Infection by *Strongyloides stercoralis* in immigrants with Chagas disease: evaluation of eosinophilia as screening method in primary care

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Abstract

Objectives: To evaluate co-infection of *Strongyloides stercoralis* and *Trypanosoma cruzi* and to assess eosinophilia as a screening test for detection of *S. stercoralis* infection in patients with Chagas disease (CD).

Methods: A retrospective diagnostic validation study was performed on serum samples from primary care patients diagnosed with CD in the southern Barcelona metropolitan area. All samples with eosinophilia (n=87) and a random sample of non-eosinophilic sera (n=180) were selected. Diagnosis of CD was based on positive serology by means of two tests: ORTHO® *T. cruzi* ELISA test, and BIO-FLASH® Chagas or Bioelisa CHAGAS. SCIMEDX ELISA STRONGY-96 was used to diagnose strongyloidiasis.

Results: *S. stercoralis* serology was positive in 15% of patients of whom 95% showed eosinophilia, vs. 21% of those with negative serology ($p<0.001$), with differences in the mean eosinophil count (0.49 vs. 0.27 x 10^9/L). Only 1.1% of patients with CD but without eosinophilia presented positive serology for *S. stercoralis*; whereas 44% of patients with CD and eosinophilia did ($p<0.001$). Sensitivity and specificity values for eosinophilia were thus 95% and 79% respectively. PPV was 42.5% and NPV, 98.9%.

Conclusions: The prevalence of co-infection by *T. cruzi* and *S. stercoralis* is not negligible and has probably been underestimated for years in many areas, due to frequently subclinical infections. Therefore, serology seems mandatory for these patients and the use of eosinophilia as initial screening could facilitate the

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task, decreasing the number of analyses to be performed.

Keywords: Chagas disease, *T. cruzi*, *S. stercoralis,* eosinophilia, co-infection, retrospective diagnostic validation, Spain

Introduction

The neglected tropical diseases (NTDs) are a group of parasitic, viral, fungal and bacterial infections that also include snakebites. So far NTDs had received miniscule treatment and research funding compared to better-known infections such as HIV/AIDS, tuberculosis and malaria. According to WHO, NTDs affect more than one billion of the world's poorest people each year, causing an estimated 534 000 deaths, mainly in socioeconomically disadvantaged areas with tropical and subtropical climates. [1] One of these conditions is Chagas disease (CD): a protozoan infection produced by *Trypanosoma cruzi*, which in 80% of cases is transmitted by triatomine insects, with other transmission routes being oral, blood-borne, vertical, via organ transplantation and as a result of laboratory accidents. The disease is endemic in 21 countries of Latin America with the greatest presence in the Southern Cone. [2] Its prevalence is estimated to be between 6 and 7 million people, most of whom are unaware of their infection. [3] Chagas disease presents an acute phase and an indeterminate asymptomatic chronic phase which in 30%-40% of cases evolves to the symptomatic phase, mainly affecting cardiac or digestive function. [4]

Strongyloidiasis is a soil-transmitted helminthiasis produced by the intestinal nematode *Strongyloides stercoralis*, which will soon be included in the NTD group. Its transmission occurs in tropical and subtropical regions and occasionally in temperate climates (the south of Europe) when skin comes into contact with contaminated wet soils. According to WHO, between 30 and 100 million people are infected around the world. [5] Although there are few studies on the prevalence of this infection in Latin America, estimations suggest that it is higher than 20% in Argentina, Bolivia, Brazil, Ecuador, Peru and Venezuela. [6, 7] In Spain, several autochthonous cases have been reported, [8] but the vast majority are imported and their incidence is increasing. [9,10] Chronic infections by *S. stercoralis* can produce skin, gastrointestinal and cardiopulmonary manifestations, and when coinciding with immunosuppressive conditions, hyperinfestation syndrome can occur, which is lethal in up to 85% of cases. [11]

