

Serologic Monitoring of Public Health Interventions against *Strongyloides stercoralis*

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Abstract. Northwestern Argentina is endemic for soil-transmitted helminths, and annual deworming programs are carried out in prioritized areas. High prevalence of *Strongyloides stercoralis* was reported in this area; therefore, control programs including ivermectin are being evaluated. The NIE-enzyme linked immunosorbent assay (ELISA) was used for this purpose. In this community trial, two groups of patients, classified according to housing and living conditions were evaluated. Simultaneous with baseline survey, Group 1 was moved to new households with access to improved water and sanitation facilities (W and S), where deworming (MDA, massive drug administration) took place within 1 month; whereas Group 2 received MDA but remained living with unimproved W and S. The mean time interval between baseline and the follow-up was 331 days for Group 1 and 508 for Group 2. Anti-NIE levels were measured for each individual before and after interventions and follow-up optical density (OD) ratios were calculated to quantify the variation. A significant decrease of the anti-NIE levels between baseline and follow-up was observed in both groups. Nonetheless, the number of patients that achieved the cure criteria (OD ratio < 0.6) was higher in Group 1 than Group 2 with values of 72.7% (24/33) and 45.0% (18/40), respectively ($P = 0.0197$). Our results support the conclusion that a combined intervention including deworming and improvements in life conditions is more effective, in terms of the proportion of subjects cured than deworming alone. Furthermore, we found that NIE-ELISA is a useful test for assessing the response to treatment and to evaluate the outcome of control intervention programs.

INTRODUCTION

Strongyloidiasis is one of the soil-transmitted helminth (STH) infections, which are among the most common infections around the world affecting the poorest and most deprived communities frequently in developing zones.^{1,2} *Strongyloides stercoralis* infection is globally distributed, affecting an estimated 30–370 million people worldwide. It is associated with poverty and the lack of basic services and sanitary conditions and has been recognized as one of the most neglected of the neglected tropical diseases (NTDs) (not officially listed as NTDs).^{3–6} It is particularly endemic in tropical and subtropical areas like northern Argentina, where prevalence rates as high as 30–50% have been reported, with socioeconomic, and environmental conditions favorable for its transmission and persistence.^{7–9}

The particularities of the life cycle of *S. stercoralis*, which include an autoinfective step where L3 larvae reinfect the host through the intestinal mucosa and the perianal skin, can lead to persistent and lifelong infections, which could develop into fatal complications like hyperinfection syndrome and disseminated infection. In this scenario, anthelmintic therapy should be curative; therefore, the evaluation of response to treatment and management of strongyloidiasis have become a major concern in strongyloidiasis, which needs to be studied and more deeply addressed.^{5,10–12}

Nowadays, ivermectin is the treatment of choice for strongyloidiasis. Its superior efficacy compared with albendazole and its better safety profile compared with thiabendazole have been demonstrated. Furthermore, there is some evidence that

endemic communities might benefit from regular ivermectin administration in populations where it was provided for onchocerciasis and lymphatic filariasis control, but further evaluations are needed to demonstrate the impact and effectiveness of mass drug administration addressed particularly to *S. stercoralis*. Currently, ivermectin donations are limited to the control of onchocerciasis and also to programs for the control of lymphatic filariasis in areas coendemic with onchocerciasis.^{5,13}

The coproparasitological tests that are conventionally used to diagnose *Strongyloides* (direct smear, formalin ether concentration, Baermann, and agar plate culture) are not sensitive enough, particularly in chronic infections where a high percentage of the cases are not detected.^{3,12,14–16}

Because of the problems with conventional parasitological tests, efforts have focused on the development and evaluation of novel serological techniques, but these show variable sensitivity and specificity depending on the source of antigen and the population tested. Enzyme-linked immunosorbent assay (ELISA) appears to be a convenient alternative for diagnosis; ELISA methods using crude antigen preparations of *Strongyloides* spp. achieve excellent sensitivity, but have some shortcomings, due to difficulties in antigen preparation as well as cross-reactions with other helminth infections.^{12,14,15,17}

These tests are also being implemented in nonendemic regions for follow-up (F/U) evaluation after treatment and have shown that IgG levels against *S. stercoralis* tend to decline over time after treatment, but in the cases of endemic population the serological evaluation has not been evaluated in depth. Nonetheless, there is considerable variability between results depending on the antigen used, the intervals of time after treatment and the drug chosen for treatment.^{12,16,18–26}

