

Short Communication

Seroepidemiological aspects of human infection by *Strongyloides stercoralis* in Alfenas, southern Minas Gerais, Brazil

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Abstract

Introduction: In most *Strongyloides stercoralis* infected individuals, nematodiasis occurs asymptotically, but in immunocompromised patients, it can cause hyperinfection. Serological techniques seem to be a good alternative for detecting this parasite. **Methods:** The frequency of seropositivity for strongyloidiasis in Alfenas, MG, was estimated using the enzyme linked immunosorbent assay on blood samples, between May and August of 2015. **Results:** Out of 258 samples tested, 53.9% were positive, and the frequency of seropositive individuals was higher in the peripheral districts of the municipality. **Conclusions:** The results indicate high seropositivity rates for strongyloidiasis among the residents of Alfenas city.

Keywords: Strongyloidiasis. Helminth, Seropositivity. Alfenas.

Human strongyloidiasis is a helminth disease caused by *Strongyloides stercoralis*, infecting 30-100 million people worldwide¹. This infection is more common in hot and humid regions, where sanitary conditions are unsatisfactory or non-existent and most infected people present a chronic asymptomatic infection. Due to this autoinfection cycle, individuals with *S. stercoralis* infection can remain infected lifelong under normal conditions, if not properly treated and are generally asymptomatic. However, immunosuppressed individuals, especially those on corticosteroids can develop a hyperinfection syndrome with symptoms of severe disease, which can be fatal^{2,3}.

The prevalence of strongyloidiasis has increased, particularly in the southern, eastern and central Europe, Caribbean islands, Southeast Asia, Latin America, and sub-Saharan Africa⁴. In non-endemic regions, it is mainly diagnosed in migrants from endemic countries³. In many tropical and subtropical countries, the prevalence of *S. stercoralis* infection ranges from 10% to 40%¹, reaching 50% in areas where moist soil and inadequate

disposal of human waste coexist, such as certain tropical regions of Brazil⁴.

Compared to other soil-transmitted helminths (STHs), *S. stercoralis* infection is difficult to diagnose. The slow release of larvae in the feces leads to a low parasite load in most infected individuals making parasitological methods less sensitive^{4,5}. The infection is systematically missed by diagnostic methods recommended by the World Health Organization (WHO), such as the Kato-Katz method¹. Besides, the parasitological methods are labor-intensive, and difficult to perform due to the presence of infective live larvae, especially in field studies⁵.

Several immunological tests, such as the enzyme-linked immunosorbent assay (ELISA), western blot, immunofluorescence, and the luciferase immunoprecipitation system, have been described for the diagnosis of strongyloidiasis, and have been demonstrated to have better sensitivity than the parasitological techniques. ELISA has a sensitivity ranging from 57% to 100%, and specificity from 60% to 100% depending on the characteristics of the serum sample and the antigenic preparation⁴. Although problems related to cross-reactivity with other nematodes must be considered, these immunological methods are promising as screening tools in clinical and epidemiological studies^{4,5}.

The present study aimed to estimate the serological frequency of strongyloidiasis in the municipality of Alfenas,

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Received 4 March 2018

Accepted 15 May 2018



Minas Gerais, using ELISA on blood samples collected from patients who attended the Central Laboratory of Clinical Analyses (Laboratório Central de Análises Clínicas, LACEN) of the Federal University of Alfenas, UNIFAL-MG.

The study was carried out in the municipality of Alfenas, located in the southern region of the state of Minas Gerais, Brazil. Alfenas is located 343 km away from Belo Horizonte, the capital of the state, and was reported to have a population of 73,774 inhabitants in the most recent census (2010) and an estimated population of 79,707 inhabitants in 2017⁶.

A total of 258 serum samples were selected between May and August 2015. The samples diluted with an equal volume of buffered glycerin (neutral pH) to improve preservation, were identified based on their LACEN database code and were stored at -20°C until further use. Anonymized information regarding the gender, age and residence address of the patients was collected from the LACEN database and organized in a Microsoft Office Excel® (Microsoft Corporation, Redmond, WA, USA) worksheet for further analysis.

