Published in final edited form as:

Am J Trop Med Hyg. 2006 January ; 74(1): 97–102.

SEROEPIDEMIOLOGY OF STRONGYLOIDIASIS IN THE PERUVIAN AMAZON

PABLO P. YORI, MARGARET KOSEK^{*}, ROBERT H. GILMAN, JULIANNA CORDOVA, CARYN BERN, CESAR BANDA CHAVEZ, MARIBEL PAREDES OLORTEGUI, CARMEN MONTALVAN, GRACIELA MEZA SANCHEZ, BEVELLE WORTHEN, JAMES WORTHEN, FAY LEUNG, and CARLOS VIDAL ORÉ

Asociacion Benefica PRISMA, Lima, Peru; Department of International Health, Johns Hopkins University School of Public Health, Baltimore, Maryland; Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Peruvian Health Ministry, Maynas, Peru

Abstract

A stool and serosurvey for *Strongyloides stercoralis* was conducted in a community in the Peruvian Amazon region. *Strongyloidiasis stercoralis* was identified in the stool of 69 (8.7%) of 792 participants. Six hundred nine sera were tested using by an enzyme-linked immunosorbent assay (ELISA), which had a sensitivity of 92% and a specificity of 94%; 442 (72%) were positive. In multivariable logistic regression models, having *S. stercoralis* in stool was associated with hookworm in the same specimen (odds ratio [OR] = 4.44, 95% confidence interval [CI] = 2.02-9.79), occasionally or never wearing shoes (OR = 1.89, 95% CI = 1.10-3.27), and increasing age (OR = 1.012 for each one-year increase, 95% CI = 1.00-1.03). Similarly, occasionally or never wearing shoes (OR = 1.54, 95% CI = 1.01-2.37) and increasing age (OR = 1.04 for each one-year increase, 95% CI = 1.02-1.06) were associated with an increased risk of a positive *S. stercoralis* ELISA result. The ELISA had a negative predictive value of 98% and is an excellent screening test for strongyloidiasis.

INTRODUCTION

Current estimates indicate that at least 100 million people are infected with *Strongyloides stercoralis* worldwide. With the increased prevalence of infection with human immunodeficiency virus (HIV) in disease-endemic areas, the importance of this soil-transmitted nematode has grown. The entire life cycle of *S. stercoralis* can be completed in the soil, leading to long-term persistence in disease-endemic areas. Similarly, the life cycle can be completed in the host (autoinfection), which enables both prolonged maintenance of active infection and amplification of the parasite load. Amplification of *S. stercoralis* in its most severe form leads to a relatively rare but life-threatening hyperinfection syndrome in

*Address correspondence to Margaret Kosek, Department of International Health, Johns Hopkins School of Public Health, 615 North Wolfe Street, Room 5515, Baltimore, MD 21205. E-mail: mkosek@jhsph.edu

Financial support: Margaret Kosek was supported by Fogarty International Center International Research Scientist Development Award KO1TW005717. The project received additional support from grants T35AI107646 and Tropical Medicine Research Center Grant P01AI52976-01.

Authors' addresses: Pablo P. Yori, Julianna Cordova, Cesar Banda Chavez, Maribel Paredes Olortegui, Carmen Montalvan, Graciela Meza Sanchez, Bevelle Worthen, James Worthen, Fay Leung, and Carlos Vidal Oré, Asociacion Benéfica PRISMA, Morona 452, Iquitos, Maynas, Peru, Telephone and Fax: 51-6-524-2226, E-mail: iqtlab@jhsph.edu. Margaret Kosek and Robert H. Gilman, Department of International Health, Johns Hopkins School of Public Health, 615 North Wolfe Street, Room 5515, Baltimore, MD 21205, Telephone: 410-995-4195, Fax: 410-510-1284, E-mails: mkosek@jhsph.edu and gilmanbob@yahoo.com. Caryn Bern, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway NE, Atlanta, GA 30341, Telephone: 770-488-7654, Fax: 770-4887761, E-mail: cbern@cdc.gov.

immunocompromised hosts. The consequences of milder infections are less clear. However, a recent study in Tanzania identified *S. stercoralis* infection in HIV-infected pregnant women as a strong risk factor for the delivery of a low birth weight child,¹ and it is a relatively common cause of diarrhea in HIV-infected individuals² and severely malnourished children.^{3,4} Despite the high prevalence of this infection, knowledge of its epidemiology and seroepidemiology is limited. We conducted a survey in a periurban village in the Peruvian Amazon with stool examinations and serologic testing for *S. stercoralis*. Our objectives were to compare stool and serologic screening, to assess the degree of serologic cross-reaction with other geohelminths, and to characterize the community epidemiology of the parasite.

