



## Use of serology in a systematic screening programme for strongyloidiasis in an immigrant population



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### ABSTRACT

**Objectives:** The aim of this cross-sectional study was to describe the results of a systematic serological screening programme for strongyloidiasis.

**Methods:** A prospective serological screening programme for strongyloidiasis was performed between 2009 and 2014 for all immigrant patients attending the Tropical Medicine Unit. Three formalin-ether concentrated stool samples and an ELISA for anti-*Strongyloides stercoralis* antibodies were used as screening tools.

**Results:** Of 659 patients screened, 79 (12%) were positive for *S. stercoralis* regardless of the diagnostic method used. The prevalence of infection was 42.9% in East African patients, 16.3% in Central African patients, 10.9% in those from South America, and 10% in the case of West Africa. Univariate analysis showed that infection by *S. stercoralis* was significantly more frequent in patients from Central Africa ( $p = 0.026$ ; OR 1.72, 95% CI 1.03–2.85) and East Africa ( $p < 0.001$ ; OR 5.88, 95% CI 1.75–19.32). Taking West Africa as the reference (as the area of lowest prevalence among the positive prevalence areas), the statistical analysis showed that the risk of infection was higher in East Africa ( $p = 0.001$ ; OR 6.750, 95% CI 2.127–21.423) and Central Africa ( $p = 0.065$ ; OR 1.747, 95% CI 0.965–3.163).

**Conclusions:** Due to the potential complications of strongyloidiasis infection, we recommend that immigrant patients from developing countries be routinely screened for *S. stercoralis*, especially those from East Africa. A serological test is a highly appropriate screening tool.

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### Introduction

Strongyloidiasis is a soil-transmitted helminth infection caused by the nematode *Strongyloides stercoralis* and is endemic mainly in tropical and subtropical regions, but also in temperate regions (Schär et al., 2013). This parasitosis is widely extended all over the world. It is estimated that at least 370 million individuals are infected worldwide, with a high degree of endemic burden in the

tropical zones, especially Southeast Asia, Sub-Saharan Africa, and Latin America (Schär et al., 2013; Monge-Maillou et al., 2018; Bisoffi et al., 2013).

Distinctive characteristics of this parasite are its ability to persist and replicate within a host for life and its potential to cause life-threatening infection in the immunocompromised host. In normal healthy individuals, the infection is usually asymptomatic or causes no specific or intermittent clinical symptoms (Bisoffi et al., 2013). However, in the presence of certain predisposing conditions such as immunosuppression due to the use of steroids, kidney allograft recipients, and patients with lymphoma, the disease may change to forms of hyper-infection or disseminated types of strongyloidiasis (Bisoffi et al., 2013).

As strongyloidiasis persists for life, it can cause serious morbidity or death long after an immigrant resettles in a new country. For this reason it has been suggested that it is necessary to conduct screening of newly arrived (i.e., within 5 years), high-risk

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immigrant populations and that serology is the most useful technique (Monge-Maillo et al., 2018; Boggild et al., 2016; Díaz-Menéndez et al., 2012).

Since 2009, the Unit of Tropical Medicine of the Hospital Universitario Central de Asturias (Spain) has performed a screening test for *S. stercoralis* infection for all immigrant patients.

The aims of this cross-sectional study were to describe the results of a systematic serological screening programme for strongyloidiasis and to identify the geographic areas with the greatest risk of infection in order to optimize the screening programme.

## Materials and methods

### Patients

This prospective screening programme for strongyloidiasis was performed within the screening programme for parasitic diseases in the adult immigrant population. All immigrant patients attending the Tropical Medicine Unit of the Hospital Universitario Central de Asturias, a reference centre in a region of northern Spain where 3.8% of the population is foreign-born, were screened, regardless of their origin (Spanish Statistical Institute, 2019). Screening took place between January 2009 and December 2014. All patients were given an epidemiological questionnaire that included demographic variables such as sex, age, country of origin, classical risk factors for the disease (walking with bare feet, contact with human waste or contaminated soil, immunosuppression), presence of symptoms, and time from arrival in Spain to first consultation at the Tropical Medicine Unit.

The patients were classified into seven groups according to their geographical area of origin (Central Africa, East Africa, West Africa, North Africa, Mexico and Central America, South America, and South Asia), following the US Centers for Disease Control and Prevention (CDC) criteria (CDC, 2019).

Only subjects who had not received any anthelmintic treatment within the previous 2 years were included in the study.

### Laboratory diagnosis protocol

Three formalin-ether concentrated stool samples and an ELISA for anti-*S. stercoralis* antibodies were used as the screening tools. The qualitative detection of IgG antibodies to *S. stercoralis* was done using an ELISA (DRG Diagnostics) with microwells coated with *Strongyloides* antigen.

