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Epidemiology and diagnostics of intestinal parasitic infections in Italy: a multicentric survey

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Summary

Background and Aims: parasitic infections are becoming more common in non-endemic countries because of intensified immigration and international travels; however, the parasitic disease burden is often underestimated because of underdiagnosis and lack of surveillance in industrialized countries. Also, in Italy, epidemiological data on intestinal parasites affecting humans are scarce and scattered in different areas of the country. The aim of this retrospective, observational study was to evaluate the prevalence of intestinal parasitic infections employing Parasitological Stool Examination (PSE) and to verify the quality of the diagnostic methodologies adopted to identify intestinal parasites in Italian laboratories.

Materials and Methods: the study involved 28 Italian diagnostic laboratories and 36389 patients, from June 2015 to May 2016.

Results: our data showed that 3173 out of 36389 subjects (8.7%) were carriers of one or more pathogenic or non-pathogenic protozoa or helminths. When only organisms known to cause intestinal disease were considered, an overall positive rate of 3.8% was found (1400/36389). Among the 1400 patients carrying one or more pathogenic species, protozoan infections predominated and were identified in 1138 subjects (3.1%); *Dientamoeba fragilis* and *Giardia duodenalis* represent the main intestinal pathogens. Concerning parasitic worms, only 262 individuals (0.7%) exhibited helminth infection; the most frequent

finding was represented by *Enterobius vermicularis*, followed by *Taenia* spp.

Conclusions: this survey also indicated that not all diagnostic laboratories can guarantee proper performance for parasitological diagnosis. In this context, close collaboration between physicians and clinical microbiologists is warranted to improve the diagnostics of intestinal parasitic infections, thus ensuring good quality service.

Introduction

Intestinal parasites, including protozoa and helminths, are among the major contributors to the burden of infectious diseases worldwide [28,30]. In fact, most infections and deaths from parasitic diseases affect people in low- and middle-income countries, but they also cause significant illness in the Western world [15,19]. A wide variety of intestinal parasites is prevalent in different parts of the world, and populations face distinct parasitic challenges [1].

Intestinal parasitic infections exhibit a low prevalence in Italy [5,10,12]: among the helminths *Enterobius vermicularis* and *Strongyloides stercoralis* are the most common; the first is frequent in children of school and preschool age and the second in elderly subjects [8,9,22]. These two nematodes need targeted tests in order to be diagnosed [21].

As for protozoa, *Giardia duodenalis* and *Dientamoeba fragilis* represent the most frequent intestinal pathogens in Italy, although their prevalence is underestimated [10,11,13,14]. Reporting of non-pathogenic protozoa is sporadic, thanks to the high hygiene standards now achieved all over the country [11,13,14].

In Italy and more generally in Europe, intestinal parasitic infections are neglected and for this reason, the facilities and expertise of laboratories are often limited. Furthermore, physicians overlook parasite-related etiology, especially if asymptomatic or paucisymptomatic. In addition, parasitic infections are not remunerative in the current national health landscape [5,10,12].

However, neglected tropical diseases are highly prevalent in tropical and subtropical areas, they are spread worldwide due to human migration, international travel, animal movement, international food trade, and global climate change that extend the area of presence and competence of insect vectors, mammalian hosts and birds, capable of spreading these pathogens.

All the above-mentioned factors lead to an underestimation of the prevalence of human parasitic infections, which on the other hand exhibit public health relevance for various reasons, including the origin of infected patients from endemic areas, the raised number of immunocompromised patients, the rising life expectancy and international adoptions. In addition, the potential risk of local outbreaks should be considered, as recently shown by two epidemics in northeastern Italy that were caused by *Giardia duodenalis* and *Cryptosporidium* spp., respectively [16,23].

By virtue of these considerations and on the basis of similar past experiences, in particular the pilot multicentric study of 1992-1993, the first and second multicentric survey of 1994-1995 and 2005-2008 respectively [5,12], the Study Committee for Parasitology of the Association of Italian Clinical Microbiologists (AMCLI-CoSP) has undertaken the current epidemiological investigation on human intestinal parasitosis in Italy through a multicentric survey involving laboratories distributed throughout the national territory.

The aim of the study was to evaluate the prevalence of intestinal parasitic infections employing PSE and other direct tests and to verify the quality of the diagnostic methodologies adopted to identify intestinal parasites in Italian laboratories.

