endemic area for some of them. Indeed, before the year 2000, different studies reported the circulation of numerous protozoa and helminths in our country (e.g. *Entamoeba histolytica*, *Giardia duodenalis*, *Dientamoeba fragilis*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Hymenolepis nana*, *Taenia saginata/Taenia solium*, *Echinococcus granulosus*, and *Enterobius vermicularis*), some of which are associated with major illnesses.[2–5]

The current opinion is that the incidence of intestinal parasitosis in Italy is low, with only sporadic cases identified, while the occurrence of disease outbreaks represents a rare event.

However, since the reporting of most intestinal parasitosis is not mandatory in Italy, there is little information on recent clinical–epidemiological features.[6–9] Furthermore, there are no data concerning the impact that massive migration flows during the last two decades, mostly from endemic areas such as Africa and southern Asia, may have had on the distribution of intestinal parasitosis in Italy. Indeed, a prevalence of intestinal parasites ranging from 20% to 60% has been reported in immigrants, depending on the country of origin and difficulties encountered during the migrant's travel, with the highest prevalence rates observed in those recently migrated and in those coming from Sub-Saharan Africa.[10–18]

Since, at present, immigrants represent 8% of the Italian population,[19] it might be justified to assume that a high prevalence of chronic carriers of intestinal parasites among immigrants could result in an increase in the circulation and transmission of these infectious agents.

The aim of this study was to investigate the prevalence and the spectrum of current intestinal parasites in Campania, a region of southern Italy, and to assess the potential epidemiological consequences of migration from endemic countries to this region. Campania is the second most populous (5 769 750 inhabitants) and the most densely populated region of Italy (428 inhabitants/km^2).[20] The population of Campania region includes a not negligible percentage of immigrants, estimated to be approximately 3% (200 000 individuals) of the resident population.[15] In order to achieve the objective of the study, a group of patients observed during 2009–2010 including both native and immigrant people, and a group of a native patients observed in 1996–1997 (when immigration was not present yet or was minimal), were compared and analyzed for intestinal parasitosis at four healthcare centres in Campania.

2. Materials and methods

This prevalence study was conducted on two groups of patients observed during different time-periods. The first group, observed during 2009–2010, included 1776 consecutive patients (without adopting inclusion or exclusion criteria) with intestinal symptoms who were referred to a healthcare service for immigrants and the regional hospital for infectious diseases in Naples, where they were tested for intestinal parasites. Throughout this article, all patients recruited during 2009–2010 are referred to as the current patient group. Among these, 720 were native (44% female, mean age 38 ± 16 years) and 1046 were immigrants (27% female, mean age 24 ± 9 years), who were mostly from Central Africa (46%), the Indian sub-continent (23%), and North Africa (16%).

All patients showed clinical signs, mainly chronic, compatible with intestinal parasitosis (fever, bloody diarrhoea or diarrhoea, irritable bowel syndrome, anaemia, eosinophilia, itching, and dermatitis). In most of the patients, these symptoms represented the primary cause of hospitalization, while in about a third of patients, these symptoms coexisted with other pathological conditions. In particular, 96 patients were HIV-positive (54 natives and 42 immigrants) and all of them but 10 (two natives and eight immigrants) were undergoing highly active anti-retroviral therapy (HAART). The time of arrival in Italy was known for all immigrants participating in the study.

The results found in this group of patients were compared to those obtained in a group of 771 consecutive native patients (38% female, mean age 39 ± 10 years; 60 HIV-positive, of whom only eight were undergoing HAART) referred for gastrointestinal symptoms to the participating units during the period 1996–1997, when immigration was not present yet or was minimal in this area. In the present study, these 771 patients are referred to as the historical patient group.

No patient in either the current or historical group was aged less than 12 years.

In a further investigation to analyze the potential inter-human transmissibility of parasites, we studied the households of 48 parasitized immigrants, for a total of 246 subjects.

