

## Prevalence of *Strongyloides stercoralis* infection among HIV-positive immigrants attending two Italian hospitals, from 2000 to 2009

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In patients with *Strongyloides stercoralis* infection, a dysregulation of host immunity can lead to hyperinfection syndrome (HS) and disseminated strongyloidiasis (DS), characterized by high fatality rate. HS has been reported in HIV-positive patients following use of corticosteroids or during immune reconstitution inflammatory syndrome (IRIS). A retrospective study was conducted to estimate the prevalence of *S. stercoralis* infection among HIV-positive immigrants, attending two Italian hospitals. From January 2000 to August 2009, 138 HIV-positive immigrants were systematically screened for strongyloidiasis, as a part of their routine care, with an indirect immunofluorescent antibody test (IFAT) developed at the Centre for Tropical Diseases, Sacro Cuore Hospital of Negrar, Verona. The majority were also submitted to stool examination. Fifteen (11%) resulted infected by *S. stercoralis*, of whom four (27%) had a negative serology (diagnosis made with stool examination). A higher eosinophil count ( $0.94$  versus  $0.24 \times 10^9/l$ ,  $P < 0.01$ ) and more frequent gastrointestinal and cutaneous symptoms (odds ratio: 4.8 and 5.8, respectively) were found in patients with strongyloidiasis compared with controls. The IFAT is more sensitive than direct parasitological methods. The proportion of false negative results was higher than expected based on the theoretical test sensitivity. Considering the high prevalence detected and the apparent, lower sensitivity of serology, we propose a systematic screening for *Strongyloides* infection, with both serology and stool culture, for all HIV-positive immigrants coming from endemic areas.

### INTRODUCTION

Strongyloidiasis is a chronic, soil-transmitted, intestinal helminth infection that is currently believed to affect 30–100 million people primarily in tropical and subtropical regions (Olsen *et al.*, 2009).

Most infections are asymptomatic or cause aspecific or intermittent clinical symptoms; nevertheless, a dysregulation of host

immunity can lead to hyperinfection syndrome (HS) and disseminated strongyloidiasis (DS), characterized by high fatality rate (Marcos *et al.*, 2011). The two conditions are overlapping and frequently used indifferently to characterize the severe and life-threatening forms of strongyloidiasis. Some authors define HS as an infection confined to lungs and gastrointestinal tract, but with signs/symptoms of severe diseases in relation to elevated number of larvae, while DS defines the presence of larvae also in additional organs and tissues (Segarra-Newnham, 2007). Among immunocompromising conditions, corticosteroid treatment

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is the most commonly associated with HS and DS. Although it was initially hypothesized that immunosuppression secondary to HIV infection would result in an increased incidence of severe presentations, such rise in incidence was not observed in endemic areas (Petithory and Derouin, 1987). Therefore, DS, initially included by CDC among AIDS defining criteria, has been subsequently removed, owing to a general lack of correlation between HIV and *Strongyloides* infection (CDC, 1986, 1987). Nevertheless, the relationship between strongyloidiasis and HIV infection seems to be complex and not completely understood. Cases of HS and DS in HIV positives are periodically reported (Orem *et al.*, 2003). In the last 10 years, some cases of HIV-associated immune reconstitution inflammatory syndrome (IRIS) due to *Strongyloides stercoralis* infection have been described (Lanzafame *et al.*, 2005; Brown *et al.*, 2006). The main aim of the present study was to retrospectively determine the prevalence of *S. stercoralis* infection among HIV positives immigrants attending two Italian hospitals.

## PATIENTS AND METHODS

A retrospective study was conducted to estimate the prevalence of *S. stercoralis* infection among HIV-positive immigrants, aged >18 years, coming from Africa, South and Central America, South East Asia and Oceania. All HIV immigrant patients attending the Centre for Tropical Diseases, S. Cuore Hospital of Negrar or the Infectious Diseases Department of Verona University were systematically screened for strongyloidiasis since the year 2000, as a part of their routine care, with an indirect immunofluorescent antibody test (IFAT) developed and validated at the Centre for Tropical Diseases (Boscolo *et al.*, 2007). The majority had also been submitted to direct parasitological tests and/or stool culture in agar for *S. stercoralis*. All patient

records from January 2000 to August 2009 were retrospectively reviewed. Patient records were analysed for laboratory and parasitological findings and clinical features suggestive of *S. stercoralis* infection such as: gastrointestinal symptoms (abdominal pain, diarrhoea, nausea, vomiting), skin problems (pruritus, dermatitis) or respiratory symptoms (cough, asthma, dyspnoea). Data were analysed anonymously. Univariate analysis was used to describe the study sample. Laboratory results, which are continuous variables, were also categorized and results presented as percentages in addition to means and standard deviation (SD). Ninety-five per cent confidence intervals for odds ratio were calculated by Epi Info Statcalc (EpiInfo software, Atlanta CDC, 3.3.2 version). Differences in laboratory examination results between infected and non infected patients were examined using one-tailed *t*-test.