CD and strongyloidiasis appear in non-endemic countries due to migratory flows. The parasites causing both conditions can persist in the host for decades, even throughout the whole of life, often resulting in asymptomatic disease. [12-14] Recently, in Spain, studies have been published linking CD and infection by *S. stercoralis*. (15)

In initial care protocols for immigrants and travellers arriving from the tropics, the blood count is a

basic test that is routinely performed. A result showing unexplained eosinophilia could constitute a valuable screening tool for the detection of *S. stercoralis*, as it appears in 75% of cases. [16] In a British Infection Society clinical guide, it is recommended that all travellers returning from tropical areas with eosinophilia should be tested by means of concentrated microscopy of faeces and *Strongyloides* serology, among other techniques. However, the same guide warns that the first method presents low sensitivity, while serology may present problems of cross-reaction with soil-transmitted helminths. [17] The low sensitivity of standard stool examination is related to the fact that larvae are excreted intermittently, especially in patients with a low burden of infection. Serological diagnosis by enzymatic-immune assay (EIA) presents sensitivity of around 85%, depending on the different tests, and a specificity >95%, demonstrating a high negative predictive value. [11,18,19]

In Spain, in general terms, 70% of the population visit their primary care centre at least once a year, [20] so this offers an excellent framework within which to screen, diagnose, treat and follow-up immigrant patients, due to its accessibility and the continuity of care. Therefore, the main objective of the present work was to conduct a study in our area, where primary care serves a high proportion of immigrant patients, to evaluate co-infection of *S. stercoralis* and *T. cruzi* and furthermore to assess eosinophilia as a screening test for *S. stercoralis* infection in CD patients.

Material and Methods

Design

We performed a retrospective diagnostic validation study. In the design and presentation of the results of the study, we have followed the recommendations for reporting studies on diagnostic accuracy: Standards for Reporting Diagnostic Accuracy (STARD). [21]

Subjects

This study was carried out on patients who attended primary care centres between 2011 and 2018 in the southern area of metropolitan Barcelona with a population of app. 1.2 million, of whom some 5% are Latin American migrants.

Eligibility of the sample and source population

Surplus anonymous samples of serum from patients diagnosed with CD were analysed. The collection of sera is registered and authorized by the *Instituto de Salud Carlos III* with access number CO0041 9. All samples were processed in the *Laboratori Clínic Territorial Metropolitana Sud* of the *Institut Català de la Salut* (Barcelona, Spain).

Of the total number of sera from patients with CD (n= 887) with available eosinophil count information (n=766), all samples with eosinophilia were selected (n=87), plus a random sample of non-eosinophilic sera (n=180) (Figure 1). This sample was calculated taking into account an estimated proportion of 14% of patients with CD being co-infected with *Strongyloides*, with an accuracy of +/- 5% and a confidence level of 95%. [15,22] All selected sera were tested for *S. stercoralis* IgG, taking it as a reference test for the diagnosis of *Strongyloides* infection and then using it as the benchmark for comparison with the results of the eosinophil count.

All samples were collected, stored and processed according to standard recommendations for serology. Each sample received an identification code in the laboratory, which corresponded to an encrypted identifier to guarantee blind analysis without the possibility of identification of any patient.

Processing of laboratory samples

Diagnosis of CD was based on positive serology by means of two tests: the ORTHO® *T. cruzi* ELISA test (Ortho Clinical Diagnostics Inc., Raritan, USA) based on the *T. cruzi* whole-cell lysate antigen, and either BIO-FLASH® Chagas (automated chemiluminescent immunoassay; Biokit S.A., Barcelona, Spain) or Bioelisa CHAGAS (Biokit S.A., Barcelona, Spain), which employ recombinant *T. cruzi* proteins as antigens. Samples that returned discordant results (after three tests: one with native antigen and two with recombinant ones) or those judged inconclusive were excluded.

