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Affiliations

Giulia Martelli*

Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy

Via Massarenti, 40138, Bologna, Italy

giuliamartelli84@gmail.com

Telephone number: +393398088116

Fax number: +390516363983

Chiara Di Girolamo*

1) Centre for International Health, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy
2) Cancer Survival Group, London School of Hygiene and Tropical Medicine, London, UK

Lorenzo Zammarchi

1) Infectious and Tropical Diseases Unit, Careggi University Hospital, Florence, Italy

2) Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Andrea Angheben
Centre for Tropical Diseases, Sacro Cuore – Don Calabria Hospital, Negrar, Verona, Italy

Matteo Morandi
Infectious Risk Area - Social and Health Agency of Emilia-Romagna Region, Bologna, Italy

Stefano Tais
Service of Epidemiology and Laboratory for Tropical Diseases, Sacro Cuore – Don Calabria Hospital, Negrar, Verona, Italy

Monica Degani
Service of Epidemiology and Laboratory for Tropical Diseases, Sacro Cuore – Don Calabria Hospital, Negrar, Verona, Italy

Issa El Hamad
Outpatient clinic for undocumented immigrants - Local Health Authority of Brescia, Italy

Silvio Caligaris
Clinic of Infectious and Tropical Diseases, ASST Spedali Civili di Brescia, Brescia, Italy

Anna Ciannameo
Centre for International Health, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy

Elisabetta Grilli
Clinical and Research Department, National Institute for Infectious Diseases “Lazzaro Spallanzani”, IRCCS-Rome, Italy

Lucia Urbinati
Clinic of Infectious and Tropical Diseases, ASST Spedali Civili di Brescia, Brescia, Italy

Geraldo Badona Monteiro
Centre for Tropical Diseases, Sacro Cuore – Don Calabria Hospital, Negrar, Verona, Italy

Carmelo Scarcella
Outpatient clinic for undocumented immigrants - Local Health Authority of Brescia, Italy

Nicola Petrosillo
Clinical and Research Department, National Institute for Infectious Diseases “Lazzaro Spallanzani”, IRCCS-Rome, Italy

Margherita Digaetano
1) Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy
2) Infectious Disease Clinic, Azienda Ospedaliero-Universitaria of Polyclinic of Modena, Modena, Italy

Lorenzo Rabbi

Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy

Noemi Bazzanini

1) Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy
2) Unit of Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria of Parma, Parma, Italy

Francesca Cacciatore

Centre for International Health, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy

Brigida Lilia Marta

Centre for International Health, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy

Maria Luisa Moro

Infectious Risk Area - Social and Health Agency of Emilia-Romagna Region, Bologna, Italy

Alessandro Bartoloni

1) Infectious and Tropical Diseases Unit, Careggi University Hospital, Florence, Italy
2) Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Pierluigi Viale

Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy

Gabriella Verucchi

Infectious Diseases Unit, Department of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, Bologna, Italy

*GM and CDG equally contributed to the writing of the paper

Abstract

Objective: This multicentre cross-sectional study aims to estimate the prevalence of five neglected tropical diseases (Chagas disease, filariasis, schistosomiasis, strongyloidiasis, toxocariasis) among immigrants accessing health care facilities in five Italian cities (Bologna, Brescia, Florence, Rome, Verona).

Methods: Individuals underwent a different set of serological tests, according to country of origin and presence of eosinophilia. Seropositive patients were treated and further followed up.

Results: A total of 930 adult immigrants were enrolled: 477 men (51.3%), 445 women (47.9%), 8 transgender (0.8%); median age was 37.81 years (range 18-80). Most of them were coming from the African continent (405/930, 43.5%), the rest from East Europe, South America and Asia. A portion of 9.6% (89/930) were diagnosed with at least one of the infections under study. Seroprevalence of each specific infection varied from 3.9% (7/180) for Chagas diseases to 9.7% (11/113) for toxocariasis. Seropositive people were more likely to be 35 to 40 years-old male and to come from South East Asia, Sub-Saharan Africa or South America.

Conclusions: The results of our study confirm that neglected tropical diseases represent a substantial health problem among immigrants and highlight the need for addressing this emerging public health issue.

Introduction

The World Health Organization (WHO) defines the neglected tropical diseases (NTDs) as a diverse group of infections mainly affecting poor populations, increasing poverty, and having a low priority in the political and scientific agenda [1].