MATERIALS AND METHODS

Study population. The study site, Santo Tomas, is a rural community on the Nanay River in the northeastern Amazon in Peru (Figure 1). The town is located 15 km from the urban center of Iquitos (population = 500,000; 3.7°S, 73.2°W). During August 2002, a house-to-house census and survey of the community was completed. The survey collected data on basic sociodemographic indicators and risk factors for infection with intestinal parasites, including source and storage methods of water used for consumption, human waste disposal, and the frequency of the use of shoes. All participants were asked to provide one stool sample and allow collection of 3-5 mL of blood. The study protocol was reviewed and approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health, Asociacion Benéfica PRISMA, and by the Department of Public Health of Loreto Peru. All participants provided informed consent, and all those found to have helminth infections were treated according to current recommendations by the World Health Organization.

Examination of stool specimens. Stool specimens were evaluated by three methods: direct examination, the Baermann method, and simple sedimentation. The latter two techniques were modified as described in this report to be more suitable for the screening of a large number of samples daily. In addition, 515 samples (65% of all analyzed stool samples) were analyzed by the agar-plate method for quality control because this is currently considered the gold standard for stool diagnostics.^{5,6}

Modified Baermann method. Approximately 10 grams of stool were placed in gauze and partially submerged in water (40°C) in a 50-mL centrifuge tube and left for two hours. Five drops of sediment from the conical tip were removed with a transfer pipette and examined microscopically.

Simple sedimentation. Fifteen grams of sample were homogenized in water and gauze filtered into 400-mL plastic cylinders. Water was then added to the volume of the cylinder and the mixture was allowed to settle for 30 minutes or until the supernatant was nearly clear. Two-thirds of the volume of water was decanted and five drops of the sediment from the cylinder based was removed with a transfer pipette and examined microscopically.

Agar plate culture. Agar media consisting of 1.5% agar, 0.5% meat extract, 1.0% peptone, and 0.5% NaCl was prepared and plated on 90-mm petri plates. Approximately three grams of stool was placed at the center of the plate and the plate was covered and left for three days in the laboratory at ambient temperature (30°C). Plates were examined daily for trails and larval canals with a stereoscopic microscope.

Serologic assays. All blood specimens were centrifuged, and the serum was separated and frozen at -20°C pending examination by an enzyme-linked immunosorbent assay (ELISA) for IgG to *S. stercoralis*. Serologic response to *S. stercoralis* is known to cross-react with that for *Wuchereria bancrofti* and *Onchocerca volvulus*, but neither of these filarial worms has been described in the study region. However, in a malaria survey in a neighboring village, 4 (0.3%)

of 1,200 thick smears were positive for *Mansonella perstans*. Since we assumed that the response to *M. perstans* might also have cross-reactivity with that to *S. stercoralis*, a subset of 96 randomly selected sera were evaluated by ELISA using several filarial antigens. An extract of *Brugia pahangi* adult worms, chosen for the extensive cross-reactivity it displays with other filarial parasites, was used for screening. Sera with positive response to the polyclonal extract were then assessed for their responsiveness to the recombinant *Brugia malayi* antigen Bm14 to identify true filarial infection, as opposed to cross-reactivity between *S. stercoralis* and *Brugia* antigens.⁷

Possible serologic cross-reactions with hookworm species were evaluated by determining the prevalence of seropositivity by the *S. stercoralis* ELISA in individuals who were negative for *S. stercoralis* in stool, but positive for hookworm. This likely substantially overestimates the true cross-reactivity because individuals who previously were infected with *S. stercoralis* and subsequently eliminated it will also be included in this subgroup.