The development of an ELISA based on a 31-kDa recombinant antigen, NIE, isolated from a *S. stercoralis* L3 cDNA library, has been reported to overcome the difficulties of

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the crude larval extracts, being over sensitive (75.4% [67.5–83.3]) without cross-reactivity with other helminthic infections (specificity 94.8% [90.7–98.9]) as well as being easily purified and produced in large scale.^{8,27,28} Furthermore, NIE-ELISA and luciferase immunoprecipitation system were recently evaluated as part of a clinical trial, and NIE-ELISA proved to be the best indicator of seroconversion after successful therapy compared also to an in-house indirect fluorescent antibody technique and two commercial serologic tests.^{29,30}

In our study, the NIE-ELISA test was used to assess the serological response to treatment in endemic areas. This study was conducted as part of a deworming project against STHs in a hyperendemic area of northwestern Argentina, where annual interventions of massive drug administration (MDA) are carried out not only with albendazole (as it is commonly executed) but also with ivermectin, in the context of a research intervention carried out by the group at Universidad Nacional de Salta, the Provincial primary care program and the nongovernmental Fundación Mundo Sano. The aim of this study was to evaluate the serological response to interventions (MDA program versus a MDA + W and S program) through NIE-ELISA among other parameters.

MATERIALS AND METHODS

This study was conducted as part of a MDA program against STH conducted by the Instituto de Investigaciones en Enfermedades Tropicales-Universidad Nacional de Salta in collaboration with Fundación Mundo Sano and the Primary Health Care System. For this program, free treatment with ivermectin (200 µg/kg of body weight) and albendazole (400 mg), both in a single dose, was delivered to the entire study population.

The study included two urban and one rural communities from Pichanal, Orán, and Tartagal, all pertaining to Salta Province. The characteristics of the area and study population were previously detailed by Echazú and others.⁶ From September 2010 to March 2014, fecal and venous blood samples were randomly collected (through a random generated list with the household as the unit of randomization) for copro-parasitological tests (sedimentation concentration, Baermann, Harada Mori and McMaster, as described elsewhere),³¹ complete blood counts (CBCs), and a serological assay (NIE-ELISA).

The study population samples were classified in two groups according to the World Health Organization/United Nations International Children's Emergency Fund recommendation for monitoring sanitation and water supply: Group 1 (G1): Simultaneous with baseline (B/L) survey, was moved to new households at 2 km from the original settlement (through a Provincial program initiated for safety reasons to accommodate a major gas duct) with access to improved water and sanitation facilities (W and S) where deworming took place within 1 month (MDA) (G1 = MDA + W and S); whereas Group 2 (G2) remained living with unimproved W and S (G2 = MDA alone). No other significant differences in terms of geographic, socioeconomic, cultural, or behavioral conditions were identified between communities in both groups.

The second (F/U) serum sample was collected for post-treatment surveillance and to evaluate the outcomes by NIE-ELISA and CBC.

NIE-enzyme linked immunosorbent assay. The antibody levels were measured in all serum samples by an ELISA test using the recombinant antigen, NIE, for the diagnosis, estimation of prevalence of *S. stercoralis* infection, and to monitor treatment outcomes.

The NIE-ELISA was performed as has been previously described,³² with minor modifications. A final concentration of 0.125 µg/well/100 µL NIE antigen in buffer coating (pH = 9.6) was added to polystyrene plates and incubated at 4°C overnight, afterward, this plate was blocked with phosphate-buffered saline (PBS)-Tween 20-5% skim milk. Sera were added at a dilution of 1:100 followed by Human anti IgG-Fc (Jackson Immuno Research, West Grove, PA) in a dilution of 1:20,000. Finally, tetramethylbenzidine substrate was added and allowed to react for 15 minutes and read at 450 nm. Each step was incubated at 37°C for 1 hour, and plates were washed five times with PBS-Tween 0.05% in between. Patient sera were tested in duplicate and means of results were calculated and corrected for background reactivity (no serum added).

The cutoff value was optimized by constructing receiver operating curves (ROCs) based on ELISA results from 20 patients with parasitological evidence of larvae as positive controls and 20 healthy controls from Salta Capital, a non-endemic area. NIE-ELISA achieved a specificity and sensibility of 85% and the area under the curve ROC = 0.92, parameter interpreted according to Swets (1988)³³ as a high accuracy test. The cutoff was selected on basis of this optimal combination of sensitivity and specificity.