Faecal specimens collected from all patients participating in the study were analyzed through direct microscopic examination, as well as after fixation and concentration. For direct microscopic examination, 1–2 g of faecal specimen were dissolved in 1–2 ml of normal saline to show the mobile forms, if present, and with Lugol's solution for staining of nuclei and protozoa intracellular structures. Smears were prepared from samples in normal saline for staining by modified Ziehl–Neelsen method (for Coccidia), Weber method (for Microsporidia), and Giemsa colouration for protozoa. Specific fluorescent monoclonal antibodies were also used for the identification of *Cryptosporidium spp*, *G. duodenalis*, and *E. histolytica/dispar*.[21]

For the concentration, 2–3 g of faecal specimen were fixed in 5% formalin (dilution ratio 1:4). All samples were then analyzed with the classical concentration technique of sedimentation. Moreover, flotation through the FLOTAC dual pellet 400 technique was also performed for the concentration of specimens from the current prospective group of patients, using two floating solutions (SF4: watery solution of sodium nitrate (specific weight = 1200); SF7: watery solution of zinc sulphate (specific weight = 1350)); the pellet was first treated with diethyl ether (2 ml diethyl ether + 10 ml saline solution).[22–27] No DNA-based methods were carried out to differentiate *Entamoeba histolytica* from *E. dispar*. Because this differentiation cannot be made on a morphological basis, it was based only on clinical criteria (i.e., the presence of specific clinical patterns of intestinal amoebiasis for *E. histolytica* that are lacking for *E. dispar*, this latter species being non-pathogenic).[28]

Only one sample per patient was available in 62% of the cases, two samples were available for 22%, and three or more samples were available for 16%, for a total of 3815 stool specimens examined. The numbers of samples were homogeneously distributed among all groups of patients.

The United Kingdom National External Quality Assessment Service (UKNEQAS; Department of Clinical Parasitology, Hospital for Tropical Diseases, London) provided specimens for faecal parasitology quality control. All samples were processed and analyzed by the same team of parasitologists.

2.1. Statistical analysis

Statistical analyses were performed using the Student's *t*-test for comparisons between means. For categorical variables, differences between groups were calculated by Chi-square test and Fisher's exact test, when necessary. A two-sided *p*-value of less than 0.05 was considered statistically significant. Analysis for linear trends in proportions was conducted using the extended Mantel–Haenszel test. All statistical analyses were performed using Stata version 11.2 (StataCorp LP, College Station, TX, USA).

The study was approved by the Ethics Committee of Cotugno Hospital. All participating patients provided signed informed consent.

3. Results

3.1. Overall prevalence of intestinal parasitosis

Considering all patient groups (current natives, current immigrants, and historical natives), a total of 1065 intestinal parasites were found in 664 patients. Most of the parasites identified were protozoa considered as non-obligate pathogens, which were often found in conjunction with pathogenic species

Table 1
Distribution of detected parasites in the three groups of patients (immigrants, current natives, and historical natives) participating in the study