Enzyme-linked immunosorbent assay for *S. stercoralis*. *Strongyloides stercoralis* third-stage (L3) antigen was kindly provided by Dr. Franklin Neva (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) and ELISAs were done with small variations from that previously described.⁸ Briefly, after performing a checkerboard the initial antigen was diluted to a concentration of 5 µg/mL with 0.06 M carbonate buffer, pH 9, and placed in polyethylene, round-bottom, 96-well plates (Immulon-2HB; Dynex, Chantilly, VA) and incubated for three hours at 37°C. Plates were washed serially with phosphate-buffered saline (PBS) with 0.05% Tween and blocked with 1% skim milk at 37°C for one hour. Sera were diluted 1:32 in PBS-Tween solution and 50 µL of test sera was incubated in wells at 37°C for one hour. Following serial washes (all washes done with PBS-Tween), peroxidase-labeled antibody to human IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was diluted 1:4,000 in PBS-Tween, 50 µL was added to each well, and the plates were incubated at 37°C for one hour. Following serial washes, 100 µL of the substrate solution (0.01% *o*-phenylenediamine plus 0.03% hydrogen peroxide in phosphate citrate buffer) was added and the plates were left in the dark for eight minutes. The reaction was stopped with 50 µL of 2N sulfuric acid and optical densities were read at 490 nm. To improve the precision of the assay, four control sera with a pre-defined target optical density were included on each ELISA plate and the plate correction-4 (PCF4) method was used. These four pools were from sera derived from children from tropical areas not endemic for nematode infections (low negative), adults from areas not endemic for helminth infections (high negative), HIV-infected adults with stools positive for *Strongyloides* (low positive), and HIV-negative adults from a disease-endemic area (high positive). These sera were chosen to span the optical density range likely to be encountered on each ELISA plate. The ratio of the measured optical density to the pre-defined optical density was calculated for each of the control sera as a correction factor and the mean correction factor (the PCF4 value) was multiplied by measured optical densities for all test sera as previously described.⁹

The cutoff value was optimized by constructing receiving operator characteristic (ROC) curves based on ELISA results from 19 patients stool positive for *S. stercoralis* from the Iquitos area (positive controls) and 37 adults from Lima (tropical negative controls), an area with frequent intestinal protozoal infections but no geohelminth infections (Gilman R, unpublished data).

Enzyme-linked immunosorbent assay for filarial species. An ELISA for filarial species using *Brugia* extract and recombinant Bm14 were done using the methods of Muck and others.⁷

Data analysis. Data were analyzed using SPSS version 12 (SPSS Inc., Chicago, IL). Pearson's chi-square test was used for dichotomous values comparing sampled and non-sampled

subpopulations, and for univariate analyses of risk factors for *S. stercoralis* infection. Univariate and multivariable logistic regression analyses were performed with SAS version 8 (SAS Institute, Cary, NC). Models were constructed using generalized estimating equations to account for intra-household correlation.

RESULTS

Study population. The total population of the study site was 1,293 individuals living in 263 households. The mean age was 23 years, and the most commonly reported occupational categories were non-agricultural manual laborers, housewives and children less than 18 years of age. Most of the population (78.2%) relied on shallow wells for drinking water and used latrines for waste disposal (77.7%). Self-reported use of foot-wear was predominantly described as frequent (42.2%) or occasional (31.7%) as opposed to always (25.6%) or never (0.5%). A total of 908 individuals participated in the study; 492 participants provided both stool and blood specimens, while 116 provided only stool specimens and 299 provided only blood. Compared with study participants, those who did not provide specimens were significantly more likely to be male (47% of participants versus 62% of non-participants; $P < 0.001$) and more than 18 years of age (46% of participants versus 66% of non-participants; $P < 0.001$).

Stool examination and ELISA results. The most prevalent parasitic pathogens were *Ascaris lumbricoides* (41.9%), *Trichuris trichura* (16.9%), *S. stercoralis* (8.7%), *Hymenolepis nana* (7.1%), *Giardia intestinalis* (5.2%), and hookworm (4.5%) (Table 1). A stool was classified as positive for *S. stercoralis* if larvae were identified by any of the three diagnostic stools tests performed. When each *S. stercoralis* diagnostic technique alone was compared with the gold standard of all three results taken together, the sensitivity of simple sedimentation was 79.7%, that of the modified Baermann method was 40.5%, and that of direct examination was 37.7%. The agar plate method used in a subset of samples had a sensitivity of only 60.9% because of an exuberant growth of mold on 180 (35%) of 515 specimens that impeded test interpretation.