The ELISA was performed according to a previously described method⁷. Briefly, polystyrene microplates were coated with detergent extract of *Strongyloides venezuelensis* (50 µL/well) at a final concentration of 5 µg/mL in 0.06 M carbonate-bicarbonate buffer (pH 9.6). After overnight incubation at 4°C, the plates were washed three times for 5 min with phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 (PBS-T), and then blocked with PBS-T plus 3% non-fat milk (PBS-TM) for 45 min at 37°C. Serum samples were diluted 1:100 in PBS-TM and incubated for 45 min at 37°C. After washing the plates, peroxidase-conjugated goat anti-human IgG antibodies (Sigma, St. Louis, MO, USA) diluted in PBS-TM at a previously determined dilution were added to each well and incubated for 45 min at 37°C. The reaction was visualized by the addition of the enzyme substrate (0.03% H₂O₂) and chromogen (ortho-phenylenediamine) in 0.1 M phosphate-citrate buffer (pH 5.0). After incubation in the dark for 15 min at room temperature, the reaction was stopped by the addition of 2 N H₂SO₄ solution. Optical densities (OD) were determined at 492 nm using an ELISA reader (Anthos-Labtec Zenyt 200rt, Cambridge, UK). Cutoff values were calculated each day using the arithmetic mean OD of three negative control samples, performed in duplicate, plus three standard deviations. The reactivity index (RI) was determined by calculating the relationship between the OD of the sample and the cutoff value for that day according to the formula: $RI = OD/cutoff$. Values of $RI > 1$ were considered positive. The positivity rates were determined according to gender, age, and place of residence.

Data were analyzed using the Epi Info™ software (version 7.2.1.0; Centers for Disease Control and Prevention, Atlanta, GA, USA). The data were reported as percent seropositive (positivity rates), and the respective 95% confidence intervals were calculated to verify the significance of the differences observed among the diverse groups.

The strongyloidiasis seropositivity data were evaluated in the districts/neighborhoods of Alfenas which were organized into seven regions, as shown in **Figure 1**. Region A represented the

central area of the city and region G included two rural areas, the Gaspar Lopes district and the Community of Barbaras.

Of the 258 serum samples tested, 139 (53.9%) were positive for strongyloidiasis. The positivity rates in women and men were 52.4% and 56.7%, respectively, with no significant difference between genders.

Table 1 shows the distribution of strongyloidiasis seropositivity in regions A to G of Alfenas (**Figure 1**). The frequency of seropositivity was higher in the neighborhoods/districts at the periphery of the municipality (E, F, and G) compared to those within the central region (A, B, C, and D), though the differences were not significant. The highest positivity rates were observed in the age groups of 31 to 40 years (62.5%) and 41 to 50 years (60%) (**Table 2**). However, the differences between age groups were not significant (**Table 2**).

Soil-transmitted helminth (STH) infections continue to represent a major public health issue in many developing countries. STHs are transmitted via the eggs or larvae in the feces of infected individuals, which in turn contaminate the soil in areas with poor sanitation. It is estimated that approximately 1.5 billion people are infected with STHs worldwide, but this could be an underestimation due to a large number of undiagnosed individuals⁸. From a clinical-epidemiological standpoint, strongyloidiasis is relevant because if not treated properly, it can persist for life in various severe forms some of which can be fatal. Many epidemiological aspects of *S. stercoralis* infection are poorly understood, with all the available information coming from studies on other STHs which mostly use diagnostic methods with low-sensitivity for *S. stercoralis*¹. The Kato-Katz technique is based on the presence of helminth eggs, but cannot detect larvae, which is the basis for the diagnosis of strongyloidiasis. Some parasitological methods that are based on the parasite load such as spontaneous sedimentation or formalin-ether centrifugation, also have low sensitivity for detecting larvae. However, the Baermann technique and its variations, as well as agar plate culture can detect larvae, the latter being more sensitive. These have been shown to be more effective methods for the parasitological diagnosis of strongyloidiasis⁹. However, the low parasitic burden in most patients with chronic infections caused due to the intermittent larval excretion results in a high number of false negatives³.

Like in other countries, strongyloidiasis is considered a neglected disease in Brazil. Based on parasitological methods, its occurrence in humans is 5.3%, 7.9%, 6.6%, 3.9% and 4% in the north, northeast, midwest, southeast and the south of Brazil¹⁰. Few serological surveys for *S. stercoralis* have been conducted in Brazil, which have focused on the state of Minas Gerais in southeast Brazil. Using an immunofluorescence antibody test (IFAT), the mean rate of positivity in different populations has been reported to range from 7.7% to 41.1%, and from 18.5% to 40.0% as measured by ELISA-IgG^{11,12}.

