Intestinal parasitoses in a tertiary-care hospital located in a non-endemic setting during 2006-2010.

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ABSTRACT

Background.
The aim of this study was to assess the epidemiology of intestinal parasitoses during a 5-year period in patients attending a tertiary-care hospital in a non-endemic setting.

Methods.
In the period 2006-2010, 15,752 samples from 8,886 patients with clinically suspected parasitosis were subjected to macroscopic and microscopic examination, to parasitic antigen detection assays, and to cultures for protozoa and nematodes. Real-time PCR assays for the differentiation of Entamoeba histolytica and E. dispar and for the detection of Dientamoeba fragilis, respectively, were also used. A statistical analysis evaluating the demographic data of the patients with intestinal parasitic infections was performed.

Results.
Intestinal parasitic infections were diagnosed in 1,477 patients (16.6% prevalence), mainly adults and immigrants from endemic areas for fecal-oral infections; protozoa were detected in 93.4% and helminths in 6.6% of the cases, the latter especially in immigrants. Blastocystis hominis resulted the most common intestinal protozoan, and among pathogenic protozoa G. intestinalis was the most frequently detected, prevalent in immigrants, males, and pediatric patients. Both single (77.9%) and mixed (22.1%) parasitic infections were observed, the latter prevalent in immigrants.

Conclusions.
Despite the importance of the knowledge about the epidemiology of intestinal parasitoses for appropriate control measures and adequate patient care all over the world, data regarding industrialized countries are rarely reported in the literature. The data presented in this study indicating that intestinal parasitic infections are frequently diagnosed in our laboratory could
make a contribution to put the attention of physicians working in non-endemic areas on the importance of suspecting intestinal parasitoses.

Keywords: intestinal parasitosis, protozoa, helminths, epidemiology, diagnosis, Italy.
BACKGROUND

Gastrointestinal diseases caused by pathogenic protozoa and helminths are related to a significant amount of morbidity and mortality worldwide, particularly in children; 58 million infections by protozoa were registered every year in children, especially in developing countries as a consequence of the deficiencies in sanitation and limited access to drinking water [1-3]. In industrialized countries the populations at greatest risk for severe enteric parasitic infections are immunocompromised subjects [1,2,4].

On 2004, *Giardia* and *Cryptosporidium* were included in the “Neglected Diseases Initiative” of WHO that consists of a heterogeneous group of parasitic, bacterial and viral diseases mostly occurring in developing countries [1].

Although the prevalence of parasitic infections is higher in developing countries, intestinal parasitoses represent frequent diseases also in industrialized ones probably in association with globalization of the food supply, to immigration/adoption from endemic regions, and to travels through the same areas [5]. The risk of contracting parasitic infections, in particular from food, in the developed world is certainly lower than in developing countries because of the accompanying features of poverty. Nevertheless, the relatively mild or non-specific symptoms, long incubation periods, and unavailable or inadequate laboratory methods contribute to underestimate the prevalence of these infections also in industrialized regions [6]. Furthermore, in Europe, the control strategies are limited and only concern a few of the pathogens and most of the parasitic diseases are subjected to notification in some countries only [7]. Moreover, the preparation of physicians is often poor about these diseases as they are considered neglected [5].

The increased movements from/through run-down areas for immigration, tourism, work or religious mission could influence the epidemiology of intestinal parasitoses in the area in which our laboratory is located (Italy). Interestingly, the incidence of immigrants in Italy in 2008 was demonstrated to be 7.2% [8].
The aim of this study was to assess the epidemiologic picture of parasitic intestinal infections in our area during a 5-year period (2006-2010), using data obtained by the routinely diagnostic practice, also in order to make a contribution to the definition of the scenario in a non-endemic setting for intestinal parasitic diseases.
METHODS

Study area and population

The study was conducted at the University Hospital of Parma, a 1,218-bed tertiary care centre with more than 50,000 admissions registered in the year 2012 [9]. The province of Parma, located in the Northern Italy, has 445,283 inhabitants [10]; the population attending this hospital was estimated in 207,594 inhabitants, 10% of whom were immigrants from developing countries [11].

Patients, samples and conventional parasitologic assays

The laboratory diagnosis of intestinal parasitosis was performed on 15,722 faecal samples belonging to 8,886 patients, including both hospitalised and outpatients, sent during the period 2006-2010 to our laboratory with the suspicion of intestinal parasitosis. Neither healthy subjects nor people for screening of migrants were included in the study.