Based on ROC curves of the ELISA results for 19 true positives and 37 true negatives, the *S. stercoralis* ELISA performance was optimal at a cutoff optical density value of 0.248. At this cutoff, the sensitivity was 92% and the specificity was 93.7%. Of 609 serum specimens from study participants, 442 (72%) were ELISA positive. The mean $\pm$ SD absorbance was 0.68 ± 0.42 for positive specimens and 0.19 ± 0.05 for negative ones. We compared the stool and ELISA results for the 493 participants who provided both types of specimens (Table 2). Of these, 41 (8.3%) were positive both by stool examination and ELISA, and 138 (28%) were negative by both assays. Most (311, 63.1%) had positive serologic results, but negative stool examination results; this group may include both individuals with previous strongyloidiasis and those for whom a single stool examination failed to detect an active infection. Three individuals (0.6%) were stool positive, but serologically negative; all three were positive for *S. stercoralis* on the agar plate test. If stool examination is taken as a gold standard for current infection, these results correspond to an ELISA sensitivity of 93% (41 of 44) and a negative predictive value of 98% (138 of 141).

Evaluation for serologic cross-reactivity with filariasis and hookworm. The ELISA to detect IgG4 using *Brugia* extract was performed with 90 randomly selected study sera (74 positive and 16 negative by the *S. stercoralis* ELISA). Only seven specimens were positive by the *Brugia* extract ELISA; six of these were positive for *S. stercoralis* by the ELISA. These seven specimens were further evaluated using the recombinant *Brugia* antigen Bm to identify filarial infection; only one was positive. This specimen was from an individual who was stool negative, but seropositive for *S. stercoralis*.

Sera were available from 18 individuals whose stool specimen was positive for hookworm, but negative for *S. stercoralis*. Fourteen of these individuals (77.8%) had a positive ELISA result for *S. stercoralis*. The *S. stercoralis* seroprevalence did not differ among those with and without hookworm in their stool ($P = 0.79$). Hookworm infection was not significantly associated with positive *S. stercoralis* ELISA results in a multivariable logistic regression model adjusted for age ($P = 0.38$).

Risk factors for *S. stercoralis* infection by stool examination and ELISA. In univariate analyses, males were more likely than females to be stool positive for *S. stercoralis* (relative risk [RR] = 1.64, 95% confidence interval [CI] = 1.00-2.71). The prevalence of *S. stercoralis* in stool increased rapidly in early childhood from 2.9% positive for children less than two years of age to 8.9% for children 2-5 years of age (Figure 2). In a univariate logistic regression analysis, the odds of being *S. stercoralis* stool positive increased with age (OR = for each one-year increase, 95% CI = 1.00-1.03). Those who occasionally or never wore shoes were almost twice as likely to have stools positive for *S. stercoralis* than those who frequently or always wore shoes (RR = 1.97, 95% CI = 1.20- 3.25). Occupation was categorized as predominantly outdoor or indoor; participants who worked in predominantly outdoor occupations were at higher risk than those with predominantly indoor occupations (RR = 1.70, 95% CI = 0.90-3.21), but this association failed to reach statistical significance. There was no significant association between risk of *S. stercoralis*-positive stool and the location of human waste disposal or water source for the household. Participants with stool positive for hookworm were more than four times as likely to have *S. stercoralis* identified in the same stool sample as those negative for hookworm (RR = 4.54, 95% CI = 2.10-9.87). In the multivariable logistic regression model, increasing age, presence of hookworm in the stool, and occasionally or never wearing shoes were associated with an increased risk of having *S. stercoralis* in stool (Table 3).

Epidemiologic findings for the serologic data were consistent with those for the stool results. The seroprevalence for *S. stercoralis* increased with age (Figure 2), and the serologic pattern, like that for *S. stercoralis* in stool, showed a rapid increase in prevalence during early childhood. Although no children less than two years of age were seropositive, 39% of 2-5-year-old children and 62.5% of 6-14-year-old children were seropositive. The odds of being seropositive increased with age (OR = 1.04 for each one-year increase, 95% CI = 1.02-1.05). In univariate models, there was a trend for wearing shoes occasionally or never to be associated with increased risk (RR = 1.37, 95% CI = 0.92-2.02). Univariate analysis showed that those who worked in predominantly outdoor occupations were more likely to be seropositive than those working in indoor occupations (RR = 1.89, 95% CI = 1.09-3.25). Waste disposal location and water source were not associated with altered risk of seropositivity. In the multivariable model, increasing age and occasionally or never wearing shoes remained significantly associated with an increased risk of positive serologic results for *S. stercoralis* (Table 3).