In the present study, which included patients attending a clinical laboratory in Alfenas, a positivity rate of 53.9% (139/258) was observed using a detergent extract of *S. venezuelensis* as the antigen in the ELISA-IgG test. According to da Silva et al.⁷ this test has a sensitivity of 83% and a

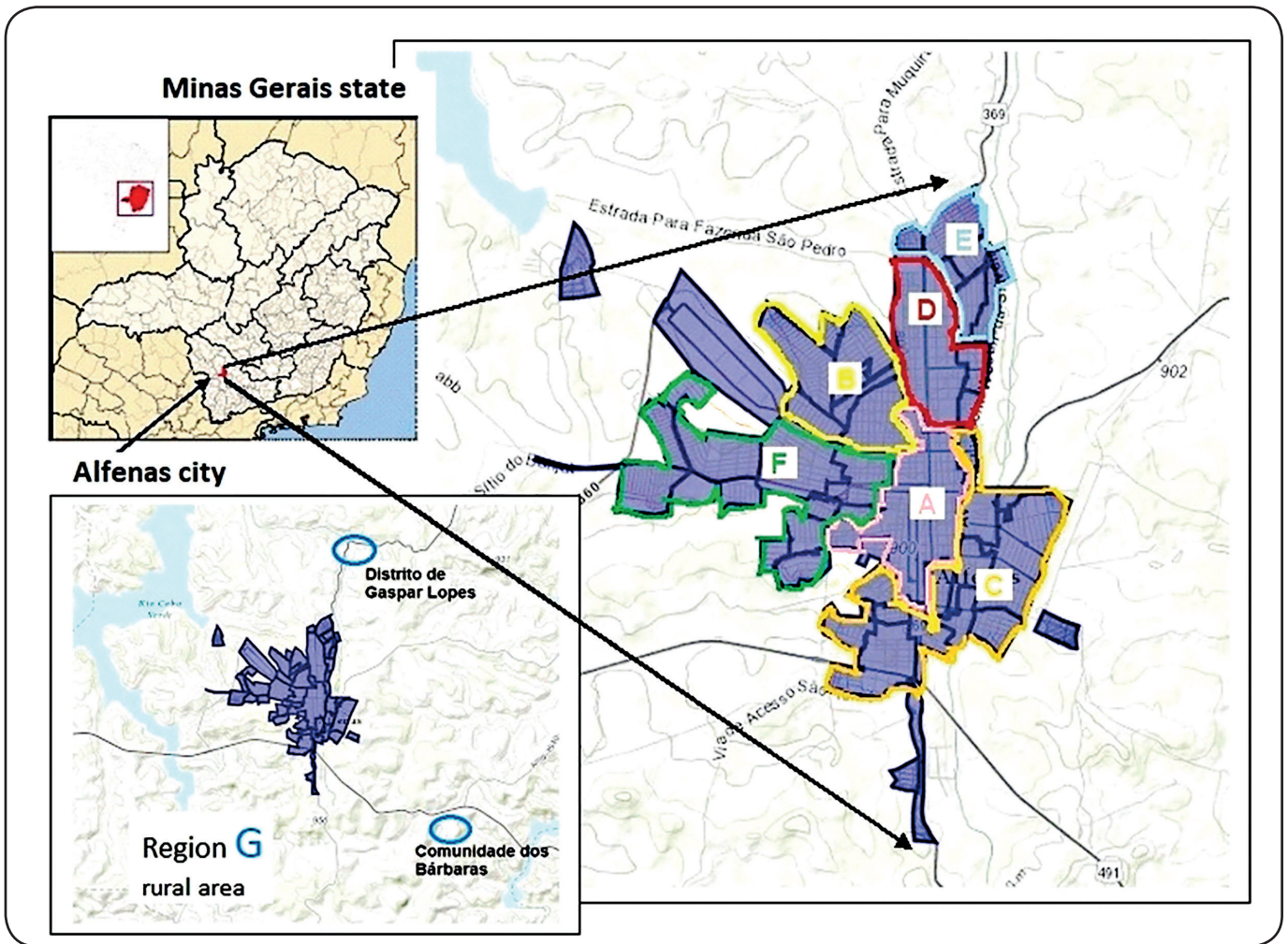


FIGURE 1: Localization of Alfenas city in the south region of Minas Gerais state, and organization of its different neighborhoods/districts as regions (A to G).

Fonte: <<https://www.arcgis.com/home/webmap/viewer.html?webmap=5a0f0edb7c05451b8dc9b8dbc9e7e519>> and <<https://pt.wikipedia.org/wiki/Alfenas>>, with some modifications introduced by the authors.

TABLE 1: The occurrence of anti-Strongyloides seropositivity in different regions of Alfenas.