Materials and Methods

Study design and data collection

This is a retrospective, observational study that involved 28 Italian diagnostic laboratories and 36389 patients, from June 2015 to May 2016.

At the beginning of 2015, the AMCLI National Secretariat sent a letter to all 960 members of AMCLI explaining the intent of the study and encouraging them to take part in the third national survey on intestinal parasitosis.

The data collection was performed by filling in a series of dedicated forms: i) techniques employed for PSE; anamnestic data with particular focus on the area of origin of the subject, travel abroad, eating habits, and any therapies; ii) techniques used by each laboratory to identify *E. vermicularis* and *S. stercoralis*, for which PSE has low sensitivity; iii) tests used in each laboratory for the identification of *Dientamoeba fragilis*, *Cryptosporidium* spp. and *Entamoeba histolytica/dispar*, respectively, and on how many samples per subject were tested for the detection of these three pathogens.

In fact, the presence of the three above-mentioned protozoa cannot be confirmed with certainty by PSE alone, and additional tests are needed, including specific staining techniques, immunochromatographic tests, and/or molecular methods [6,17,21,27].

Laboratory methods

PSE must include macroscopy and microscopy (wet mount) direct sample examination. The second time, the examiner performs microscopy analysis after Lugol or Dobell stain and/or permanent stain with Giemsa or Trichromic solution. Smear analysis includes concentration by different methods. The suggested examination is by a 10x phase-contrast objective and a 40x phase-contrast objective. Permanent smears could be prepared from fresh stool without fixative and examined with a 100x oil-immersion objective after specific staining. Antigenic tests and/or molecular tests are carried out employing commercially available kits, according to the organization of the different laboratories.

As reported above, the procedures were not carried out in the same way by the enrolled laboratories.

Indirect tests were not included.

Results

From June 2015 to May 2016, data were collected from all involved laboratories; the distribution is shown in Figure 1. More than 200 Italian laboratories were invited, but only 28 participated in the survey (~15%).

Each enrolled laboratory used different diagnostic tools. Data of PSE are summarized in Table 1, concerning the number of samples examined for each patient, only (Table 2).

Firstly, data obtained from 36389 patients were analyzed. As shown in Table 3, 3173 out of 36389 examined subjects (8.7%) carried one or more pathogenic or non-pathogenic protozoa or helminths in their gut. When only parasites known to cause intestinal disease were considered, an overall positive rate of 3.8% was found (1400/36389).

Among the 1400 patients carrying one or more pathogenic species, protozoan infections predominated and were identified in 1138 subjects (3.1%), while 262 (0.7%) exhibited helminth infection (Table 3).

Protozoa infections

Concerning infections caused by pathogenic protozoa the species identified were *D. fragilis* 933/36389 (2.6%), *G. duodenalis* 357/36389 (1.0%), *Cryptosporidium* spp. and *Cystoisospora belli* 5/36389 (<0.1%), while no infection caused by *Cyclospora cayetanensis* or *Balantidium coli* was reported in Figure 2.

In 1773 patients, non-pathogenic protozoa were detected (Figure 2), among which *Blastocystis* spp. was the most frequently reported (1599/36389, 4.4%).

Laboratories employed various targeted diagnostic tools for the identification of intestinal parasites.

In this survey, only 19 out of 28 (67.9%) laboratories performed confirmation tests for *D. fragilis* infection: 17 of them carried out permanent Giemsa stain, 1 laboratory carried out Trichome stain, 1 laboratory employed in-house Polymerase Chain Reaction (PCR). By restricting data to those obtained from the 19 laboratories that performed confirmation tests for *D. fragilis*, 933 out of 21263 patients (4.4%) tested positive for *D. fragilis* (Table 4).

Targeted identification of *E. histolytica/dispar* was performed by 15 out of 28 (53.6%) participating laboratories; 9453 patients were specifically examined for this protozoan infection (Table 4). Different methodologies were used for the targeted identification of *E. histolytica/dispar* in different laboratories, including immunochromatographic tests (10/15), immunoenzymatic assays (5/15), or in-house PCR (2/15). Ninety-eight out of 9453 patients tested positive for *E. histolytica/dispar* with a positive rate of 1%. Only two laboratories could indeed identify this parasite at the species level with molecular tests, differentiating between *E. histolytica* and *E. dispar*; thus the number of confirmed *E. histolytica* infections was 10/9453 (0.1%).