Follow-up. Serum samples were stored at -20°C and tested paired in parallel on the same run to eliminate run-to-run variation. To monitor the response to the intervention, we measured the anti-NIE antibody levels (optical density [OD]) and the eosinophil count (absolute eosinophil count cell/µL) at B/L and F/U. To quantify the variation in anti-NIE antibody levels, a F/U OD ratio (OD_{F/U}/OD_{B/L}) was calculated as the posttreatment values divided by the B/L ones for each subject, following the criteria of Kobayashi and others.³⁴ An OD ratio < 0.6 was considered to be an effective response to treatment (serological cure).

Statistical analysis. For the assessment of the change between B/L and F/U, the changes in mean antibody levels (OD) were assessed by the Wilcoxon matched pairs test, and the difference in OD ratios between the two groups was analyzed by a Mann-Whitney test for intergroup comparisons of continuous data. Differences between specified patient groups were analyzed by the Kruskal-Wallis test and differences in proportions of patients who seroconverted and achieved the cure criteria (OD ratio < 0.6) were evaluated through χ^2 test. Results were considered statistically significant with $P < 0.05$. The analyses were performed using software Prism 5 GraphPad Demo (San Diego, CA) and Info stat, version 1.1. (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) and EPIDAT3.1 (<http://dxsp.sergas.es>).

Ethical approval. Ethical approval was obtained from the Bioethics Committee of College of Physicians of Salta Province, Argentina (N°14.200), and the Faculty of Health Sciences from the Universidad Nacional de Salta. Participation was

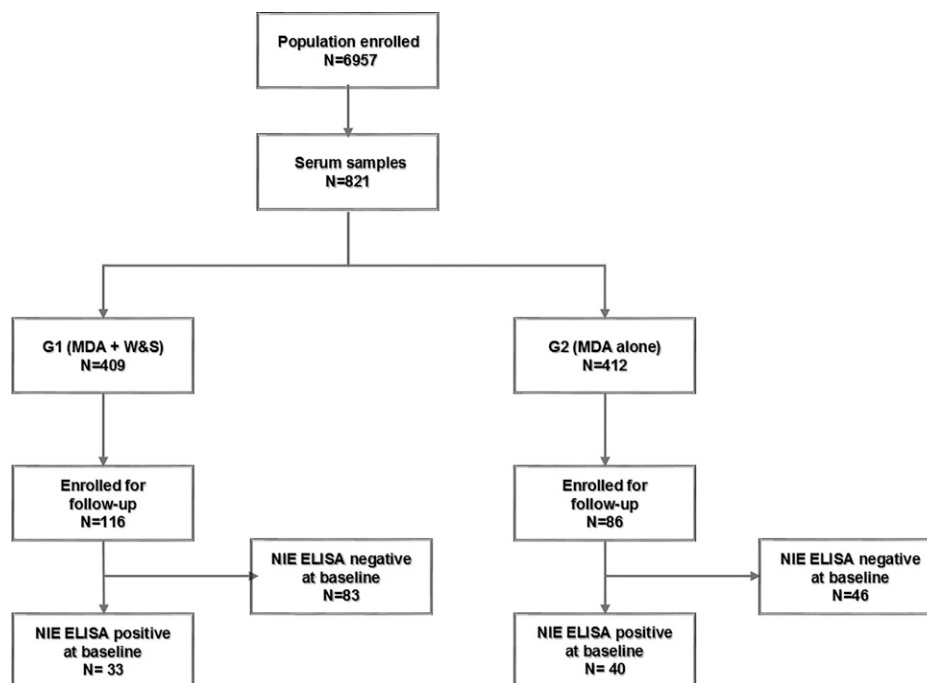


FIGURE 1. Flow chart. The flow of participants involved in the project.

voluntary and an informed consent was obtained from all patients involved in the study.

RESULTS

The population under treatment in the three communities included 6,957 individuals; the randomly selected survey sample group included 821 blood samples at B/L, 409 belonging to G1 and 412 to G2. Of these, the F/U group of samples included 116 and 86 individuals with paired samples in Groups 1 and 2, respectively (Figure 1). The main characteristics of the population are summarized in Tables 1 and 2.

The mean interval between B/L and the F/U measurements was 331 days for G1 and 508 days for G2. The number of patients who had a NIE-ELISA positive at B/L (from the 116 and 86 individuals with paired serum samples) was 33 for G1 and 40 for G2. See details of sample selection (Figure 1). Of the patients NIE-ELISA negative at B/L, 1.2% (1/83) became positive at the time of F/U in G1 and 8.7% (4/46) in G2; a significant difference in the incidence between groups $P = 0.0347$.