It is known that *Strongyloides* serology may have cross-reactions with filarial worms and other intestinal helminths such as hookworm species (Yori et al., 2006). For this reason, a blood sample was taken from all patients of Sub-Saharan origin, for the detection of microfilaremia. The diagnosis of filariasis was made through observation of the parasite after a concentration technique with 2% formalin (Knott technique). The species diagnosis was done by Giemsa staining.

A patient was considered as positive if at least one of the diagnostic tests used for screening (stool samples or/and ELISA) was positive. The patients with *S. stercoralis* and mixed infection with other filarial worms or nematodes were excluded from the study. An eosinophil blood count was performed for all patients. Eosinophilia was defined as  $>500$  eosinophils/mm<sup>3</sup>. All positive patients were treated with ivermectin (200 µg/kg for 2 days).

### Statistical analysis

Age was described by mean and standard deviation, while the time in Spain was described using the median and range. The non-parametric Mann-Whitney test was used for comparisons.

Categorical variables were described by absolute and relative frequencies. In the analysis of geographical distribution, only regions that registered positive for the *S. stercoralis* parasite were considered. The region with the smallest observed prevalence was used as the reference region.

The exact Chi-square test was used to contrast relationships between categorical variables. In addition, exact binomial 95% confidence intervals (CI) were computed for the *Strongyloides* prevalence rates. Univariate and multivariate logistic regression analyses were used to compute crude and adjusted odds ratios (OR), respectively.

### Ethics statement

The study presented in this paper forms part of an overall project entitled “Study of prevalence of imported diseases in an immigrant population”, which was approved by the Ethics Committee of Clinical Investigation of Asturias.

## Results

During the study period, 659 patients were screened (mean age  $34 \pm 12$  years; 50.4% female). Seventy per cent of patients had lived in Spain for more than 1 year. The areas of origin were Central Africa (30.9%), West Africa (30.4%), South America (28%), North Africa (5.3%), East Africa (2.1%), Mexico and Central America (1.8%), and South Asia (1.5%). The most frequent countries of origin were Equatorial Guinea (28.7%), Senegal (19.3%), and Ecuador (12%).

Table 1 shows the geographical distribution and prevalence of strongyloidiasis by geographical area. The univariate analysis showed that a positive result for *S. stercoralis* was significantly more frequent in patients from Central Africa ( $p=0.026$ ; OR 1.72, 95% CI 1.03–2.85) and East Africa ( $p<0.001$ ; OR 5.88, 95% CI 1.75–19.32).

Taking West Africa as the reference (as the area of lowest prevalence among positive prevalence areas), the statistical analysis showed that the risk of infection was higher in East Africa ( $p=0.001$ ; OR 6.750, 95% CI 2.127–21.423) and Central Africa ( $p=0.065$ ; OR 1.747, 95% CI 0.965–3.163). When the risk of infection was adjusted for sex and age, it was observed that it decreased significantly: from 6.750 to 5.070 in East Africa and from 1.747 to 1.314 in Central Africa. When adjusting for underlying diseases, the risk decreased in Central Africa (1.151,  $p=0.702$ ), and only East Africa remained as a risk area (5.298,  $p=0.007$ ) (Table 2).

Seventy-nine patients (12%, 95% CI 9.43–14.54%) had a positive serological test for *S. stercoralis*; microscopic visualization of larvae of *S. stercoralis* by formalin-ether concentration of faeces was positive for only four of them. There was no statistically significant difference in relationship with age or time in Spain between the positive and negative patients. On the other hand, females had more strongyloidiasis than males ( $p=0.005$ ). The characteristics of *Strongyloides* and non-*Strongyloides* infected patients are shown in Table 1.

When information about risk factors was examined, it was seen that all *Strongyloides*-positive patients had been walking with bare feet, most of them in areas with mud. One patient had an HIV infection with a CD4+ count of 366 cells/mm<sup>3</sup> at the time of diagnosis. The most frequent symptoms were abdominal pain (60%) and diarrhoea (18%). Three patients described itching and one patient had an urticariform rash. No patient described respiratory diseases. Twenty-two patients (28.6%) were asymptomatic, although 10 of them had eosinophilia. Overall, 30% of patients had eosinophilia, with a mean cell count of  $1063 \pm 2076$  cells/mm<sup>3</sup>. All patients diagnosed with *S. stercoralis* were treated with ivermectin and none of the patients developed clinical complications.