Targeted identification of *Cryptosporidium* spp. infection was performed by 21 out of 28 (75.0%) laboratories with different techniques, such as immunochromatographic tests (11/21), direct

immunofluorescence (1/21), modified Ziehl-Neelsen staining (7/21) Kinyoun staining (2/21) commercially available PCR (1/21) or in-



Figure 1. Distribution of the 28 participating laboratories in different regions within the country.

Table 1. Data on techniques employed for Parasitological Stool Examination (PSE) and on anamnestic data collection that were carried out by the 28 laboratories involved in the analysis.

Direct examination + concentration	Extemporary staining (Lugol or Dobell)	Permanent staining (Giemsa/Trichrome stain)	Anamnestic data collection
28/28	21/28	18/28	10/28; 15/28*

*Not always.

Table 2. Number of samples examined for each patient and per specific diagnostic question.

Diagnostic question	≥3 samples	1-2 samples	1 sample
Direct examination + concentration	5/28	22/28	1/28
Scotch tape test	0/28	25/28	3/28
<i>S. stercoralis</i> ¹	1/11	8/11	2/11
<i>D. fragilis</i> ¹	0/17	10/17	7/17
<i>E. histolytica</i> ²	0/12	7/12	5/12
<i>Cryptosporidium</i> spp.	0/20	12/20	8/20

¹Two laboratories did not disclose this information; ²three laboratories did not disclose this information.

Table 3. Overall results of the standard Parasitological Stool Examination (PSE).

	Positive n (%)		Helminths	Pathogen sn (%)		Negative n (%)	Total n (%)
	Pathogen	Non pathogenic		Protozoa	Mixed		
Patients	1400/36389 (3.8%)	1773/36389 (4.9%)	262/36389 (0.7%)	1138/36389 (3.1%)	591/36389 (1.6%)	33216/36389 (91.3%)	36389 (100%)

house PCR (1 /21). Of the 6850 patients that were analyzed, 21 tested positive for *Cryptosporidium* spp, with a positive rate of 0.3%.

Helminth infections

Examining in detail infections caused by helminths (262/36389, 0.7%), we observed different data between nematodes, cestodes, and flukes. *Dicrocoelium dendriticum* was detected in three cases, but it could be considered in transit, so not a pathogen (Table 5).

Among nematodes, the most frequent finding was represented by *E. vermicularis* (108/262, 41.2%), followed by *S. stercoralis* (41/262, 15.6%) and hookworm (31/262, 11.8%), while *Taenia* spp. (34/262, 13.0%) and *Schistosoma mansoni* (18/262, 6.9%) were the most frequent findings among cestodes and flukes, respectively (Figure 3). The retrieval of *Schistosoma* eggs took place exclusively in subjects from endemic areas (former residents or international travelers).

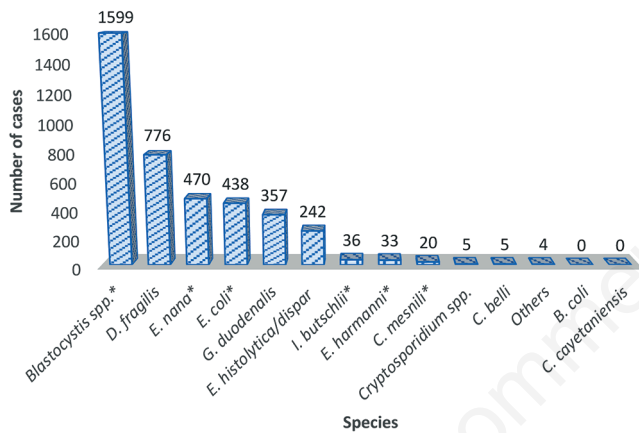
Targeted identification of *E. vermicularis* was performed by all participating laboratories; in 27 out of 28 (96.4%) diagnostic units, the scotch tape test was carried out, while the remaining laboratory

employed the perianal swab. A total of 5,047 patients were examined by targeted diagnostic tools and 838 turned out to be positive, with a positive rate of 16.6%, which is much higher than that resulting from the fortuitous discovery of *E. vermicularis* eggs in the feces with FEA (0.3%).

Targeted detection of *S. stercoralis* larvae was carried out by 13 out of 28 laboratories (46.4%) performing three different techniques: agar culture (n=9), traditional or modified Baermann method (n=3), with only one laboratory employing both the agar culture and the Baermann method. Thirty patients out of a total of 511 (5.9%) tested positive for the presence of *S. stercoralis* larvae. Without the employment of targeted techniques, occasional retrieval of *S. stercoralis* larvae occurred in only 41 patients (0.1%).