Human migration is a key factor in the appearance/re-appearance of NTDs in non or former endemic contexts [2] [3]. We live in an era of unprecedented human mobility, with approximately 232 million international migrants and 740 million internal migrants worldwide [4]: Eurostat estimated that a total of 3.4 million people migrated to one of the European Community countries in 2013; half of them came from non-member countries [5]. In 2014, immigrants accounted for the eight per cent of the total Italian resident population [6]; another 326,000 undocumented immigrants and refugees were present in Italy. [7].

Immigrants are generally young and in good health conditions [8], nevertheless, the prevalence of some infectious diseases may be significant among immigrants, as a result of the wide diffusion of these conditions in their countries of origin [9] and the further exposure during migration [10] [3]. Many infections, including several NTDs, may be asymptomatic and hence remain undiagnosed [11]. As a consequence, seropositive individuals can develop chronic forms (e.g. Chagas disease, schistosomiasis), fatal complications (e. g. Chagas disease, schistosomiasis or strongyloidiasis) and can potentially transmit the disease [11].

The research on the burden of communicable diseases among immigrants in Western countries mainly focuses on HIV, tuberculosis and viral hepatitis [8] [12]. NTDs were rarely addressed, possibly because they are often asymptomatic and have a relatively low transmission in the absence of environmental and biological reservoirs/vectors. Moreover in most of the European countries there is no systematic mandatory regulation regarding NTDs reporting and surveillance [13]. Given the considerable immigration flows to Italy and the scarce information on the relevance of the NTDs, seldom considered at hospital level, this study aims to estimate the seroprevalence of five NTDs (Chagas disease, filariasis, schistosomiasis, strongyloidiasis and toxocariasis) among immigrants attending

hospitals and relative outpatient clinics in five Italian cities: Bologna, Brescia, Florence, Rome, and Verona.

Methods

Study population, data collection and patient management

A cross-sectional survey was performed in five Italian infectious and tropical diseases units located in five different hospitals (Bologna University Teaching Hospital; Florence University Teaching Hospital; Hospital Sacro Cuore – Negrar, Verona; Spedali Civili General Hospital, Brescia; L. Spallanzani University Teaching Hospital, Rome) and in one outpatient clinic for undocumented immigrants (Brescia Local Health Authority outpatient clinic). In the hospital setting, patients were usually referred from primary care, Emergency Departments or other secondary care services; they were either inpatients or individuals with chronic infections followed-up in specialised outpatient clinics.

Individuals who attended any of the above mentioned centres for any reason in the study period (November 2012 to November 2014) and who were born in an endemic country (see online appendix Annex 1 for details), older than 18 years and with sufficient knowledge of Italian or a timely access to a linguistic mediator, were eligible. In each clinical centre one or two investigators were responsible for offering participation to the project to each eligible patient seeking care during any of their routine clinical activities. After signing the informed consent, enrolled patients underwent a different set of serological tests according to the criteria reported in online appendix Table 1. The choice of the infections was based on the most common areas of origin of immigrants in Italy, the potential severity of the disease if not treated, the availability and quality of diagnostic tools, the amenability to treatment and the potential for spreading in the community. The definition of endemic country for a certain infection was based on the WHO geographical

188 classification of NTDs [14] (see online appendix Annex 1 for details). For filariasis, only
189 endemic countries for lymphatic filariasis, onchocerciasis and loiasis were included.
190 Serology for toxocariasis and filiariasis was limited to individuals with eosinophilia These
191 two diseases usually present with a raised eosinophil count [15]. However, in order to
192 increase case detection, eosinophil cut-off level was set at 300/µL instead of 450-500/µL,
193 as routinely suggested [15], for its good positive predictive value for helminthiases [16].
194 Concurrently, clinical and socio-demographic information, including country of origin, list of
195 visited countries, time since arrival, and educational level were collected. The investigators
196 offered treatment and follow-up to seropositive patients, while they supplied seronegative
197 individuals with the results of their tests. The centres elaborated operational guidelines for
198 the management of each disease which were made available on the study website.
199 Study protocol was approved by the ethics committee of the coordinating site (Bologna
200 University Teaching Hospital) under the resolution number 124/2012/O/Oss and by those
201 of all other participating units.
202
203 *Microbiological diagnosis procedures*
204 A sample of 12 ml of venous blood was collected from each participant. Blood samples
205 were centrally tested at the Service of Epidemiology and Laboratory for Tropical Diseases
206 of the Hospital Sacro Cuore – Don Calabria, Negrar in order to reduce inter-laboratory
207 variability. Serum samples were tested for specific antibodies using commercial
208 immunoenzymatic assays according to manufacturer's instructions. The qualitative
209 presence of antibodies for *Trypanosoma cruzi* (etiological agent of Chagas disease) was
210 tested employing two enzyme-linked immunosorbent assays (ELISA), one based on
211 recombinant antigens (“BioELISA Chagas”, Biokit, Lliça d′Almunt, Spain), the other based
212 on crude antigens (“BioELISA Chagas III”, BiosChile, Santiago, Chile). For the other
213 infections a single ELISA was used (“Filariasis ELISA kit”, Bordier Affinity Products SA,