## RESULTS

From 1 January 2000 to 30 August 2009, 138 adult immigrants affected by HIV infection underwent the serological screening for *S. stercoralis*. The characteristics of the study population are reported in Table 1. Half were females. The mean age was 38.5 (range: 20–61) years. Most patients (77.5%) came from Africa, 19.6% were from Central and South America, two were from Thailand, one was from India and one from United Arab Emirates. The mean time of residence in Italy was 7 years. Twenty-one (15%) patients reported history of previous corticosteroid treatment. Five (3.6%) reported a previous treatment with drugs effective (at least partially) against *S. stercoralis* (ivermectin, thiabendazole or albendazole). As shown in Table 1, half of the patients were on antiretroviral therapy (HAART) and 39% had a HIV viral load <50 copies/ $\mu$ l. The CD4<sup>+</sup> cell count was  $\geq$ 350 cells/ $\mu$ l in 47% of patients. As shown in Table 2, 50 patients (36%) reported

TABLE 1. Characteristic of HIV-positive immigrants: all, patients with strongyloidiasis (cases) and patients without strongyloidiasis (controls)

	All	Cases	Controls
	n=138	n=15	n=123
<b>GENERAL CHARACTERISTIC</b>			
Female, n (%)	69 (50)	7 (47)	62 (50.4)
Age <sup>a</sup>	38.5 (8), 37	36.6 (5.4), 38	37.2 (8.4), 36
<b>ORIGIN, n (%)</b>			
Africa	107 (77.5)	14 (93.3)	93 (75.6)
Central and South America	27 (19.6)	1 (6.7)	26 (21.2)
Asia and Oceania	4 (2.9)	0	4 (3.2)
Length of stay in Italy <sup>b</sup>	87.1 (61.1), 73	86 (66), 97	87.3 (75.7), 68.8
<b>PREVIOUS TREATMENTS, n (%)</b>			
Anti <i>S. stercoralis</i>	5 (3.6)	1 (6.6)	4 (3.2)
Corticosteroids	21 (15.2)	3 (20)	18 (14.6)
<b>HIV INFECTION</b>			
CD4 <sup>+</sup> ( $\mu$ l) <sup>c</sup>	373 (244), 342	373 (210), 309	408 (259), 36
CD4 <sup>+</sup> $\geq$ 350/ $\mu$ l, n (%)	65 (47.1)	6 (40)	59 (48)
HIV viral load <50 copies/ $\mu$ l, n (%)	54 (39.1)	5 (33.3)	49 (39.9)
HAART, n (%)	67 (48.5)	5 (33.3)	62 (50.4)

<sup>a</sup>Mean (SD), median in years.

<sup>b</sup>Mean (SD), median in months.

<sup>c</sup>Mean (SD), median.

symptoms consistent with a *S. stercoralis* infection, at the time of diagnosis. Twenty-three (16.7%) reported skin problems for a mean time of 3.5 years; 21 (15%) reported gastrointestinal problems, for a mean duration of 10 months. Respiratory problems were present in 19 (14%) patients for a mean time of 1.6 years (Table 2). Among symptomatic patients, only 11 (22%) resulted affected by strongyloidiasis. Strongyloidiasis was diagnosed in seven patients (30%) with skin problems and in three patients (16%) with respiratory problems.

### Cases of Strongyloidiasis

Fifteen (11%) patients resulted affected by strongyloidiasis. Seven (47%) were females. The mean age was 36.6 (range: 26–46) years. Most came from West Africa (93%): Nigeria (5), Ivory Cost (4), Ghana (2), Guinea Bissau (1), Congo (1) and Cameron (1); one was from Brazil (Table 1). The mean time of residence in Italy was 2.8 years. Three patients (20%) had a history of previous corticosteroid treatment. Eleven patients (73%) had a positive serology for *S. stercoralis* (titre  $\geq$ 1:20), while

TABLE 2. Clinical findings in HIV-positive immigrants with strongyloidiasis (cases) compared with those without strongyloidiasis (controls)

	All	Cases	Controls	OR	CI
	n (%)	n (%)	n (%)		
Symptoms	50 (36.2)	11 (73.3)	39 (31.7)	5.92	1.60–23.96, $P < 0.01$
Skin problems	23 (16.7)	7 (46.6)	16 (13)	5.85	1.63–21.16, $P < 0.01$
Gastrointestinal problems	21 (15.2)	6 (40)	15 (12.2)	4.80	1.29–17.73, $P < 0.01$
Respiratory problems	19 (13.8)	3 (20)	16 (13)	1.67	0.33–7.44, $P = 0.45$

four (27%) were negative and the diagnosis was based on a positive stool microscopy (2) or a positive stool culture (2). Only one patient reported a history of previous treatment with a drug effective against *S. stercoralis*. He had a negative serology and a positive stool culture.