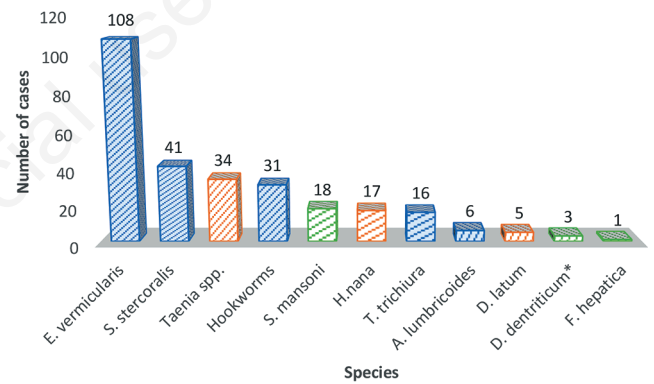
Discussion

Parasitic infections are becoming more common in non-endemic countries because of intensified immigration and international travel [15,19,29]; however, estimation of the parasitic disease burden is often complicated by the absence of reliable data due to underdiag-



*Non-pathogenic species.

Figure 2. Intestinal protozoa detected in stool samples by number of cases and by species.



Blue histograms, nematodes; orange histograms, cestodes; green histograms, flukes. *Not pathogen species.

Figure 3. Number of patients infected by helminths.

Table 4. Number of cases and frequency of detection of specific parasites by employing Parasitological Stool Examination (PSE) or targeted techniques.

Parasite	PSE test n (%)	Target test n (%)
<i>D. fragilis</i>	776/36389 (2.1%)	933/21263 (4.4%)
<i>E. histolytica/dispar</i>	242/36389 (0.7%)	98/9453 (1.0%)
<i>E. vermicularis</i>	108/36389 (0.3%)	838/5047 (16.6%)
<i>S. stercoralis</i>	41/36389 (0.1%)	30/511 (5.9%)

Table 5. Number and frequency of helminth infections.

Helminths	Number of positive patients (%)	Number of positive patients/total of positive for helminth (%)
Nematodes	202/36389 (0.6%)	202/262 (77.1%)
Cestodes	56/36389 (0.2%)	56/262 (21.4%)
Flukes	22/36389 (<0.1%)	22/262 (8.4%)

nosis and lack of monitoring programs in industrialized countries. This is also the case for Italy, where epidemiological data on intestinal parasites affecting humans are scarce and scattered in different areas of the country [3,4,12,18,20,24].

The dissemination of information about the present multicentric survey has allowed a good response from the laboratories, at least in northern Italy (24/28), while in the central and southern parts of the country as well as in the islands there was less participation. As a result, this multicenter study collected a large amount of data, mainly reflecting the epidemiological situation in Northern Italy.

We observed that not all laboratories involved in the study carried out a correct and complete parasitological examination of the feces, which should consist of at least one direct microscopic examination, a microscopic examination after concentration plus Lugol or Dobell stain and a permanent staining (Giemsa stain, Trichromic stain or ferric hematoxylin stain) the latter being necessary to correctly identify *D. fragilis* [17,30].

In addition, diagnostic tools for culture, antigenic tests, and/or molecular biology to detect *E. histolytica*, *Cryptosporidium* spp., and *S. stercoralis* should be carried out, and different methods are used according to the organization of the different laboratories. Finally, a tape test for *E. vermicularis* should be performed, and anamnestic and clinical data should be provided for each patient, including travelling history and history of immigration [17,30].

In the current survey, we observed that all the contributing laboratories possessed targeting diagnostic methods for the identification of *E. vermicularis*, while only 35.7% of the diagnostic centers routinely collected anamnestic and clinical data for each patient. Moreover, PSE should be performed on at least three fecal samples emitted spontaneously and preferably every other day as the emission of parasites is discontinuous; we found that only 5 out of 28 (17.9%) laboratories carried out PSE on 3 fecal samples; by examining less than three samples the probability of detecting parasites decreases significantly [17,30].

The overall positivity for protozoa (pathogens or not) and helminths was 8.7%, while the positivity for pathogenic protozoan and helminths was 3.8%. In line with the literature [14,20], the most frequently identified protozoa were *Blastocystis* spp., followed by *D. fragilis* and *G. duodenalis*.