Crissier, Switzerland, for filariasis; "*Schistosoma mansoni* ELISA kit", Bordier Affinity Products SA, Crissier, Switzerland for schistosomiasis; "Strongyloidiasis ELISA kit" based on *Strongyloides ratti* antigens, Bordier Affinity Products SA, Crissier, Switzerland for stronglyloides; "DRG *Toxocara canis* ELISA", DRG Instruments GmbH, Marburg, Germany, for toxocariasis).

Statistical analysis

Categorical variables were described through frequencies and the median and the ranges were used to describe age. Countries of origin were subsequently grouped into 11 regions following the Geosentinel classification [17]. This choice relies on the fact that Geosentinel system splits the globe into a higher number of regions (eleven) than WHO (six), with more precise identification of risk areas.

Prevalence point estimates and their 95% confidence intervals were obtained. Chi-square tests were performed to assess differences between groups. Data were managed and analysed using STATA 14.1.

Results

Description of the study population

From November 2012 to November 2014 a total of 930 individuals were enrolled across the six centres. Two thirds of them were outpatients. Due to refused consent or scarce knowledge of Italian/lack of linguistic mediator, 4.9% (48/978) of the individuals were not enrolled.

Socio-demographic information of the enrolled population is summarized in online appendix Table 2. The male-to-female ratio was 1:1, and the median age was 37.8 years (range 18-80); almost half of the participants had been living in Italy for more than 10

240 years. Individuals coming from the African region represented 43.5% of the total (405/930);
241 other frequent areas of origin were Eastern Europe (197/930, 21.2%), South and Central
242 America (177/930, 19.0%) and Asia (142/930, 15.3%). More than a half of patients
243 declared a medium or high level of education (high school diploma or degree). The socio-
244 demographic profile of the individuals varied slightly across the six centres. In the clinic for
245 undocumented immigrants in Brescia, enrolled subjects were younger than the total
246 population (median age of 35.2 versus 37.8 years, age range of 18-64 versus 18-80) and
247 their time since arrival was slightly shorter (50.9%, 56/110, of them arrived in the last four
248 years versus 31.3%, 290/930, in the total). Differences across the centres in terms of
249 origin might mirror the differing immigrant flows to the Italian cities: despite the high
250 presence of African immigrants in the whole sample, individuals enrolled in the Roman
251 hospital were mainly South Americans and the ones enrolled in Bologna mainly came from
252 Eastern Europe.
253 A white blood cell count was available for 583 individuals: among them, 19.4% (113) had
254 eosinophilia.
255
256 *Seroprevalence of the neglected infectious diseases*
257 Among the 930 enrolled individuals, 96 new infections were detected: 42 cases of
258 strongyloidiasis, 31 of schistosomiasis, 11 of toxocariasis, 7 of Chagas disease, and 5 of
259 filariasis. Eighty-nine patients were diagnosed with one or more NTDs, which leads to an
260 overall seroprevalence of 9.6% (95%CI 7.8-11.6) in the study population. Seven
261 individuals had two infections simultaneously. Across the centres the prevalence varied
262 between 6.3%, 7/110, (in Brescia clinic for undocumented immigrants) and 15.3%, 30/193,
263 (in Verona).
264 Seropositive individuals were mostly men (M:F=2:1) with a median age of 38.8 years
265 (range 21-78). The seroprevalence was twice as high in men as in women (p-value<0.05)

for all infections except for Chagas disease. The Geosentinel region with the highest NTDs prevalence was South East Asia, followed by Sub-Saharan Africa and South America.

