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Seroprevalence of five neglected parasitic diseases among immigrants accessing five infectious and tropical diseases units in Italy: a cross-sectional study

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Seroprevalence of five neglected parasitic diseases among immigrants accessing five infectious and tropical diseases units in Italy: a cross-sectional study

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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

classification of NTDs [14] (see online appendix Annex 1 for details). For filariasis, only endemic countries for lymphatic filariasis, onchocerciasis and loiasis were included. Serology for toxocariasis and filariasis was limited to individuals with eosinophilia. These two diseases usually present with a raised eosinophil count [15]. However, in order to increase case detection, eosinophil cut-off level was set at 300/ μ L instead of 450-500/ μ L, as routinely suggested [15], for its good positive predictive value for helminthiases [16]. Concurrently, clinical and socio-demographic information, including country of origin, list of visited countries, time since arrival, and educational level were collected. The investigators offered treatment and follow-up to seropositive patients, while they supplied seronegative individuals with the results of their tests. The centres elaborated operational guidelines for the management of each disease which were made available on the study website. Study protocol was approved by the ethics committee of the coordinating site (Bologna University Teaching Hospital) under the resolution number 124/2012/O/Oss and by those of all other participating units.

Microbiological diagnosis procedures

A sample of 12 ml of venous blood was collected from each participant. Blood samples were centrally tested at the Service of Epidemiology and Laboratory for Tropical Diseases of the Hospital Sacro Cuore – Don Calabria, Negrar in order to reduce inter-laboratory variability. Serum samples were tested for specific antibodies using commercial immunoenzymatic assays according to manufacturer's instructions. The qualitative presence of antibodies for *Trypanosoma cruzi* (etiological agent of Chagas disease) was tested employing two enzyme-linked immunosorbent assays (ELISA), one based on recombinant antigens ("BioELISA Chagas", Biokit, Lliça d'Almunt, Spain), the other based on crude antigens ("BioELISA Chagas III", BiosChile, Santiago, Chile). For the other 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

years. Individuals coming from the African region represented 43.5% of the total (405/930); other frequent areas of origin were Eastern Europe (197/930, 21.2%), South and Central America (177/930, 19.0%) and Asia (142/930, 15.3%). More than a half of patients declared a medium or high level of education (high school diploma or degree). The socio-demographic profile of the individuals varied slightly across the six centres. In the clinic for undocumented immigrants in Brescia, enrolled subjects were younger than the total population (median age of 35.2 versus 37.8 years, age range of 18-64 versus 18-80) and their time since arrival was slightly shorter (50.9%, 56/110, of them arrived in the last four years versus 31.3%, 290/930, in the total). Differences across the centres in terms of origin might mirror the differing immigrant flows to the Italian cities: despite the high presence of African immigrants in the whole sample, individuals enrolled in the Roman hospital were mainly South Americans and the ones enrolled in Bologna mainly came from Eastern Europe.

A white blood cell count was available for 583 individuals: among them, 19.4% (113) had eosinophilia.

Seroprevalence of the neglected infectious diseases

Among the 930 enrolled individuals, 96 new infections were detected: 42 cases of strongyloidiasis, 31 of schistosomiasis, 11 of toxocariasis, 7 of Chagas disease, and 5 of filariasis. Eighty-nine patients were diagnosed with one or more NTDs, which leads to an overall seroprevalence of 9.6% (95%CI 7.8-11.6) in the study population. Seven individuals had two infections simultaneously. Across the centres the prevalence varied between 6.3%, 7/110, (in Brescia clinic for undocumented immigrants) and 15.3%, 30/193, (in Verona).

Seropositive individuals were mostly men (M:F=2:1) with a median age of 38.8 years (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,

given the limited sample size. The prevalence of all infections was twice as high in men as in women ($p\text{-value}<0.05$) but Chagas disease. A potential higher environmental and working exposure risk for intestinal parasites and other vector-borne infections among male immigrants can contribute to explain this finding [22]. Age and time since arrival in Italy were not associated with the presence of infections.

These results are in line with the findings of similar studies carried out in Spain [8] [23], except for Chagas disease prevalence, which was much higher in the Spanish samples. This difference may be explained by the larger proportion of enrolled Latin American subjects in the Spanish studies, as a consequence of a different migration pattern and the availability of widespread screening programs. A cross sectional study carried out in Australia [11] among recent immigrants and two others conducted in the United States in refugees [9] [24] reported a higher prevalence of intestinal parasitic infections than in our study. These figures may be explained by the different population under study and by our diagnostic approach based on antibody detection. The mentioned studies mainly enrolled refugees and recent immigrants and used microscopic examination of the stools, identifying also parasites for which no serological test is available.

As already reported by others [25], we noticed a significant seroprevalence of NTDs in the subgroup of patients with a known HIV infection. In this subgroup of patients treatment should be strongly recommended, because of the risk of severe complications especially in the case of strongyloidiasis and Chagas disease [25].

In our study, the proportion of individuals with an increased eosinophil count was in line with other studies [26] [15], despite a possible overestimation due to the lower cut-off level for an abnormal eosinophil count. Eosinophilia generally occurs in approximately 10% of individuals returning from the tropics [15], and a prevalence up to 30% is often reported among immigrant populations [26]. In this last group helminthic infections are the commonest identifiable cause of eosinophilia, accounting for 14% to 64% of the total

cases [15]. Among those who were screened for strongyloidiasis, positive patients were more likely to have a high eosinophil count (data not shown). This result confirms what was previously found and emphasizes the importance of investigating the presence of *S. stercoralis* among immigrants, particularly in presence of raised eosinophil levels [15].

Whilst for Chagas disease and schistosomiasis serology is deemed to be a valid screening tool [8], there is no standard method for the detection of other intestinal parasites [27]. When stool microscopy had been used for screening purpose, the prevalence was found to be relatively low [8] [23]. Amongst intestinal parasites we focused on strongyloidiasis, because of its potentially fatal complications in immunosuppressed populations and to its long-term persistence in the host [28].

The main limitation of the study is its generalizability. The enrolment took place at hospital level and not at the community level; this means that our results may not be entirely valid for the general immigrant population. Individuals who access the health services may be those who have been in the host country for a longer time; they may differ from the general population in terms of socio-demographic characteristics and, therefore, may have a different risk profile for the infections. Moreover, no formal sample size calculation was performed; some prevalence estimates on specific infections and subpopulations showed a great uncertainty due to the small sample size of these groups.

Additionally, a selection bias cannot be ruled out. Enrolment in the study completely relied on the staff dedicated to the project. It is likely that a proportion of patients eligible for the study had been missed; however, they should not have been systematically different from those enrolled because the physicians responsible for the enrolment covered different care settings within their units. Moreover, not all the patients who were asked to take part in the study gave their consent. The proportion of patients who did not participate despite their eligibility was less than five percent in all the centres suggesting 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 anthelmintic 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		
				<i>Geosentinel Region</i>	<i>Numerator and denominator</i>	<i>Region-specific prevalence (95% CI)</i>
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