	Immigrants (n = 1046)			Current natives (n = 720)				Historical natives (n = 771)			
	n	% of isolated parasites	% of patients	n	% of isolated parasites	% of patients	p-Value[a]	n	% of isolated parasites	% of patients	p-Value[b]
Helminths											
Trichuris trichiura	40	4.8	3.8	0	0.0	0.0	<0.0001	1	1.0	0.1	<0.0001
Ancylostoma duodenale	33	3.9	3.2	0	0.0	0.0	<0.0001	2	1.9	0.3	<0.0001
Hymenolepis nana	15	1.8	1.4	0	0.0	0.0	<0.001	0	0.0	0.0	<0.001
Schistosoma mansoni	9	1.1	0.9	0	0.0	0.0	0.01	0	0.0	0.0	0.01
Dicrocoelium dendriticum	8	1.0	0.8	0	0.0	0.0	0.02	0	0.0	0.0	0.02
Ascaris lumbricoides	7	0.8	0.7	1	1.1	0.1	0.02	0	0.0	0.0	0.04
Strongyloides stercoralis	5	0.6	0.5	0	0.0	0.0	0.08	0	0.0	0.0	0.07
Trichostrongylus spp	4	0.5	0.4	1	1.1	0.1	0.6	0	0.0	0.0	0.1
Enterobius vermicularis	3	0.4	0.3	2	2.1	0.3	1.0	1	1.0	0.1	0.6
Taenia spp	2	0.2	0.2	1	1.1	0.1	1.0	1	1.0	0.1	1.0
Pathogenic protozoa											
Entamoeba histolytica	52	7.3	5.0	8	8.9	1.1	<0.0001	3	3.0	0.4	<0.0001
Dientamoeba fragilis	4	0.5	0.4	2	2.1	0.3	1.0	5	4.8	0.6	0.5
Giardia duodenalis	35	4.2	3.3	9	9.5	1.3	0.005	5	4.8	0.6	<0.0001
Isospora belli	2	0.2	0.2	1	1.1	0.1	1.0	1	1.0	0.1	1.0
Cryptosporidium spp	0	0.0	0.0	1	1.1	0.1	0.4	40	38.1	5.2	<0.0001
Facultative pathogenic protozoa											
Entamoeba coli	130	15.4	12.4	8	8.4	1.1	<0.0001	2	1.9	0.3	<0.0001
Endolimax nana	107	12.7	10.2	5	5.3	0.7	<0.0001	5	4.8	0.6	<0.0001
Entamoeba hartmanni	47	5.6	4.5	1	1.1	0.1	<0.0001	0	0.0	0.0	<0.0001
Entamoeba dispar	20	2.4	1.9	3	3.2	0.4	0.1	0	0.0	0.0	0.002
Iodamoeba bütschlii	8	1.0	0.8	0	0.0	0.0	0.02	0	0.0	0.0	0.02
Chilomastix mesnili	6	0.7	0.6	5	5.3	0.7	0.7	0	0.0	0.0	0.04
Parasites of different taxonomic classification[c]											
Blastocystis spp	323	38.4	30.9	50	52.6	6.9	<0.0001	37	35.2	4.8	<0.0001

[a] Difference between immigrants and current natives; a two-sided p-value of <0.05 was considered statistically significant.
[b] Difference between immigrants and historical natives; a two-sided p-value of <0.05 was considered statistically significant.
[c] Blastocystis spp is thought to be a protist, belonging to the stramenopiles, a branch of the Chromalveolata.

(Table 1). No associations between age and/or sex and prevalence of the different parasites were found (data not shown).

Among all patient groups, Blastocystis spp was the most frequently detected parasite (410 total cases), and in 48% of cases it coexisted with one or more other agents.

3.2. Prevalence of intestinal parasitosis among native patients

Current and historical native patients were parasitized in 9.6% (69/720) and 11.4% (88/771) of cases, respectively (p = 0.28). E. histolytica was detected in three patients in the historical group and in eight subjects in the current group (Table 1). All these patients had a clinical picture of intestinal amoebiasis and all of them reported recent travel to an endemic area.

Of note, considering patients with HIV infection, Cryptosporidium spp was detected at a significantly lower frequency in the current group than in the 1996–1997 group (1 case vs. 40 cases, respectively; p < 0.001) (Table 1). With the exception of Cryptosporidium spp, no other significant difference was found in

Table 2
Prevalence of intestinal parasitic infection among immigrants according to their geographical area of origin

Geographical areas of origin	Tested patients	Infected patients	Prevalence	p-Value[a]
Native	720	69	9.6	-
East Europe	82	15	18.3	0.27
Central-South America	49	10	20.4	0.20
North Africa	165	64	38.8	0.017
Central Africa	486	307	63.2	<0.01
Asia	264	111	44.7	<0.01

[a] Statistical differences were calculated using the group of native patients as the reference category. A two-sided p-value of <0.05 was considered statistically significant.

the prevalence of the different parasites between the two groups of native patients, nor was any parasite detected that had previously been absent in our territory.

Among native patients, those belonging to the current group reported a significantly higher frequency of recent travel to endemic areas in comparison to patients in the historical group (12% vs. 7%; p < 0.005). Also, the detection of at least one parasite was significantly associated with recent travel to endemic areas in both the current and historical native groups. In fact, travel to endemic areas was reported in 38% of parasitized patients and in only 7% of non-parasitized patients (p < 0.0001). The same pattern was noted in the historical native group, in which travel to endemic areas was reported by 21% of parasitized patients compared to 4.5% of the non-parasitized (p < 0.001).