Among the 189 patients who were known to be HIV positive, 14 (7.4%) were also seropositive for at least one of the NTDs under study (8 cases of strongyloidiasis, 4 cases of schistosomiasis, 2 cases of toxocariasis and 1 case of Chagas disease).

Global seroprevalence, women to men ratio and regions with highest prevalence are shown in Table 1. Detailed prevalence estimated by infections and Geosentinel regions are listed in online appendix Table 3.

Discussion

Approximately one out of 10 individuals in our study was seropositive for at least one of the infections. This figure represents a considerable burden given the potential consequences of these conditions. In particular, strongyloidiasis and Chagas disease can lead to chronic infections, which might represent a serious threat for the individual and the health systems [18]. Strongyloidiasis is responsible of the hyperinfection syndrome, a rare life-threatening complication that mainly affects immunosuppressed individuals, thus early detection and treatment are particularly relevant [19]. Similarly, the potential transmissibility of Chagas disease outside endemic areas, through blood transfusions, organ or tissue transplants, or mother-to-child, highlights the importance of its early detection [20]. Furthermore, most of these infections are treatable with affordable and generally well-tolerated therapies, especially when compared to the severity of the untreated consequences [21].

Interestingly, we noticed a higher prevalence of the five neglected infections among patients coming from South East Asia, Sub-Saharan Africa and South America, suggesting that immigrants coming from these areas are most at risk. However, this broad geographic subdivision may mask differences at country level which we were not able to account for,

292 given the limited sample size. The prevalence of all infections was twice as high in men as
293 in women (p-value<0.05) but Chagas disease. A potential higher environmental and
294 working exposure risk for intestinal parasites and other vector-borne infections among
295 male immigrants can contribute to explain this finding [22]. Age and time since arrival in
296 Italy were not associated with the presence of infections.
297 These results are in line with the findings of similar studies carried out in Spain [8] [23],
298 except for Chagas disease prevalence, which was much higher in the Spanish samples.
299 This difference may be explained by the larger proportion of enrolled Latin American
300 subjects in the Spanish studies, as a consequence of a different migration pattern and the
301 availability of widespread screening programs. A cross sectional study carried out in
302 Australia [11] among recent immigrants and two others conducted in the United States in
303 refugees [9] [24] reported a higher prevalence of intestinal parasitic infections than in our
304 study. These figures may be explained by the different population under study and by our
305 diagnostic approach based on antibody detection. The mentioned studies mainly enrolled
306 refugees and recent immigrants and used microscopic examination of the stools,
307 identifying also parasites for which no serological test is available.
308 As already reported by others [25], we noticed a significant seroprevalence of NTDs in the
309 subgroup of patients with a known HIV infection. In this subgroup of patients treatment
310 should be strongly recommended, because of the risk of severe complications especially
311 in the case of strongyloidiasis and Chagas disease [25].
312 In our study, the proportion of individuals with an increased eosinophil count was in line
313 with other studies [26] [15], despite a possible overestimation due to the lower cut-off level
314 for an abnormal eosinophil count. Eosinophilia generally occurs in approximately 10% of
315 individuals returning from the tropics [15], and a prevalence up to 30% is often reported
316 among immigrant populations [26]. In this last group helminthic infections are the
317 commonest identifiable cause of eosinophilia, accounting for 14% to 64% of the total

318 cases [15]. Among those who were screened for strongyloidiasis, positive patients were
319 more likely to have a high eosinophil count (data not shown). This result confirms what
320 was previously found and emphasizes the importance of investigating the presence of *S.*
321 *stercoralis* among immigrants, particularly in presence of raised eosinophil levels [15].
322 Whilst for Chagas disease and schistosomiasis serology is deemed to be a valid screening
323 tool [8], there is no standard method for the detection of other intestinal parasites [27].
324 When stool microscopy had been used for screening purpose, the prevalence was found
325 to be relatively low [8] [23]. Amongst intestinal parasites we focused on strongyloidiasis,
326 because of its potentially fatal complications in immunosuppressed populations and to its
327 long-term persistence in the host [28].
328 The main limitation of the study is its generalizability. The enrolment took place at hospital
329 level and not at the community level; this means that our results may not be entirely valid
330 for the general immigrant population. Individuals who access the health services may be
331 those who have been in the host country for a longer time; they may differ from the general
332 population in terms of socio-demographic characteristics and, therefore, may have a
333 different risk profile for the infections. Moreover, no formal sample size calculation was
334 performed; some prevalence estimates on specific infections and subpopulations showed
335 a great uncertainty due to the small sample size of these groups.
336 Additionally, a selections bias cannot be ruled out. Enrolment in the study completely
337 relied on the staff dedicated to the project. It is likely that a proportion of patients eligible
338 for the study had been missed; however, they should not have been systematically
339 different from those enrolled because the physicians responsible for the enrolment
340 covered different care settings within their units. Moreover, not all the patients who were
341 asked to take part in the study gave their consent. The proportion of patients who did not
342 participate despite their eligibility was less than five percent in all the centres suggesting
343 that this source of bias is not substantial.