At the time of performing the serology for *S. stercoralis*, the CD4<sup>+</sup> cell count was  $\geq 350/\mu\text{l}$  in six patients (40%) and  $< 200$  cells/ $\mu\text{l}$  in three patients (20%). Five patients had a HIV viral load  $< 50$  copies/ $\mu\text{l}$ . Five were on antiretroviral therapy (Table 1). As shown in Table 2, 11 patients (73%) were symptomatic for *S. stercoralis* infection at the time of diagnosis. Seven (46%) reported skin problems for a mean duration of 3 years. Six (40%) reported gastrointestinal symptoms for a mean duration of 4 months. Respiratory problems were reported by three patients (20%) for a mean duration of 5 years. As shown in Table 3, the mean eosinophil count was  $0.94 \times 10^9/\text{l}$ , the mean IgE level was 948 UI/ml. Ten patients (67%) had eosinophilia (eosinophil count  $\geq 0.45 \times 10^9/\text{l}$ ), and five had a normal eosinophil count: one of them was on corticosteroids.

Patients with a positive serology had a mean CD4<sup>+</sup> count of 401/ $\mu\text{l}$  (SD: 215), a mean CD4% of 23.3 (SD: 12.5) and a mean

CD4<sup>+</sup>/CD8<sup>+</sup> ratio of 0.57 (SD: 1.18); those with a negative serology had a mean CD4<sup>+</sup> count of 296/ $\mu\text{l}$  (SD: 201), a mean CD4% of 15.9 (SD: 13.5) and a mean CD4<sup>+</sup>/CD8<sup>+</sup> ratio of 0.15 (SD: 0.07). All had CD4<sup>+</sup> cell count  $> 100/\mu\text{l}$ .

No case of DS or HS occurred. Treatment (ivermectin 200  $\mu\text{g}/\text{kg}$  orally, single dose) was well tolerated by most patients. Only two developed mild side effects as pruritus and transient hypotension.

### Comparison between HIV-Positive Immigrants with (Cases) and without (Controls) Strongyloidiasis

The two groups were similar in terms of sex, age, provenience and length of stay in Italy. No significant differences were identified in the mean CD4<sup>+</sup> count, CD4<sup>+</sup> percentage, CD4<sup>+</sup>/CD8<sup>+</sup> ratio and HIV viral load. Gastrointestinal and cutaneous symptoms were significantly more frequent in patients with strongyloidiasis than in controls, odds ratios 4.8 and 5.8, respectively (Table 2). Their positive predictive values were 28% and 30%, while their negative predictive values were 92% and 95%, respectively. Patients with strongyloidiasis presented significantly higher absolute and relative eosinophil count compared with controls (Table 3).

TABLE 3. Main laboratory findings in HIV-positive immigrants with strongyloidiasis (cases) compared with those without strongyloidiasis (controls)

	All <i>n</i> =138	Cases <i>n</i> =15	Controls <i>n</i> =123	
	Mean (SD)	Mean (SD)	Mean (SD)	<i>t</i> -test
	Median	Median	Median	
White blood cells ( $\times 10^9/\text{l}$ )	5.0 (1.7)	5.5 (1.8)	4.9 (1.7)	<i>P</i> =0.13
	5.0	5.2	4.9	
IgE (UI/ml)	517 (997)	948 (981)	456 (990)	<i>P</i> =0.06
	137	376	115	
Eosinophil count ( $\times 10^9/\text{l}$ )	0.32 (0.39)	0.94 (0.72)	0.24 (0.24)	<i>P</i> <0.01
	0.19	0.71	0.17	
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	OR (CI)
Eosinophil count $\geq 0.45 \times 10^9/\text{l}$	31 (22.5)	10 (67)	21 (17)	9.7 (1.08–40.56)
$< 0.45 \times 10^9/\text{l}$	107 (66.7)	5 (33)	102 (83)	<i>P</i> <0.01