The eosinophil count was performed in an ABX Pentra DX120® autoanalyzer (Horiba Medical, Kyoto, Japan); eosinophilia was classified as mild (> 0.50-1.5 x 10^9 eos/L), moderate (1.5-3.0 x 10^9 eos/L) or high (> 3 x 10^9 eos/L). [23]

To diagnose strongyloidiasis, we used SCIMEDX ELISA (STRONGY-96, SciMedx Corporation, Denville, NJ, USA), which employs a soluble fraction of *S. stercoralis* filariform larvae as an antigen. Briefly, serum was diluted to 1:64 in dilution buffer, and 100 µl was pipetted into microtitre wells coated with *Strongyloides* antigen and incubated at 15ºC to 25ºC for 10 min. The wells were washed three times, and 100 µl of the supplied horseradish peroxidase-conjugated protein A was added and incubated for 5 min at 15ºC to 25ºC. The wells were washed three times and 100 µl of chromogen tetramethylbenzidine (TMB) was added and incubated for 5 min at 15ºC to 25ºC, followed by the addition of 100 µl of stop solution (0.73 M phosphoric acid). The optical density (OD) was subsequently measured at a wavelength of 450 nm (BEST 2000®, Biokit, Werfen Group Barcelona, Spain) and divided by the cut-off indicated by the manufacturer (0.2) to give an index value (IV): IV >1.1 was considered positive; from 0.9 to 1.1, indeterminate; and <0.9, negative.

Anonymized sociodemographic data were obtained from the patients: age at the time of the diagnostic

test for *T. cruzi*, gender and country of origin.

Statistical analysis

First, univariate descriptive analysis was carried out by frequency distribution and percentages for the qualitative variables (gender, age group, country of origin, eosinophilia (YES/NO), degree of eosinophilia, positive *S. stercoralis* serology (YES/NO)). Data analysis was conducted using the IBM SPSS Version 25.0. (IBM Corp, Armonk, NY, USA).

For the quantitative variables (age, eosinophil count, and reactivity index for *S. stercoralis*), the analysis consisted of calculating the mean, median, standard deviation (SD) and percentiles. Subsequently, bivariate analysis was performed, relating the presence or absence of IgG anti-*S. stercoralis* and eosinophilia with the other variables in the study. Finally, the association between *S. stercoralis* infection and eosinophilia in *T. cruzi*-positive individuals was studied by calculating the odds ratio (OR) using cross-product ratios of 2 x 2 tables with 95% confidence intervals (CI). *p*-values of 0.05 or less were used in the 2-sided tests as criteria for statistically significant differences. The SP, S, and positive and negative predictive values (PPV and NPV) with 95% CI were calculated by comparing the results of the eosinophil count with the *S. stercoralis* serology as the reference test. For the comparison, indeterminate values were excluded.

Ethics

This study was reviewed and approved by the Institutional Review Board (IRB) at IDIBELL (PR57/19), Barcelona, Spain. All patient data were anonymized for this analysis. The implementation of the research project did not alter usual patient care. Patient information was protected according to the General Data Protection Regulation (2016/679) of the Council of the European Union, which came into force on 25/05/2018 on the protection of natural persons with regard to the processing of personal data; and Spanish Organic Law 15/1999.

Results

Of the 267 patients with CD, 73% were women. All cases except four were adults (18 or more years old) with an average age of 37.2 years (SD 10.5). Of the 197 patients whose country of origin was known, 95% were Bolivian. Of the 87 patients with eosinophilia, 77 (89%) presented mild eosinophilia and 10 (11%) with moderate, with no cases of high eosinophilia. The mean eosinophil count was 0.4157 eosinophils x 10^9/L (SD 0.41698) (Table 1).

The serology for *S. stercoralis* was positive in 40 patients (15%) and equivocal in 4 cases (1.5%) that

were excluded (3 with eosinophilia and 1 without). Regarding the data of patients in whom *S. stercoralis* serology was performed, the proportion of men was higher in positive cases (16, 40%) than in negative ones (54, 24%) (p=0.038). Patients with antibodies to *S. stercoralis* showed eosinophilia in 38 cases (95%), vs. 46 (21%) patients without (p<0.001), also showing statistically significant differences in the mean eosinophil counts (Table 1).