Another limitation of the study lies in the exclusive use of serology to estimate the prevalence of selected NTDs in immigrants. As a matter of facts, these tests may not distinguish between prior infection and active disease since antibodies may persist for many months to years after successful treatment in most of the NTDs evaluated and these tests are prone to cross-reactions with other parasite antigens [29]. Additional tests on stool or urine samples would have certainly increased the diagnostic sensitivity but were deemed not be feasible, given the logistic arrangement of the study that relied on a centralised laboratory. Since the therapies for the infections under study are mostly short-term and well-tolerated, we opted for treating all seropositive patients, except those affected by *T. cruzi* infection, who underwent disease staging before treatment according to current agreements [30].

Despite these limitations, our findings highlight the importance of tackling the NTDs challenge in a non-endemic setting. The call for systematic detection and appropriate management is even more urgent because it has been reported that health professionals rarely consider these diseases. As a consequence, NTDs are highly likely to be underdiagnosed at present, or diagnosed too late or inefficiently managed [31]. As previously suggested in the European context, screening protocols seem to be a sensible option [32]. A presumptive anthelminthic therapy for immigrants coming from areas at high risk had been previously demonstrated to be cost effective in certain setting [27]. However, this approach is not free from drawbacks, including toxicity, under-treatment of certain infections [33] and risk of focusing on a single medical intervention while neglecting a proper follow-up and a more comprehensive approach to migrants' health.

The importance of diagnosing and treating these infections is crucial among immunosuppressed patients (for example those receiving chemotherapy, chronic steroid or immunosuppressive treatment) and donors/recipients of solid organ and hematopoietic stem cell transplantation, as well as blood transfusion [34]. Indeed, the rising success and

adoption of transplantation reasonably increases the proportion of the immigrant population who will become donor/recipient of organ transplantation and blood/hemoderivates in destination countries. Many of the pathogens that cause NTDs can be either reactivated during immunosuppression or transmitted via organ graft or blood transfusions [34], making a screening approach in these contexts life-saving.

We declare that we have no conflicts of interests.

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Infection	Numerator and denominator	Overall prevalence (95% CI)	Women to men ratio	Geosentinel Regions with highest prevalence		
				Geosentinel Region	***Numerator and denominator***	***Region-specific prevalence (95% CI)***
Strongyloidiasis	42/939	4.51% (3.35-6.05)	1:2	North East Asia	2/31	6.45% (1.48-23.93)
				Sub-Saharan Africa	20/330	6.06% (3.93-9.22)
				South Central Asia	5/90	5.55% (2.29-12.85)
Schistosomiasis	31/519	5.97% (4.22-8.37)	1:2.8	Sub-Saharan Africa	25/323	7.73% (5.27-11.22)
				South America	3/46	6.52% (2.02-19.05)
				South Central Asia	1/34	2.94% (0.37-19.77)
Chagas disease	7/180	3.88% (1.85-7.98)	2.5:1	South America*	7/172	4.06% (1.93-8.34)
Toxocariasis	11/113	9.73% (5.42-16.86)	1:1.8	South East Asia	2/6	33.33% (4.18-85.13)
				South America	4/19	21.05% (7.33-47.32)
				Eastern Europe	3/24	6.89% (3.73-34.48)
Filariasis	5/54	9.25% (7.83-11.63)	1:1.5	Sub-Saharan Africa*	5/34	14.70% (5.96-31.91)

* Only one region is reported because the infection was not found in people coming from other areas