## DISCUSSION

We found a 11% prevalence of *Strongyloides* infection among HIV-positive immigrants attending two Italian hospitals. In a previous study, a positive *Strongyloides* serology was found in 5/47(11%) HIV-positive African subjects with eosinophilia attending a HIV treatment Centre in London (Sarner *et al.*, 2007). Most of our patients came from West Africa where the prevalence in the general population ranges from 12% in Ghana to 25% in Nigeria and from Brazil where a 11% prevalence was reported (Kobayashi *et al.*, 1996; Agi, 1997; Yelifari *et al.*, 2005). In a recent study conducted in the USA among 128 foreign-born, HIV-infected patients, a serological evidence of *S. stercoralis* was found in 33 (26%) subjects (Hochberg *et al.*, 2011). Studies carried out in endemic countries, such as Tanzania and Ethiopia, reported a higher prevalence of strongyloidiasis in HIV positives (Gomez Morales *et al.*, 1995; Assefa *et al.*, 2009). In Brazil, a significant difference was found in some areas, but not in other (Dias *et al.*, 1992; da Silva *et al.*, 2005). The serology is considered the method of choice for screening, owing to its higher sensitivity compared with stool examinations (Loufty *et al.*, 2002; Sithithaworm *et al.*, 2003). The sensitivity of direct diagnostic methods is limited by the irregular and often low concentration of larval stages in faecal specimens and is directly proportional to the number of samples and to the amount of stool examined (Siddiqui and Berk, 2001). At a titre of  $\geq 1:20$ , the IFAT developed at the Centre for Tropical Diseases has a high sensitivity (97%) and specificity (98%) (Boscolo *et al.*, 2007). However, the proportion of false-negative results in our series was higher than expected based on the theoretical test sensitivity. While the limited diagnostic value of serological tests in HIV positives has been reported for other parasitic diseases, such as toxoplasmosis and leishmaniasis, to our knowledge, no study has assessed the diagnostic accuracy of *S. stercoralis* serology in these patients (Montoya, 2002; Alvar *et al.*,

2008). In patients with a positive serology, the mean value of CD4<sup>+</sup> cells was 401/  $\mu$ l (SD: 215) compared with CD4<sup>+</sup> 296/ $\mu$ l (SD: 201) in patients with a negative serology. Although we are aware that the small study sample does not authorize any conclusion, we suggest that further investigations should explore the serology performance in HIV-positive subjects. The prevalence of *Strongyloides* infection in HIV-positive immigrants is probably underestimated not only due to the low sensitivity of the currently available diagnostic tools but also due to the low level of clinical suspicion. In our study, 36% of all patients reported symptoms consistent with *S. stercoralis* infection; nevertheless, only 22% of symptomatic patients were found to be infected. The presence of symptoms consistent with strongyloidiasis had a limited diagnostic value. In HIV positives, symptoms like diarrhoea, pruritus and gastrointestinal symptoms are frequently reported, either as HAART side effects or caused by concomitant opportunistic infections. We found a significantly higher mean eosinophil count in HIV-positive patients with strongyloidiasis compared with those non-infected. In high-risk groups for *Strongyloides* infection, eosinophilia has been estimated to have a specificity of 93% and a sensitivity of 93.5%; nevertheless, the eosinophil count, if used alone, is not sensitive enough to screen for strongyloidiasis especially in chronic infections and more so in HS (Gill *et al.*, 2004; Keiser and Nutman, 2004). Interestingly, five infected patients (33%) had a normal eosinophil count. Among them, one was on corticosteroids. Corticosteroids are known to reduce the levels of circulating eosinophils by inhibiting their proliferation and increasing apoptosis. Lower levels of eosinophils have been described in immunocompromised patients compared with healthy subjects. Moreover, significantly lower eosinophil counts have been found in HIV-positive subjects than in immunocompromised patients for different reasons such as chronic illness or use of immunosuppressant drugs (Vaiyavatjamai *et al.*, 2008).

It is noteworthy that 15% of our patients reported previous or concomitant corticosteroid therapy. Among immunosuppressive drugs, corticosteroids are the most specifically associated with *Strongyloides* HS. HIV-infected patients are frequently treated with corticosteroids during toxoplasmosis, *Pneumocystis jirovecii* pneumonia, IRIS, haematological diseases, meningitis, pericarditis and malignancies. Some cases of HS reported in HIV positives occurred in patients treated with corticosteroids (Maayan *et al.*, 1987; Sarangarajan *et al.*, 1997). Moreover HIV positives may have additional risk factors for *Strongyloides* dissemination such as other immunosuppressive conditions or treatment, malnutrition, malignancies and alcohol abuse (Keiser and Nutman, 2004). In the last 10 years, some cases of HIV-associated IRIS due to *S. stercoralis* infection have been reported (Lanzafame *et al.*, 2005; Brown *et al.*, 2006). IRIS is now known to be associated to a range of parasitic infections including strongyloidiasis (Lawn, 2007). No cases of HIV-associated IRIS due to *S. stercoralis* occurred in our patients; nevertheless, as the access to HAART is increasing in areas where HIV and strongyloidiasis are co-endemic, this as well as other aspects of the co-infection should be better understood. Considering the high prevalence detected, albeit in a relatively small group of patients, and given the apparent, lower sensitivity of serology, we propose a systematic screening for *Strongyloides* infection, with both serology and stool agar culture, for all HIV-positive immigrants coming from endemic areas.

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