Region	N	P	% P (95% confidence interval)
A	24	22	47.8 (32.9 - 63.1)
B	22	23	51.1 (35.8 - 66.3)
C	10	4	28.6 (8.4 - 58.1)
D	23	23	50.0 (34.9 - 65.1)
E	24	37	60.7 (47.3 - 72.9)
F	9	21	70.0 (50.6 - 85.3)
G	2	7	77.8 (40.0 - 97.2)
Without information	5	2	28.6 (3.7 - 71.0)
Total	119	139	53.9 (47.6 - 60.1)

Shown are the positivity rates (% P) of anti-*Strongyloides* IgG antibodies, detected by ELISA in 258 patients living in Alfenas, MG, who attended the Central Laboratory of Clinical Analysis (LACEN) of the Federal University of Alfenas, from May to August 2015. The patients have been categorized based on the regions of their residence, within the municipality of Alfenas.

N (Negative) or P (Positive), if $RI \leq 1.0$ and $RI > 1.0$ [$RI = \text{reactivity Index} = \text{OD of the sample} / \text{Cut Off}$], respectively.

TABLE 2: The occurrence of anti-Strongyloides seropositivity in different age groups.

Age group (years)	N	P	%P (95% confidence interval)
0 - 10	8	7	46.7 (21.3 – 73.4%)
11 - 20	17	22	56.4 (39.6 – 72.2%)
21 - 30	17	24	58.5 (42.1 – 73.7%)
31 - 40	12	20	62.5 (43.7 – 78.9%)
41 - 50	14	21	60.0 (42.1 – 76.1%)
51 - 60	19	23	54.8 (38.7 – 70.2%)
61 - 70	18	12	40.0 (22.7 – 59.4%)
>70	14	10	41.7 (22.1 – 63.4%)
Total	119	139	53.9 (47.6 - 60.1)

Shown are the positivity rates (% P) of anti-*Strongyloides* IgG antibodies, detected by ELISA, in the 258 patients living in Alfenas, MG, attending at the Central Laboratory of Clinical Analysis (LACEN) of the Federal University of Alfenas, from May to August 2015. The patients have been categorized based on their age.

N (Negative) or P (Positive), if $RI \leq 1.0$ and $RI > 1.0$ [$RI = \text{reactivity Index} = \text{OD of the sample} / \text{Cut off}$], respectively.

specificity of 86%. The seroprevalence for strongyloidiasis observed in this study area is higher than that in northwestern Minas Gerais, which reported prevalence rates of 18% to 40%. Serological surveys conducted in other Latin American countries have shown contrasting results. In Peru, in a rural community of the Amazon region, a seroprevalence of 72% (442/609) was found by ELISA, whereas *S. stercoralis* was identified in only 8.7% (69/792) of the stool samples evaluated by three parasitological techniques including the Baermann method.¹³ In Chile, 0.25% (3/1200) of the samples from apparently healthy individuals were assessed by ELISA, and in some high-risk groups, such as mentally ill hospitalized patients, a positive frequency of 12.1% (82/675) was observed¹⁴. A serological prevalence of 11.2% (137/1225) can be indirectly inferred for the Bolivian population through information related to the Bolivian immigrants in European countries, in which positivity rates of 9.7% and 13.4% were reported in 739 and 486 Bolivian migrants in Italy and Switzerland, respectively¹⁵.

The particularly high seroprevalence for strongyloidiasis observed in this study when compared to other studies employing a similar diagnostic approach must be further investigated. One of the greatest challenges related to the use of serological tests for epidemiological studies is the possibility of cross-reactivity with other helminthic infections. Therefore, the limitations of this study include the lack of data related to other intestinal parasites within the study area, in addition to the relatively small sample size and no access to other clinical and laboratory data on patients. Furthermore, certain clinical conditions which are more common among patients attending the laboratory might have created a bias towards a higher prevalence.

To our knowledge, this is the first study on the occurrence of strongyloidiasis in the southern region of Minas Gerais. We provide serological evidence that strongyloidiasis is present

in Alfenas, although it is important to confirm these findings in a larger and more representative population sample. The epidemiological data used in this study was limited to those currently residing in the city, and several aspects of the study require further investigation. Future epidemiological studies should identify individual risk factors so that appropriate interventions can be implemented.

Ethical aspects

Serum samples were provided by the LACEN without patient names to preserve their anonymity. Instead only their registration code, gender, age and place of residence were provided. The project was approved by the Research Ethics Committee of the Federal University of Alfenas (approval number 968,626; 27/02/2015).

Acknowledgments

To the team of the Central Laboratory of Clinical Analyses / Federal University of Alfenas (UNIFAL-MG).

Conflict of interests

The authors declare that there is no conflict of interest.

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