Only about 18% of this reported travel was related to business purposes and had a duration of 9 ± 8 months; no significant association was found between the time of permanence and the frequency of parasitosis (data not shown).

3.3. Prevalence of intestinal parasitosis among immigrant patients

Among immigrants, 862 parasites were detected in 507/1046 subjects (48.5% of cases). The most frequent parasites identified, in decreasing order, were Blastocystis spp, Entamoeba coli, Endolimax nana, E. histolytica, Entamoeba hartmanni, T. trichiura, and A. duodenale. The prevalence of parasitized individuals with each of these parasites was significantly higher in immigrants than in both of the groups of native patients (p < 0.001 for all these differences).

3.4. Prevalence of infection with pathogenic agents

When only pathogenic agents were considered (Table 1), the prevalences of patients parasitized with helminths and protozoa (if cases of Cryptosporidium spp in HIV-positive patients in the
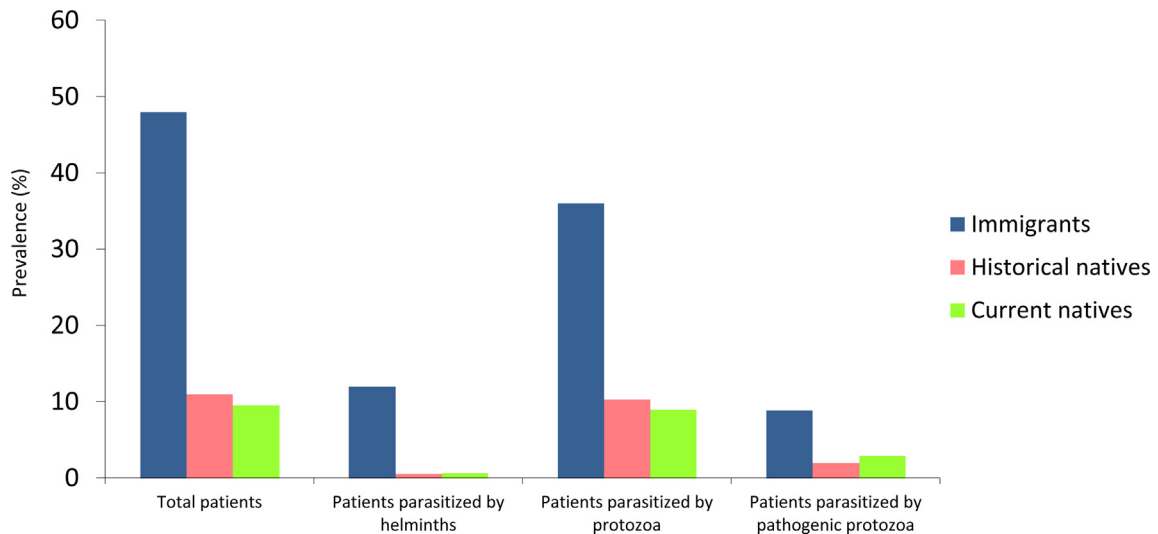
Figure 1. Prevalence of parasitized individuals among immigrants and current and historical natives according to the principal groups of parasites detected.
Note: All differences in prevalence between immigrants and each group of natives were statistically significant ($p < 0.01$); all differences in prevalence between the two groups of natives were not significant; in the group of historical natives *Cryptosporidium spp* cases were excluded; *Blastocystis spp* cases were included in protozoa.

historical group were excluded) were significantly higher among immigrants than among native patients analyzed in either 2009–2010 or in 1996–1997: 12% vs. 0.7% and 0.6%, respectively, for helminths ($p < 0.001$), and 8.9% vs. 2.9% and 1.8%, respectively, for protozoa (Figure 1).

3.5. Comparison between native and immigrant patients

The prevalence of parasitized patients was lower among current natives than among each group of different current immigrants classified on the basis of their geographical origin. In this respect, significant differences were found for immigrants from North Africa, Central Africa, and the Indian sub-continent (Table 2). The prevalence of patients with multiple simultaneous infections was significantly higher among immigrants than among native patients in both groups. In particular, the simultaneous detection of three or more parasites was observed in 20.1%, 10.1%, and 4.5% of immigrants, current natives, and historical natives, respectively ($p < 0.001$ for immigrants vs. current natives; $p < 0.05$ for immigrants vs. historical natives; no significant difference was found between current and historical native patients).