Comparing anti-NIE antibody levels between B/L and F/U within groups (B/L G1 versus F/U G1 and B/L G2 versus F/U

G2), we observed a significant decrease ($P < 0.0001$) in both groups (Figure 2). In the comparison between groups, we found no significant difference in anti-NIE antibodies levels between B/L (B/L G1 versus B/L G2) and F/U (F/U G1 versus F/U G2) (Figure 2) or in the ratio of antibodies between groups ($P > 0.05$) (OD ratio G1 versus OD ratio G2) (Figure 3).

Nonetheless, the cure rates were different in each group in terms of the proportion of individuals who reached the cure criteria for G1 (72.7% [24/33]) and G2 (45.0% [18/40]). Table 3 summarizes the results of the two F/U groups.

We found no correlation between eosinophil count and anti-NIE Ab (OD), nor between the decrease of antibody levels and eosinophil count (OD ratio with eosinophil ratio). No association was found between the age of the subjects with the B/L levels of anti-NIE and the decrease of antibodies (OD ratio); neither with eosinophilia in both groups.

DISCUSSION

Because of the potential risk of developing fatal complications and the autoinfective cycle that allows for perpetuation of infections, strongyloidiasis requires the complete eradication of the parasite. Therefore, efforts should be directed

TABLE 1
Population monitored through NIE-ELISA with paired samples

	G1 (MDA + W and S intervention) (N = 116)	G2 (MDA intervention) (N = 86)	P value
Age (year)	10 (6.3–13)*	14 (8.3–2.3)*	0.0056
Female sex (%)	56.9 (66/116)	67.4 (58/86)	0.1452
Interval between B/L and F/U (days)	331 (330–395)*	508 (282–549)	< 0.0001
Incident cases (%)† between B/L and F/U	1.2 (1/83)	8.7 (4/46)	0.0347

B/L = base line; ELISA = enzyme linked immunosorbent assay; F/U = follow-up; G1 = Group 1; G2 = Group 2; IQR = interquartile range; MDA = massive drug administration; W and S = water and sanitation facilities.

* Median (IQR).

† Based on ELISA-NIE.

TABLE 2
Parasitological and hematologic findings

	G1			G2		
	B/L	F/U	P value	B/L	F/U	P value
<i>Stst</i> community seroprevalence by NIE ELISA	20.05% (82/409)	20.5% (104/508)	0.8740	44.90% (185/412)	42.25% (139/329)	0.4693
<i>Stst</i> paired samples seroprevalence by NIE ELISA	28.4% (33/116)	8.6% (10/116)	0.0002	46.5% (40/86)	27.9% (24/86)	0.0176
Eosinophilia (%) [*]	64.3% (72/112)	42.5% (48/113)	0.0010	50.0% (42/84)	54.8% (46/84)	0.5366
Eosinophilia count [†]	593.5 (400.8–990.0)	411.5 (211.5–931.3)	0.0036	536.9 (326.5–1120)	568.0 (354.5–905.0)	0.7867
STH [‡]	58.3% (7/12)	11.8% (4/34)	0.0030	40.9% (29/71)	19.5 (8/41)	0.0231
<i>Strongyloides stercoralis</i> [‡]	16.7% (2/12)	2.9% (1/34)	0.1623	18.3% (13/71)	4.8% (2/41)	0.1612
Hookworms [‡]	8.3% (1/12)	2.9% (1/34)	0.4548	21.1% (15/71)	7.3% (3/41)	0.6550
<i>Trichuris trichura</i> [‡]	0% (0/12)	0% (0/34)	–	2.8% (2/71)	0% (0/41)	1.000
<i>Ascaris lumbricoides</i> [‡]	41.7% (5/12)	0% (0/34)	0.0006	8.5% (6/71)	14.6% (6/41)	0.3507

B/L = base line; ELISA = enzyme linked immunosorbent assay; F/U = follow-up; G1 = Group 1; G2 = Group 2; IQR = interquartile range; STH = soil-transmitted helminth.

^{*} > 500 cell/ μ L.

[†] Median (IQR).

[‡] Coproparasitological diagnosis of subjects who had provide fecal samples.

toward evaluation of the response to therapy, especially in endemic areas like northwestern Argentina.^{7,8,11} The evaluation of antibody levels among population from nonendemic countries as well as migrants has shown good results, though the information available from endemic populations is still limited.^{16,18–25,29,34–36}

In the present study, an ELISA test using the recombinant antigen NIE was applied with the aim to evaluate the serological response to interventions in groups with differential access to water and sanitation.