Concerning parasitic worms, very little is known about the prevalence of intestinal helminths in Italy. From our data, the most prevalent organisms were *E. vermicularis*, according to Guidetti *et al.* [18], followed by *Taenia* spp. and *S. stercoralis*, different from what was shown by Masucci *et al.* [20]. In fact, they found more frequently *Ascaris lumbricoides* and *Hymenolepis nana*.

While all diagnostic centers included in this study performed a direct microscopic examination and a microscopic examination after concentration, only 64% of the laboratories performed permanent staining to confirm the presence of *D. fragilis*. Our survey highlights the higher frequency of positive *D. fragilis* detection by employing a permanent stain as compared to laboratories that did not carry out any targeted stain to identify this protozoan parasite (4.4% vs 2.1%, respectively), thus confirming the importance of the employment of a targeted, permanent stain to identify *D. fragilis* [2,25,26].

In addition, targeted identification of *E. histolytica/dispar* was performed by 54% of the participating laboratories by employing immunochromatographic tests, immunoenzymatic assays, or PCR. Consistent with our findings for *D. fragilis* detection, the prevalence of *E. histolytica/dispar* was higher when the targeted diagnosis was performed (1.0% vs 0.7% respectively). Optical microscopy and antigenic tests do not allow the distinction between the pathogenic *E. histolytica* and the nonpathogenic *E. dispar* species; we observed that only two laboratories (7%) employed molecular tools, which are the most accurate methods for the segregation between these two

amoeba species [15]. However, PCR assays are expensive and require specialized equipment and dedicated molecular areas; these methods are not routinely used for the detection of parasitic protozoa, even in resource-rich settings [15]. Thus, the diagnostic performance for the highly pathogenic *E. histolytica* was not optimal in most of the contributing laboratories.

Coccidia were rarely detected in the present survey, with *Cystoisospora belli* and *Cryptosporidium* spp. being identified in 5 and 21 cases, respectively. *Cryptosporidium* spp. is considered a leading global cause of waterborne disease, which is often underdiagnosed and underreported [16]; environmental surveys have demonstrated that this protozoan parasite contaminates wastewater and surface waters in Italy [7,16] and a human outbreak of cryptosporidiosis was recently detected in the northeastern part of the country [16]. The low prevalence of cryptosporidiosis observed within this survey is in line with the huge neglect of this infection in Italy; 28% of the laboratories included in this study did not carry out targeted identification for this protozoan parasite, which implies the lack of diagnostic tools for cryptosporidiosis in 8 diagnostic units participating to the survey.

Blastocystis spp. was detected with high frequency (4.4%) in the laboratories participating in this survey. The detection of non-pathogenic protozoa must in any case be reported in the diagnostic response. In fact, the presence of non-pathogenic protozoan species, such as *Blastocystis* spp., in a stool sample suggests the ingestion of food or water contaminated with fecal material, which increases the risk of contamination of food/water with fecal parasites; thus, when detecting a non-pathogen protozoan species, it is recommended to repeat the PSE on additional samples. In addition, some non-pathogenic protozoa can be considered opportunistic, as in particular situations, they can be responsible for intestinal symptoms [13].

Enteric parasites continue to contribute to the burden of preventable infectious diseases affecting humans in industrialized settings. Therefore, parasitological investigations are necessary and the minimum level consists of PSE, to which other targeted investigations should be added based on epidemiological and clinical suspicion.

This study highlights the epidemiology of intestinal parasitic infections, based above all on PSE and other direct tests, without indirect tests, in Italy and the state-of-the-art parasitological diagnostics in Italian laboratories for the detection of intestinal parasites, which includes microscopy, but also antigen tests, culture and molecular tests as there is no single technique, whose sensitivity and specificity allows to detect all intestinal parasites (trophozoites, cysts and oocysts of protozoa and eggs and larvae of helminths) [6,17,27].

Our data highlighted that not all diagnostic laboratories are able to perform targeted identification of specific parasites and therefore cannot guarantee proper parasitological diagnostic performances.

In this context, we emphasize the importance of a close collaboration between physicians and clinical microbiologists or parasitologists, with important skills in microscopy, that is warranted to improve parasitological diagnostics, thus ensuring a good quality service. We would like to underline also the usefulness of collecting clinical and epidemiological information through a form filled by the clinician that still few laboratories use today.

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