The frequency of detected parasites changed among immigrants depending on their length of stay in Italy. As shown in Table 3, the frequency of detected parasites in immigrants was significantly inversely related to the amount of time spent in Italy (p-value for trend <0.0001, for all and each of the groups of parasites analyzed in Table 3). Thus, among immigrants with a length of stay in Italy of >5 years, the percentage of parasites

detected tended to be similar to the rate observed in the native population.

3.6. Inter-human transmissibility of parasites

Of the households of the 48 parasitized immigrants analyzed, all but three were entirely constituted by immigrants, mainly from the same geographical area. In one of these three groups that was not entirely composed of immigrants, a *Trichostrongylus spp* that is very rare in our territory[29] was identified in a native elderly person. This person likely acquired the infection from a Pakistan home care nurse, who harboured the same parasite. In all the other household groups, no case of parasite/parasites transmission from the index case to a household contact was identified. In the households, the overall prevalence of parasitized individuals did not differ significantly from that observed in the general immigrant population.

3.7. Clinical symptoms

The clinical symptoms, mainly chronic, were extremely heterogeneous for both the spectrum of manifestations and intensity.

Diarrhoea, sometimes hematic and very profuse, was mostly associated with protozoa infections, and fever was present mainly in amoebiasis and schistosomiasis. Signs of malabsorption, abdominal cramps, anaemia, and urticarial reactions with itching and eosinophilia were associated particularly with helminth infestations. It should be noted that these symptoms, even if

Table 3
Prevalence of intestinal parasitic infections among immigrants according to their length of stay in Italy

	Length of stay in Italy		
	<2 years (478 patients tested)	2–5 years (362 patients tested)	>5 years[a] (206 patients tested)
	n (%)	n (%)	n (%)
Parasitized patients[b]	317 (62.5)	165 (32.6)	25 (4.9)
Parasitized by			
Helminths	79 (62.7)	41 (32.5)	6 (4.8)
Pathogenic protozoa	86 (76.1)	22 (19.5)	5 (4.4)
Facultative pathogenic protozoa	468 (75.1)	133 (21.3)	22 (3.5)

[a] *p*-Value for trend <0.0001, for all and each of the groups of parasites analyzed.
[b] Patients were parasitized with at least one parasite.

mild, were present even in those subjects parasitized by agents considered simply parasites.

4. Discussion

In our study, the concentration of stool specimens in the more recent series of patients was performed with both the traditional and the FLOTAC systems. Some authors have reported a slightly improved sensitivity using this latter method.[25–27] In our experience, FLOTAC increases the sensitivity only minimally (0.2%) compared to the other method of concentration used. Thus, even if the methodology to detect the parasites is partially different in the two groups of patients, the overall sensitivity is not substantially changed.

About 10% of our native patients, irrespective of the date on which they were investigated, showed an intestinal parasite in their stool. In the vast majority of cases it was a protozoan, largely represented by facultative pathogens, while helminths were found in less than 1% of cases and were represented by only a few species, such as *Ascarides*, *Taenia*, and *E. vermicularis*. Most available data on the epidemiology of intestinal parasitosis in Italy are from studies focusing on selected groups of patients (e.g. children, HIV-infected individuals, and institutionalized psychiatric patients) or specific pathogens.[30–32] Very few epidemiological studies dealing with the prevalence of intestinal parasitosis in the general population have been performed so far. Among these, of particular note are two extensive surveys, also including immigrants, carried out recently in northern and central Italy.[8,9] In the study by Masucci et al., prevalence rates of 8.9% and 26.8% were found among Italian and non-Italian patients, respectively.[8] Peruzzi et al. reported very similar data, showing prevalence rates of 10% and 31% among Italians and foreigners, respectively.[9] The results of these two studies are comparable to those found in our survey, also regarding the spectrum of parasites involved, thus suggesting a substantial homogeneous epidemiological picture throughout our country.