It should be noted that of the 179 patients with CD but without eosinophilia, only 2 (1%) presented positive serology for *S. stercoralis* whereas 38 (45%) of the 84 patients with CD and eosinophilia did (p<0.001) (Table 1).

Table 2 shows the simple and age-sex-adjusted ratios of the association between eosinophilia and the presence of antibodies against *S. stercoralis*. Eosinophilia maintains a significant association with positive serology, whereas sex is not significant.

Table 3 shows the data of the eosinophil count parameter as a diagnostic screening test. The sensitivity and specificity of eosinophilia were 95% (CI 95%, 82%–99%) and 79% (CI 95%, 73%–84%) respectively. The PPV was 42% (CI 95%, 34%–56%), the NPV was 98.9% (CI 95%, 95.6%–99.8%).

Discussion

We present a study of 267 serum samples from patients with CD who attended primary care centres of the southern Barcelona metropolitan area during the period 2011 to 2018. Of these patients, 15% also presented strongyloidiasis, proportionately more men and more patients with eosinophilia; although in the adjusted analysis, only the relevance of eosinophilia was confirmed. Eosinophilia showed a sensitivity of 95% for diagnosis of *S. stercoralis* infection in patients with CD.

There is considerable prevalence of *T. cruzi* infection among the Latin-American migrant population who attend primary care centres in our area: 3.6% overall but up to 13.7% among Bolivian migrants specifically. [24] Studies have recently been published in Spain, showing that *T. cruzi* and *S. stercoralis* co-infection occurs in 16.7% of chronic CD patients. [22] In the present work, we obtained a positive result in 40 cases (15%) of CD patients: in line with the results obtained by others. [15,22] The observed differences regarding sex (greater proportion in men) probably correspond to activities of men (such as agriculture without footwear), which correlate with the chance of contact with *S. stercoralis*. [25]

Data concerning the prevalence of *S. stercoralis* in Spain, relating to native and migrant populations, are diverse. In one study of a native population infected with *S. stercoralis* in eastern Spain, a prevalence of 12.4% was found, with 83.7% of the patients presenting eosinophilia. This laboratory parameter was considered the best infection prognostic factor. [26] In another study of 70 cases of strongyloidiasis based on serological diagnosis in the northern Barcelona metropolitan area, eosinophilia was found in 90% of

cases. [14] In a review of intestinal parasitism of patients from the southern Barcelona metropolitan area (2001 to 2010), in which 78 cases of *S. stercoralis* infection were studied, 90% had eosinophilia. [27] Moreover, in a recent systematic review of endemic strongyloidiasis in Spain, eosinophilia was documented in 82% of cases. [28] However, in a case–control study of a sample of patients treated at a tropical diseases unit in Barcelona, the authors found that only 38.5% of patients with *S. stercoralis* infection had absolute eosinophilia and that patients with CD were more than twice as likely to be infected by *T. cruzi*. [29] In our study, almost half of the patients with CD and eosinophilia (45%) were infected with *Strongyloides*. Moreover, 95% of patients with *S. stercoralis* showed eosinophilia: a slightly higher prevalence than that found in previous studies, probably due to the selection of the sample for our study (only patients with CD). It is unknown whether an association between the 2 parasites exists, although it seems likely, given their close relationship when both geographical and socioeconomic risk factors are shared. Bearing in mind the regulation of host immune system by *S. stercoralis*, [30] in case of co-infection it could affect the response to the protozoan and affect the evolution of CD via an underlying modulation of cytokine responses. In a study of blood donors, it was found that 10.9% of patients with CD were co-infected with *S. stercoralis*, presenting a greater proportion of PCR-positivity for *T. cruzi* than patients with negative *Strongyloides* serology. [15]

Eosinophilia presents a high NPV (99%), corresponding quite well with the values obtained by other authors working in the area. [14,26,27] This is a high value when referring to the aim of establishing this determination as a screening test, bearing in mind that the eosinophil count is included in any haemogram. In Latin American patients with eosinophilia in whom *T. cruzi* infection is not detected, the prevalence of *S. stercoralis* infection is 28%. [31]