**Table 1**  
Comparison of the characteristics between *Strongyloides* and non-*Strongyloides* infected patients.

Characteristics	Non- <i>Strongyloides</i> group (n = 580)	<i>Strongyloides</i> group (n = 79)	Univariate analysis	
			p-Value	OR (95% CI)
Demographic characteristics				
Sex, male/female	299/281	28/51	0.005	
Age (years)	34.24 (12.085)	32.67 (12.314)	0.335	NS
Average time in Spain (days)	1133 (788)	981.71 (839.30)	0.673	NS
Area of origin				
Central Africa	171 (30%)	33 (42%)	0.026	1.72 (1.03–2.85)
West Africa	180 (31%)	20 (25%)	0.299	NS
South America	164 (28%)	20 (25%)	0.30	NS
North Africa	35 (6%)	0 (0%)	0.024	0.00 (0.00–0.99)
East Africa	8 (1.4%)	6 (8%)	0.00003	5.88 (1.75–19.32)
Mexico and Central America	12 (2%)	0 (0%)	0.196	NS
South Asia	10 (1.6%)	0 (0%)	0.239	NS
Country of origin <sup>a</sup>				
Equatorial Guinea	156	33	0.003	2.02 (1.21–3.37)
Senegal	120	7	0.012	2.68 (1.15–6.55)
Ecuador	67	13	0.210	NS
Brazil	21	1	0.274	NS
Ivory Coast	6	2	0.254	NS
Guinea Conakry	16	2	0.907	NS
Nigeria	22	6	0.116	NS
Paraguay	10	4	0.053	0.33 (0.09–1.28)
Mauritania	2	1	0.253	NS
Ethiopia	2	4	0.00003	15.41 (2.38–123.3)
Gambia	1	1	0.225	NS
Argentina	6	2	0.254	NS
Ghana	6	1	0.225	NS
Kenya	2	2	0.072	NS
Underlying diseases				
HIV infection	23	2	0.757	NS
Chronic hepatitis B	46	5	0.617	NS
Hepatitis C	18	7	0.011	3.04 (1.11–8.04)
HTLV	0	1	0.184	NS
Asymptomatic	221	22	0.018	1.751 (1.098–2.79)

OR, odds ratio; CI, confidence interval; NS, not significant; HTLV, human T-lymphotropic virus. Data are presented as n (%), or as the mean (standard deviation).

<sup>a</sup> Only countries with positive cases.

**Table 2**  
Global and adjusted risk of infection for geographic area.

Origin	Number (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
West Africa	20 (10%)	1	1	1
Central Africa	33 (16.3%)	1.747 (0.965–3.163)	1.314 (0.666–2.593)	1.151 (0.560–2.367)
East Africa	6 (42.9%)	6.750 (2.127–21.423)	5.070 (1.530–16.804)	5.298 (1.581–17.749)
South America	20 (10.9%)	1.098 (0.570–2.113)	0.867 (0.420–1.789)	0.858 (0.410–1.798)

OR, odds ratio; CI, confidence interval.

Model 1: global risk of infection by geographic area.

Model 2: risk of infection by geographic area adjusted for age and sex.

Model 3: risk of infection by geographic area adjusted for age, sex, and underlying diseases.

## Discussion

*S. stercoralis* is a nematode with a worldwide distribution, although it is particularly prevalent in regions of Africa, Southeast Asia, and Latin America (Schär et al., 2013). Its ability to propagate in the host by internal autoinfection results in chronic infection that can last for life. More than 50% of patients with chronic infection are asymptomatic, while others may show non-specific cutaneous, gastrointestinal, or pulmonary symptoms (Puthiyakunnon et al., 2014). Consequently, strongyloidiasis is frequently a chronic but unrecognized infection of immigrant populations in developed countries (Monge-Maillo et al., 2018; Díaz-Menéndez et al., 2012; Salvador et al., 2019). In previous studies performed in Spain in 14 centres, *S. stercoralis* was diagnosed in nearly 1% of travellers, 6% of immigrants, and 9.7% of visiting friends and relatives of immigrants 5]. Therefore, it appears necessary to conduct screening for *S. stercoralis* in the population coming from

endemic areas (Monge-Maillo et al., 2018; Boggild et al., 2016; Díaz-Menéndez et al., 2012; Salvador et al., 2019; Agbata et al., 2018; Requena-Méndez et al., 2017; Caruana et al., 2006). However, the selection of the method to be used for screening is controversial. Studies using stool microscopy have reported prevalence rates (0.8% to 4.3%) lower than those obtained when serological enzyme immunoassays are used (9% to 77%) (Biggs et al., 2009; Requena-Méndez et al., 2013; Lifson et al., 2002; Martin and Mak, 2006; Miller et al., 2000; Rice et al., 2003).