Since all patients investigated in this study had chronic intestinal symptoms, the influence of a positive selection bias on prevalence estimates is possible. Nevertheless, our findings show that a wide variety of intestinal parasites are still present in Campania and their possible etiological involvement had to be considered in the diagnosis of chronic intestinal disease.

No significant differences were found in the distribution of the different parasites between the two groups of natives except for the frequent identification of *Cryptosporidium spp* in the historical HIV-positive patients, which was significantly decreased in the more recent cases. However this exception cannot be ascribed to the migration flows and is more likely due to the improved control of this, as well as other opportunistic infections, induced by HAART in the last decade.[33] Thus, it appears that the recent and more intense migration flows from developing countries to Italy have not affected the spread of parasites among the native population of Campania. Our data demonstrate instead that travel to endemic geographical areas significantly influenced the prevalence of parasites among the native population of Campania. Indeed, in both the current and historical native groups, the detection of parasites was significantly associated with a history of recent travel in these areas, which have become increasingly popular and fashionable in the last few years in our country.

As in other surveys, *Blastocystis spp* was the most common parasite isolated in our study and was detected at a significantly higher frequency among immigrants than among both groups of native patients. Blastocystis is an enteric microorganism found in humans and in many animals, ubiquitous and largely present in our territory as well, and with a taxonomic position that has remained elusive for many years. At present, on the basis of its molecular features, it is thought to be a protist belonging to the stramenopiles, a branch of the Chromalveolata.[34,35] It is usually considered a non-pathogenic agent. However, recent evidence suggests that in particular conditions *Blastocystis spp* may be responsible for enteric disease; this appears to be linked to its genetic variability and to the emergence of possible pathogenic genotypes.[36–40]

Indeed, the role of many parasites considered saprophytes is controversial and should be revised in order to identify their possible pathogenicity in some circumstances.[1] Even in our study, some were detected in patients with intestinal symptoms but without any other possible cause of illness. Moreover, the intestinal symptoms tended to resolve after specific therapy in these patients.

As already reported by others,[12,13] both helminths and facultative or pathogenic protozoa were identified at a significantly higher frequency among immigrants than in the native population. In particular, the highest prevalence rates were observed in immigrants from equatorial Africa and the Indian sub-continent, who also harboured pathogens usually not detectable in our country, such as schistosomes. Also, nearly half of the immigrant patients were positive for at least one parasite and they were affected by multiple simultaneous infections with a significantly higher frequency than natives.

We observed that the frequency of parasite detection among immigrants decreased inversely to the length of stay in Italy; thus after 5 years the positive detection rate among immigrants tended to be similar to the rate observed in the native population. This finding, in addition to the lack of effects of the recent intense immigration flows upon the epidemiological profile of intestinal parasitosis among the native population of our area and the lack of parasite circulation among household contacts of parasitized subjects, demonstrates that migration from endemic countries has not induced an additional risk of parasitic infection or re-infection among people living in our geographical area. A possible explanation is that the biological prerequisites for the maintenance of a self-sustaining cycle of these agents are missing in Italy; outbreaks are not possible because of the lack of intermediate hosts (e.g., for schistosomes), or because of the lack of favourable environmental and climate conditions required by other parasites that are directly transmitted from man to man (e.g., *E. histolytica* and *E. nana*).

However, in Italy not all parasites are extinct, also because many of them are zoonotic agents.[41] Thus, in the routine protocol for the evaluation of patients with bowel symptoms, especially those with a history of travel to an at-risk area, a parasite search should always be considered. In addition, given the frequency of parasites found in immigrants, especially in those from high-risk areas and those expatriated in particularly difficult circumstances, a cycle of anti-parasitic therapy with albendazole would be advisable, without taking a coprologic test, as suggested by some public health programs.[42] This should be viewed in the context of a first impact health care service and hospitality offered by our country, reserving further evaluations only for those cases that show persistence of symptoms and peculiar clinical features.

Ethical approval: The study was approved by the Ethics Committee of Cotugno Hospital.

Conflict of interest: All of the authors declare no conflicts of interest.

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