After treatment, both groups showed a marked decline in antibody levels and had a significant difference between the B/L and F/U time points. Given, the considerable heterogeneity in the level of reactivity among patients and the different B/L prevalence between groups, we considered that the

optimal way to evaluate the change in anti NIE-antibodies between B/L and F/U was calculating a F/U ratio as was suggested by Kobayashi and others.³⁴ Using this approach, we observed that 72.7% of G1 could be considered cured in 1 year, and 45.0% of the G2 in 508 days. On the other hand, the proportion of people who seroreverted (fall under the cutoff) was very similar to the proportion of people who reached the serological cure criteria (OD ratio < 0.6), demonstrating the consistency and robustness of the NIE-ELISA technique. Our findings support others that have shown that following therapy the serum antibodies levels decrease slowly over time.^{22,23,29,34}

Even though the general mean OD values were not different between the groups (G1 [MDA + W and S] versus G2 [MDA alone]), the proportion of people who reached the cure

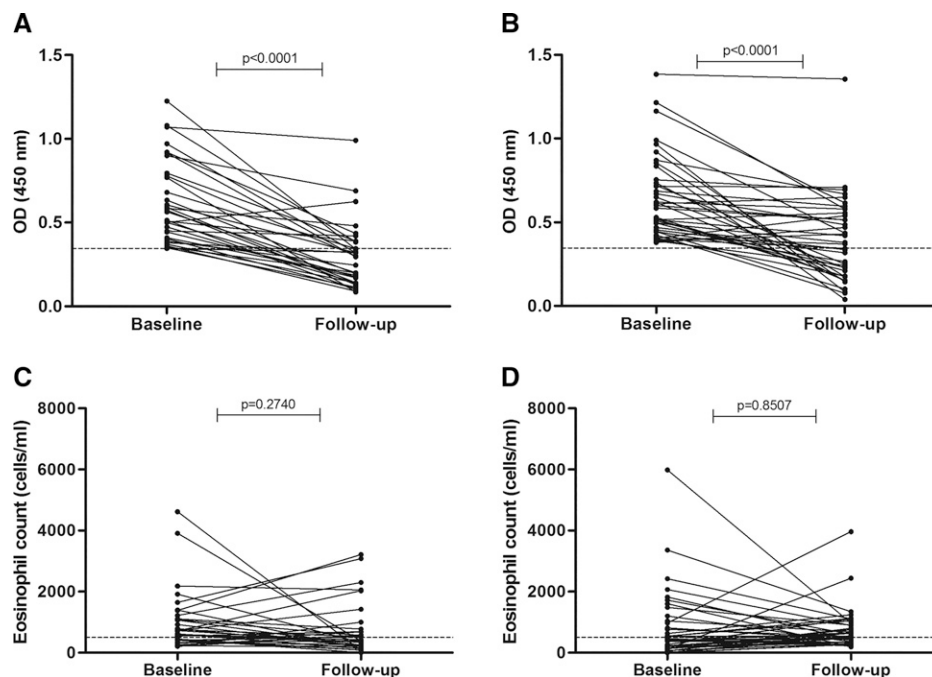


FIGURE 2. Comparison of base line and follow up. Serum anti-NIE values (OD) in (A) G1 (MDA + W and S) (N = 33) and (B) G2 (MDA alone) (N = 40), the dashed line represents the cutoff value. Absolute eosinophil count before and after treatment in (C) G1 and (D) G2. GI = Group 1; G2 = Group 2; MDA = massive drug administration; OD = optical density; W and S = water and sanitation facilities.

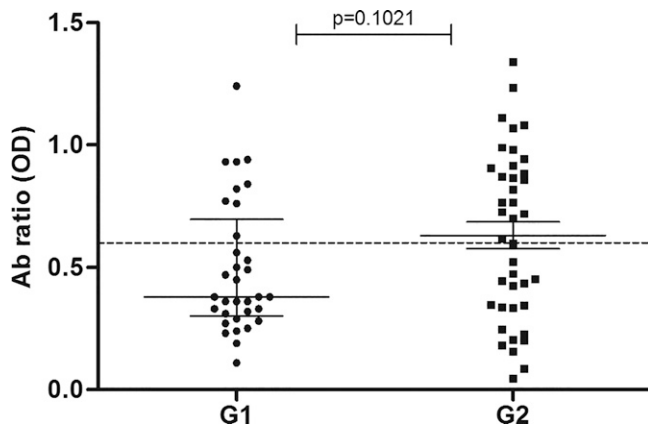


FIGURE 3. Change in antibody levels after treatment. The values are represented as OD ratio of baseline values divided by follow-up values in G1 (MDA + W and S) and G2 (MDA alone). An OD ratio < 0.6 is considered as cure. G1 = Group 1; G2 = Group 2; MDA = massive drug administration; OD = optical density; W and S = water and sanitation facilities.