The definitive diagnosis of strongyloidiasis is usually made on the basis of the detection of larvae in the stool. In patients with strongyloidiasis, larval output in stools is often low, and microscopic examination of a single stool sample is negative in up to 70% of cases (Requena-Méndez et al., 2013; Buonfrate et al., 2015; Bisoffi et al., 2014; Inês Ede et al., 2011). Several techniques have been used to discern larvae in stool samples, including direct smear of faeces in saline–Lugol iodine stain, Baermann

concentration, formalin-ethyl acetate concentration, Harada–Mori filter paper culture, and nutrient agar plate cultures. The most sensitive morphological method is the agar plate technique. However, it is expensive, requires fresh faeces, and takes several days to obtain a result (Buonfrate et al., 2015; Bisoffi et al., 2014; Inês Ede et al., 2011).

Serology has been used for screening and diagnosis, as well as post-treatment monitoring, alone or in combination with stool testing, and is considered by some authors to be the method of choice for screening due to its higher sensitivity compared to stool examination (Boggild et al., 2016; Biggs et al., 2009; Inês Ede et al., 2011; Centers for Disease Control and Prevention, 2018; Murray et al., 2019; Sudarshi et al., 2003). Nevertheless, the predictive value in high-risk populations is limited due to the cross-reactivity with other helminth infections (Murray et al., 2019). So, co-infection with filarial worms or other nematodes must be excluded.

The seroprevalence of strongyloidiasis depends on the methodology used and the origin of the population studied. Studies performed in South Asian immigrants have shown seroprevalence rates of 24–65% (Monge-Maillou et al., 2018; Díaz-Menéndez et al., 2012; Salvador et al., 2019; Lifson et al., 2002; Miller et al., 2000). An infection rate of 39% for *Strongyloides* infection was found in asymptomatic refugees with eosinophilia originating from diverse regions, who were evaluated serologically (Seybolt et al., 2006). Other series performed in African patients have reported seroprevalence rates of strongyloidiasis of 12–17% (18,19), as found in this study. In the present study, migrants from Ethiopia, Nigeria, Paraguay, and Equatorial Guinea showed prevalence rates of 18–66%, but significant differences between countries were not found, probably due to the low number of cases. In other studies, the prevalence in these areas varied from 25% in Nigeria (Sanyaolu et al., 2011) to 2% and 5% in Brazil (De Assis and de Oliveira, 2013). Although a prevalence of between 7% and 13% has been reported in Ethiopia (Getaneh et al., 2010; Berger et al., 1989), the Ethiopian immigrant population in the present study showed a prevalence of 66%, the highest of the countries studied.

Infections with *S. stercoralis* can be asymptomatic or lead to cutaneous, gastrointestinal, or pulmonary symptoms, although in many cases, eosinophilia is the only indication of its presence. Several approaches have been tried for the screening and treatment of these patients based on the presence of eosinophilia. Canadian clinical guidelines for immigrants and refugees consider testing foreign-born individuals for strongyloidiasis if they have lived in areas of the world where these parasites are endemic and (1) have compatible signs and/or symptoms of infection and/or (2) have evidence of peripheral blood eosinophilia (Boggild et al., 2016). It is noteworthy that only 30% of the present study patients presented blood eosinophilia, which was the only finding in 10 of them. These results show a poor correlation between serological positivity and the presence of blood eosinophilia, perhaps because eosinophilia typically occurs in response to tissue invasion by a parasite, so occurs intermittently and may be missed if only a single complete blood count (CBC) is examined. This suggests that the eosinophil count in peripheral blood is not sensitive enough to be used as a sole initial screening test, as has been supported by several studies (Seybolt et al., 2006).

On the other hand, due to documented high rates of infection in refugee populations and its serious clinical consequences, the CDC have recommended pre-departure treatment for strongyloidiasis with two doses of ivermectin for refugees from non-*Loa loa* endemic countries and albendazole in those from countries with endemic *Loa loa* (Centers for Disease Control and Prevention, 2018). This approach could be difficult in the case of illegal immigrants without pre-departure counselling, and treatment with albendazole in possible *Loa loa* patients is a less effective option than ivermectin for strongyloidiasis.

There are limitations to this study. It is possible that, despite the attempt to exclude the presence of other parasitic diseases, we overestimated the prevalence of *Strongyloides* due to cross-reactions with undetected infections. The presence of undiagnosed *S. stercoralis* is of particular concern, due to the potential serious clinical complications, such as hyper-infection syndrome. This syndrome is characterized by infective filariform larvae in the stool and sputum, leading to clinical manifestations of increased parasite load and migration (such as gastrointestinal bleeding, respiratory distress, and septic shock) (Buonfrate et al., 2013). Active screening and treatment of at-risk groups eradicates infection and eliminates the risk of future severe complications, including death.

Thus, we recommend that immigrant patients from developing countries be routinely screened for *S. stercoralis*, especially those from East Africa. Due to the low sensitivity of stool examination for ova and parasites arising from low larval burden and intermittent shedding in the stool, serological testing is the diagnostic method of choice for screening in immigrant populations.

### Conflict of interest

None.

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