The strengths of the present study include the fact that in our type of population, the absence of eosinophilia would allow a large number of sera not to be tested for *S. stercoralis* antibodies. This could improve cost-effectiveness at a time when active screening for *S. stercoralis* is increasingly advocated. [32]

Regarding to the cut-off used in *Strongyloides* serology, in our experience it is specific enough with no cross-reactions with other intestinal parasitic infections except hookworms. [32] The possibility of unnoticed cross-reactions with hookworms due to the lack of a copro-parasitological study, and the fact that the percentage of eosinophilic patients with CD included in our study (16%) is high due to how the sample was selected are limitations of our study.

Conclusions

It seems that when looked for actively, the prevalence of co-infection between *T. cruzi* and *S. stercoralis* is

not negligible and probably has been underestimated for years in many areas, due to the infections frequently presenting subclinically. Therefore, serology seems mandatory for the type of patients considered here, and the use of eosinophilia as a previous screening method could facilitate the task, decreasing the number of analyses to be performed.

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Figure 1. Flow Chart for sera eligibility

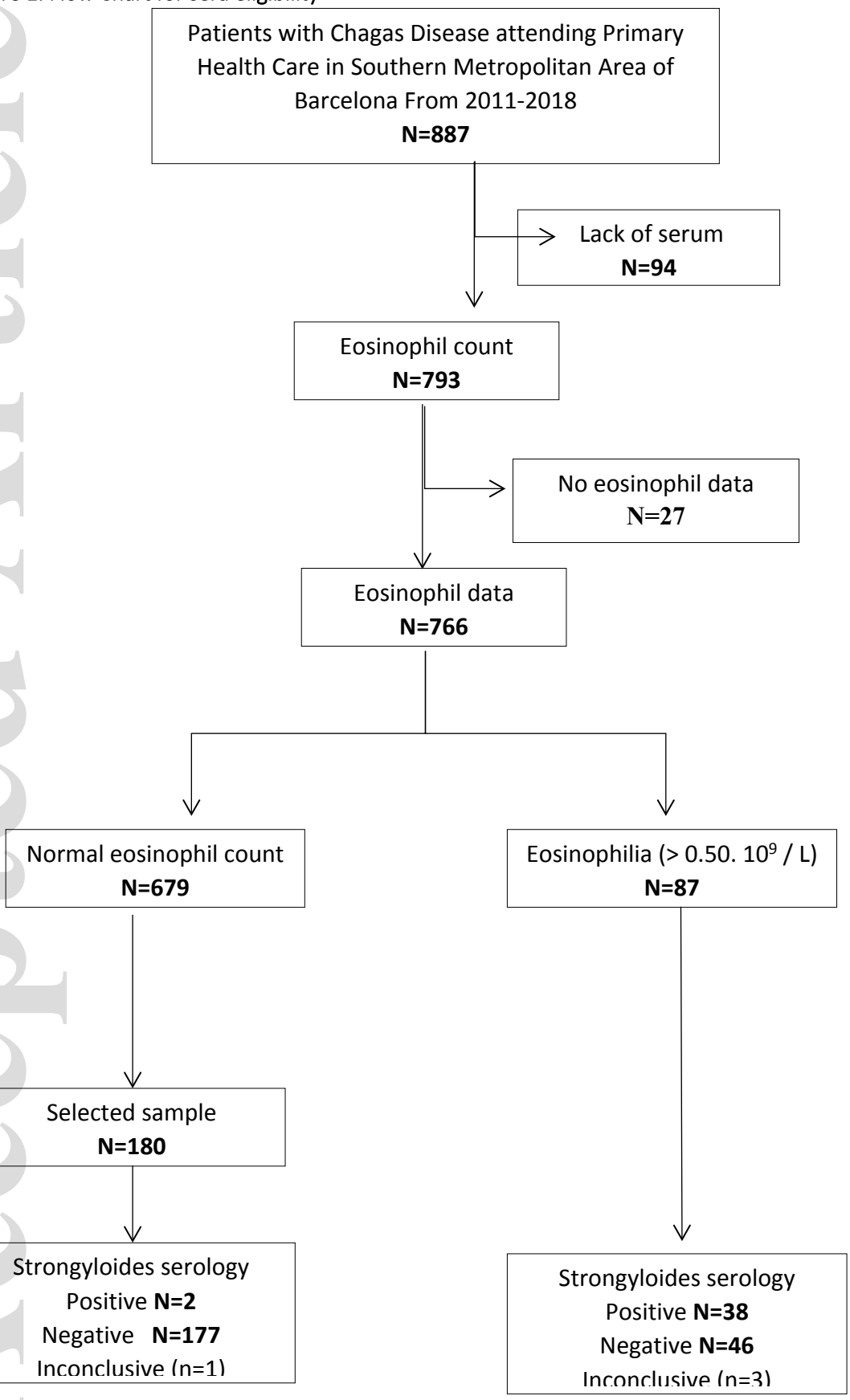

Table 1. Demographic and epidemiological characteristics of studied population and according to *S. Stercolaris* serology

Categorical	Total				***Strongyloides stercoralis* serology**					
					Negative			**Positive**		
		N	**Freq.**	**%**	**N**	**Freq.**	**%**	**Freq.**	**%**	***p***
Gender	Female	267	195	73.0	263	169	87.6	24	12.4	0.038
	Male		72	27.0		54	77.1	16	22.9	