Seven thousand-eighty seven patients out of 8,886 were Italians and 1,799 immigrants from developing countries, 6,512 were adults, 1,819 children and for 555 the age was unknown, 3,969 were male and 4,917 were female. For the most of the Italian patients a travel history and/or risk factors for infections transmitted by faecal-oral route were not reported or not available. In Table 1 one further partition of Italian and immigrant patients related to age and sex is presented.

The samples analyzed in this study had been submitted to the University Hospital of Parma for routine diagnosis and no approval by the local review committee was required.

The diagnosis of intestinal parasitosis was performed according to standard procedures [12,13] by macroscopic examination of faeces and microscopic examination of wet mounts prepared from both fresh and concentrated faecal material after formalin-ethyl acetate sedimentation, as previously described [14-16] Simultaneously, an immunocromatographic
assay (IC) was performed as previously described [15] in order to detect specific antigens of *Cryptosporidium parvum* and *G. intestinalis* in faecal samples. Positive results by IC were confirmed by an immunofluorescence assay performed as previously described [15]. One thousand-six hundred-fifty-two samples belonging to 906 patients with reported diarrhea, abdominal pain, bloody faeces, eosinophilia, and/or risk factors for parasitic infections, and/or in whose faeces diagnostic stages of intestinal parasites were detected [16], were subjected also to culture for enteric protozoa in Robinson’s medium and to culture for larval stage-nematodes according to standard procedures, as already described [13,14]. Furthermore, in 116 cases of suspected osсиuriasis, a Scotch test [17] was performed to detect *Enterobius vermicularis* ova and/or adult worms.

**Molecular assays**

The 1,652 faecal samples (906 patients) subjected to cultures were also used to perform PCR assays. DNA was extracted partly by using the manual extraction system High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) as previously described [14], and partly by the automated evolution of the manual system (MagNA Pure LC DNA extraction kit III on the MagNA Pure LC instrument-Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions [16]. The extracted DNA was immediately used for PCR assays or stored at -20°C until analyzed. A conventional PCR assay and its evolution to a FRET (Fluorescence Resonance Energy Transfer) real-time PCR assay detecting and differentiating *Entamoeba histolytica* and *E. dispar* were alternatively performed as previously described [14]. Moreover, part of the 1,652 faecal samples (959 specimens belonging to 491 patients) were also subjected to a TaqMan real-time PCR assay for the detection of *D. fragilis*, as previously described [18].
A flowchart describing the algorithm for the diagnosis of intestinal parasitoses used in our laboratory is reported in Figure 1.

**Statistical analysis**

For all the patients, demographic data (origin, age, and sex) were collected and then related to the detected parasitic infections. Statistical significance of the figures of patients with intestinal parasitic infections into each demographic group was calculated by chi-square test: a p value <0.05, calculated by two-tailed test, was considered significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the strength of the associations that emerged. As concerns age data, the group of patients with age not known (555 subjects) was not included in the statistical analysis.
RESULTS

On the total of the 15,722 analyzed samples belonging to 8,886 patients, intestinal parasites were detected in 2,630 samples belonging to 1,477 patients, corresponding to a prevalence of patients with intestinal parasitoses of 16.6%. A statistical analysis concerning the patients with intestinal parasitoses diagnosed in this study in association to origin, age and sex is reported in Table 1.

On the total of the parasites detected in this study (1,915), present in the 1,477 patients with parasitic infections either single or in mixed combinations, protozoa were 1,789 (93.4%) and helminths were 126 (6.6%) (Table 2).

Table 2 reports the percentages reflecting the occurrence of the infections by protozoa and those by helminths in the population studied, not taking into account their involvement either in single or in mixed infections but considering the parasitoses (and subsequently the respective parasites involved) once at a time.

The most commonly detected intestinal protozoan was *Blastocystis hominis*, parasite whose pathogenic role is still controversial, and, as concerns the pathogenic ones, *G. intestinalis* was the most frequently detected among protozoa and *S. stercoralis* among helminths.

The parasites found in the patients with intestinal parasitic infections with mention to origin, age and sex, and their occurrence (reported as %), with a statistical analysis, are reported in Table 2.

Out of the total of the 1,477 patients with intestinal parasitoses, single parasitic infections were observed in 1,150 cases corresponding to 77.9% (Table 3), 65.91% Italian patients and 34.09% immigrant patients. The frequency of single infections in association with origin was 85% (758/892) in Italians and 97% (392/585) (OR 2.79; CI 2.16-3.58; p=0) in immigrants.