criteria by NIE-ELISA test was highly different between them. These findings lead us to presume a decreased risk of reinfection in G1 as a result of the change in the living conditions regarding water and sanitation, since the same drug treatment was applied to both G1 and G2. Furthermore, the statistically significant difference in the percentage of incident cases between groups could be a result of this change in conditions. This is consistent with the association between the presence and prevalence of STH infection and unimproved water and sanitation that has been described in previous studies.^{6,37,38}

However, there are alternative explanations for this difference that should be taken into account: for instance, the prevalence of STH and particularly *S. stercoralis* was different between study communities at B/L. Therefore, the risk of reinfection could be not only higher in G2 due to the lack of sanitary improvements or a larger environmental burden developed to the higher B/L prevalence but also could exist different factors not evaluated in this work that can influence the antibodies levels found at F/U.

The results in G1 are similar to the findings of two novel evaluations using NIE antigen by ELISA in nonendemic areas where the rate of cure was 71–73% between 9 and 18 months.^{29,39} In G2, however, the cure rate was lower than G1 despite the fact that the same treatment was applied. An alternative explanation to this difference, which constitutes a limitation of this study is the longer interval between B/L and F/U in G2, which could potentially allow more time for reinfection. The differences in the intervals between surveys should be understood in the context of a substudy performed within a large intervention trial guided by the times and priorities of the Provincial Primary Care service.

F/U time is another factor that affects the treatment outcome, and it is difficult to define an appropriate time interval to measure antibody and detect a significant decrease in antibody level. Most authors assessed a F/U period of 9–21 months,^{18,19,21–23,29,34} and observed that the largest drop occurs between the 6 and 9 months, suggesting that ideal F/U time could be 6–12 months. An

TABLE 3

Compared results of patients enrolled for follow-up at G1 (MDA + W and S) and G2 (MDA alone)

	G1 (N = 33)	G2 (N = 40)	P value
Serological cure (OD ratio < 0.6)	72.7% (24/33)	45.0% (18/40)	0.0197
Eosinophil count decrease	42.4% (14/33)	30% (12/40)	0.3296

G1 = Group 1; G2 = Group 2; OD = optical density; MDA = massive drug administration; W and S = water and sanitation facilities.

evaluation of the NIE-ELISA against another four tests with a composite reference standard, through the application of a mixed model for predicting the drop of antibodies, supports the conclusion that 1 year is necessary to observe seroreversion.²⁹

This is similar to the findings in the G1 after approximately 331 days after treatment where the life conditions were improved. Here, 72.7% of patients got cured, whereas in G2 it was only 45%. Nonetheless in this work, it was not possible to ensure that the reinfection factor was avoided in either group and it would be necessary in future works to have a better appreciation of the precise drug efficacy without having reinfections as a confounding factor.

Despite the fact that eosinophil count has a good positive predictive value, and some studies found an association between a decrease in the eosinophil count, decrease of antibodies, and cure,^{19,20,35} our results differ from those studies. Our study population is endemic for STH and other nematode infections such as toxocariasis⁷; therefore, the elevated and persistent eosinophilia could be triggered by parasites other than *S. stercoralis*. Besides, a good comparison between groups was not possible since the G2 had a higher number of people with polyparasitosis than G1.

This study has some limitations due the heterogeneous ages of the patients between groups; and mainly due to the loss of subjects for the time of F/U since the study area is a border area with an active labor-related migration. Because of the nature of this work, we were able to have only one follow-up point and we could not obtain fecal samples of all the subjects of study. It would be ideal for further studies to extend the F/U period and the number of collection times to ensure that each patient had a serological and copro-parasitological control.

The progress in the diagnosis and treatment monitoring of *S. stercoralis* infection, along with a better understanding of the benefit of sanitary improvements, would contribute to the creation of guidelines for the control of endemic strongyloidiasis. In this study, we observed a marked decline in anti-NIE antibody levels following deworming. Our results support the body of evidence that serology is useful for the evaluation of interventions and treatment outcomes. The use of NIE-ELISA as a tool to evaluate the serological response to treatment in endemic areas is feasible and represents a potentially significant contribution to improve the monitoring of public health interventions against *S. stercoralis*.

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