Age (years)	<18	267	4	1.5	263	4	100.0	0	0.0	
	18-65		259	97.0		215	84.3	40	15.7	0.261
	>65		4	1.5		4	100.0	0	0.0	
Country of origin	Bolivia	267	187	70.0	263	156	83.4	31	16.6	0.113
	Paraguay		4	1.5		4	100.0	0	0.0	
	EL Salvador		2	0.7		2	100.0	0	0.0	
	Brazil		1	0.4		1	100.0	0	0.0	
	Argentina		1	0.4		1	100.0	0	0.0	
	Uruguay		1	0.4		0	0.0	1	100.0	
	Honduras		1	0.4		0	0.0	1	100.0	
	Unknown		70	26.2		59	89.4	7	10.6	
Eosinophil count	Normal	267	180	67.4	263	177	98.9	2	1.1	<0.001
	≥ $0.5.10^{9}$/L		87	32.6		46	54.8	38	45.2	

S. stercoralis	Negative	267	223	83.5						
	Positive		40	15.0						
	Inconclusive		4	1.5						
Continuous		**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**Mean**	**SD**	***p***
Age (years)		267	37.17	10.54	263	36.74	10.57	39.35	10.56	0.151
Eosinophil ($x10^9$/L)		267	0.4157	0.41698	263	0.2936	0.26624	1.0458	0.49419	<0.001

Freq. = Frequency; SD = standard deviation

Table 2. Simple and age-gender-adjusted ratios of association: eosinophilia and *S. stercoralis* infection

	Not Adjusted OR	95% CI	Adjusted OR	95% CI	*p*
Constant	**0.011**		**0.003**		
Eosinophil ≤ 0.5.10 ^{9} /L	**73.109**	**17.005-314.311**	**75.584**	**17.344-329.079**	**<0.001**
Gender (Female)	**2.086**	**1.033-4.214**	**1.623**	**0.674-3.907**	**0.280**
Age	**1.022**	**0.992-1.053**	**1.034**	**0.996-1.074**	**0.082**

OR. = Odds ratio, 95% CI=Confidence Interval 95%

Table 3. Test accuracy of eosinophil count to *S. stercoralis* infection

Outcomes	***Strongyloides stercoralis*** **ELISA**		
	Positive	**Negative**	
Eosinophil count ≥ 0.5.10 ^{9} /L	38	46	
Eosinophil count ≤ 0.5.10 ^{9} /L	2	177	
Total	40	223	
			IC 95%
Sensibility	95%		82-99
Specificity	79%		73-84
Positive predictive value	45%		34-56
Negative predictive value	99%		96-100