Mixed parasitic infections were observed in 327 cases corresponding to 22.1% out of the total of the patients with intestinal parasitic infections, 41% Italian patients and 59% immigrant.
patients. The frequency of mixed infections in association with origin was 15.02% (134/892) in Italians and 32.9% (193/585) (OR 2.79; CI 2.16-3.58; p<0.0025) in immigrants. Protozoa were present in single infections in 74.8% of the cases and in mixed infections in the 25.2% of the cases. Helminths were present in single infection in the 35.71% of the cases and in mixed infection in the 64.29% of the cases. Among mixed infections the most frequent combinations were \textit{B. hominis} and \textit{Entamoeba coli} (56), \textit{B. hominis} and \textit{D. fragilis} (54), \textit{B. hominis} and \textit{G. intestinalis} (44), \textit{B. hominis} and \textit{E. dispar} (20), \textit{B. hominis}, \textit{E. coli} and \textit{D. fragilis} (18). The majority of patients with mixed infections (235) had a parasitosis caused by 2 parasites. Seventy-seven patients presented with a mixed infection caused by 3 parasites. Eleven patients presented with 4 simultaneous parasitic infections, 3 patients with 5 simultaneous parasitic infections and 1 patient with 6 simultaneous parasitic infections, both by protozoa and helminths.
DISCUSSION

Many epidemiological data on the diffusion and the prevalence of intestinal parasitoses in humans are available for developing areas, but in industrialized countries intestinal parasitoses are usually not notified and few data are reported in the literature. In Italy recent epidemiological reports are restricted to the analysis of few parasitic agents [i.e. 19] or to a selected population [i.e. 8,20], except for a study describing the presence of intestinal parasites isolated in a large teaching hospital located in Rome during a period of 32 months [5].

This is the first study reporting the occurrence of intestinal parasitic infections in a non-endemic setting investigated by using data obtained by parasitological examination performed daily on samples belonging to patients with clinical suspicion of parasitosis based on several abdominal disorders, admitted to our University Hospital during a 5-year period.

These data allowed obtaining a real scenario of our area in the view of continuous changes in the composition of the population and in the habits in order to make a contribution to put the attention of both physicians and microbiologists on the importance of suspecting and diagnosing intestinal parasitoses. Furthermore the data could be representative of the whole Italian and the European scenario, that are likely comparable to our setting in terms of risk of transmission of intestinal parasites by faecal-oral route.

The prevalence of intestinal parasitic infections detected in this study (16.6%) was unexpectedly high in a non-endemic area for infections with a parasitic aetiology transmitted by faecal-oral route. Nevertheless, our laboratory receives samples from individuals who immigrate from or travel to developing countries and presenting risk factors for acquiring parasitic infections including malaria [21], proving that human flows from and to our area are correlated with importation of parasitic agents. Interestingly, 6 patients with intestinal parasitic infections (4 cases by B. hominis, 1 case by E. dispar and G. intestinalis, 1 case by A. lumbricoides and T. trichiura) reported also concomitant infections by plasmodia [22].
Among the group of patients evaluated in this study a significant difference in the prevalence of parasitic infections emerged in adults, for whom it was 1.4 times higher than in children, and in immigrants, for whom it was 3.35 times higher than in Italians. The difference between males and females was not remarkable.

In our study *B. hominis*, often reported as the most commonly detected organism in parasitological surveys [5], resulted the most frequently detected among intestinal protozoa. In our setting the prevalence of *B. hominis* is significantly higher in immigrants than in Italians (about 3 times) and in adults than in children (1.76 times) all having intestinal symptoms. Despite its role in pathogenesis is controversial [23], epidemiological data about the prevalence of *B. hominis* in the analyzed population were essential to state that a faecal-oral route subsists in our area and this study made a contribution to unravel this scenario.

*G. intestinalis* is known as the most common enteric protozoan pathogen of humans, domestic animals and wildlife, having a more relevant prevalence in warm climate and in children [15], particularly those living in developing countries and in disadvantaged community settings [24]. In Italy giardiasis is a not notifiable disease and prevalence data are based on specialised studies reporting percentage of infection ranging from 0.9% to 2.41% [19]. In this study, this epidemiological trend was confirmed being *G. intestinalis* the second parasite detected in the analyzed population with a prevalence of 1.89%, similarly to previously reported data [i.e. 5]. The prevalence of *G. intestinalis* was significantly higher in immigrants than in Italians (about 4 times) and in males than in females (1.89 times). Interestingly, as expected giardiasis was more frequent in paediatric patients than in adult ones (2.35 times).

In general, the results reported in this study showed that in the case of parasitoses by protozoa the infection rate was significantly higher in males than in females (1.20 times) and in immigrants than in Italians (4.53 times). Concerning the frequency of the protozoa, the infection rate was significantly higher in children than in adults, except for *B. hominis*. 
It is noteworthy that in this study a whole of 73 patients were diagnosed with infection by the pathogenic species *E. histolytica* or the non-pathogenic species *E. dispar*, microscopically not distinguishable: in these cases species differentiation was accomplished by PCR that revealed *E. histolytica* infection in 4 cases and *E. dispar* infection in 69 cases, becoming an essential tool allowing to focus on *E. histolytica* infections permitting the administration of a targeted therapy only in those cases and avoiding the treatment in the cases of *E. dispar* infections.

In our study helminthic infections resulted lower in frequency than protozoan ones (93.4% vs. 6.6% on the total of detected parasites); the higher prevalence of parasitoses and in particular of helminthiasis in immigrants (2.23 times as compared to that of Italians) is not unexpected since it is known that helminthic infections are most frequent in the population living in developing countries and in immigrants from those areas [25]. Whilst the most of epidemiologic research focused on the occurrence of helmintic infections depending on age, revealing that changes with age in the average intensity of infection tend to be convex, rising in childhood and declining in adulthood [5], interestingly our data did not show any difference of the prevalence of helminthiasis as concerns age group of patients.

In our setting mixed parasitic infections proved to weigh considerably on the global epidemiology (22.7%), especially in the population from developing countries. These data confirmed those reported in other industrialized countries such as North America and Europe where parasitic infections are most prevalent within immigrant and refugee communities [26]. Furthermore, statistically evaluating the association of the origin of the patients and the occurrence of mixed infections, the higher frequency (2.79 times) in immigrant group compared to that of Italian group resulted significant.

**CONCLUSIONS**

Knowledge about the epidemiology of parasitic infections becomes an essential tool in non-endemic areas in order to provide appropriate control measures and adequate patient care,
underlining the importance that intestinal parasitoses should be considered in the differential
diagnosis of gastrointestinal diseases.

The data reported herein could be useful to physicians working in non-endemic areas with the
aim of increasing their attention during the anamnesis about the concrete possibility of
intestinal parasitoses for patients reporting signs and symptoms and/or risk factors consistent
with this suspicion.

Moreover, the data reported in this study could be also useful to parasitologists to obtain
information suitable to plan the adoption of appropriate tools to achieve an accurate
laboratory diagnosis of parasitic infections.
LIST OF ABBREVIATIONS

IC: Immunocromatographic Assay
FRET: Fluorescence Resonance Energy Transfer
OR: Odds ratio
CI: Confidence Interval

LEGEND TO FIGURES:

Figure 1. Flowchart of the algorithm for the diagnosis of intestinal parasitoses used in our laboratory.

Legend: IC= Immunocromatographic assay; IF= Immunofluorescence; POS= positive; NEG= negative.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

AC, SM, SR, CG conceived the study.
AC, SM, SR, CG designed the study protocol.
AC, CC carried out the clinical assessment.
AC, CC, CG, SR, FD, MCM, MCA carried out the analysis and interpretation of the data.
AC, CG, SM drafted the manuscript.
AC, CC critically revised the manuscript for intellectual content.
All authors read and approved the final manuscript: AC, CC, MCM, FD, MCA are guarantors of the paper.
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Faecal sample

Microscopic examination (fresh and concentrated faecal material)

IC assay: - Giardia intestinalis - Cryptosporidium parvum

When reported in the medical order: Diarrhea, abdominal pain, bloody faeces, eosinophilia, and/or risk factors for parasitic infections

Diagnostic stages of intestinal parasites observed

Culture for larval stage-nematodes

Culture for enteric protozoa in Robinson’s medium

Entamoeba histolytica and E. dispar real-time PCR assay and/or D. fragilis real-time PCR assay

Figure 1
Additional files provided with this submission:

Additional file 1: Table 1 revision.doc, 36K
http://www.biomedcentral.com/imedia/8292689941215210/supp1.doc
Additional file 2: Table 2 revision.doc, 178K
http://www.biomedcentral.com/imedia/1218028975121521/supp2.doc
Additional file 3: Table 3 revision.doc, 112K
http://www.biomedcentral.com/imedia/5636027061215211/supp3.doc