DISCUSSION

The impact of *Strongyloides stercoralis* infection is poorly characterized compared with that of other common helminths such as *A. lumbricoides* and hookworm. Simple stool screening techniques for *S. stercoralis* tend to be insensitive, while the most sensitive techniques, the Baermann and the agar plate methods, are too labor-intensive to be used in extensive population screening and epidemiologic studies. The apparatus for the Baermann procedure is bulky and cumbersome to assemble, and dismounting and cleaning it exposes laboratory personnel to highly infectious larvae. We hoped the agar plate method might be more adaptable for large-scale screening and provide a reliable method to define current infection status. However, the test performance was poor because of fungal overgrowth in more than 30% of the tested samples during the three-day culture period. Introduction of fungi may have occurred when

participants defecated on the ground and then transferred the sample, contaminated with soil fungal elements, to the collection container, or may have been the result of the primary fecal passage of fungal elements. The laboratory in which the specimens were processed was not air-conditioned, with an average ambient temperature of 30°C and high humidity; fungal overgrowth may have been a result of the prevailing conditions.

Because of the poor performance of the agar plate method, we used the composite result of three less sensitive tests to define current infection status. We believe that the large amount of stool screened in these serial exams (more than 25 grams per sample) to some extent overcomes the limited sensitivity of each test performed individually. Nevertheless, some participants classified as stool negative might have been found to be positive if multiple stool specimens had been examined. Remarkably, the simple sedimentation procedure was more sensitive than the modified Baermann method or direct examination. This simple technique is appropriate for screening of large numbers of specimens, and we recommend its use for epidemiologic studies, and for the assessment of helminth control programs.

For the *S. stercoralis* ELISA, we used the PCF4 correction method to improve the precision, and to decrease within-plate and plate-to-plate variability.⁹ In epidemiologic studies or large-scale screening programs, plate-to-plate variation in particular must be minimized to accurately classify the test results. Precision in the testing of an ELISA is often assumed in assay development and serologic studies, despite the observation that plate-to-plate coefficients of variation are generally at least 10% even under ideal conditions,¹⁰ and may reach $\geq 15\%$.^{11, 12} Although the PCF4 control sera occupy 8 wells of the 96-well plate, the improved precision and simplicity of the method make this adjustment worthwhile, especially in laboratories without automated plate washers.

Although the ELISA used in this study has been available for several years, its use has been limited by significant cross-reactivity with filarial species, which are often co-endemic with *S. stercoralis*. Western blots have been used to overcome this problem, but are only partially effective in doing so¹³ and are relatively cumbersome to perform. An ELISA with additional preincubation of the test sera with *Onchocerca gutturosa* has been reported to reduce cross-reactions in patients with lymphatic filariasis and infection with *Necator americanus*.¹⁴ An ELISA based on a recombinant antigen has recently been reported to have a sensitivity of 87.5% and a specificity of 93.5%, with no cross-reactivity with *O. volvulus* or *L. loa*; however, difficulties in the concentration of the expressed protein limit the current availability of this protein.¹⁵

In our data, we used an ELISA with good sensitivity and specificity, and our study area was not endemic for most of the filarial species known to cross-react. Nevertheless, potential cross-reactions with hookworm species and other nematodes were of concern to us. In our study, participants stool positive for hookworm were four times as likely to be positive for *S. stercoralis*, an observation also noted in an earlier study.¹⁶ This is not surprising because the two nematodes share similar risk factors, such as going barefoot in contaminated soil. However, in our data, there was no demonstrable cross-reactivity between hookworm infection and seropositivity for *S. stercoralis*.

There are few previous studies of the seroepidemiology of strongyloidiasis in the literature. Some studies used sampling methods that select for higher risk individuals, and therefore cannot give valid estimates for risk factors or age-specific rates of infection.^{17,18} A study conducted in a leper colony in Thailand provides some data for comparison.¹⁹ Of 177 participants concurrently screened by stool examination and serologic tests, 11% had *S. stercoralis* identified in stool and 45% were seropositive. No association was found between age and serologic status, but the exclusion of children less than six years of age from serologic

testing limited the ability to delineate the age-specific pattern of seropositivity. The area in question was reported to be non-endemic for filariasis, but because the village was a leper colony, people from other parts of Thailand may have migrated there, and individuals with prior exposure to filariasis may have been included.¹⁹

Other studies from the Amazon region show rates of *S. stercoralis* in stool of 16–20%, demonstrating that our study area is part of a highly disease-endemic zone.^{20,21} In addition, the factors associated with higher risk of strongyloidiasis by both assays, increasing age, going without shoes, and for stool infection, concurrent hookworm, are consistent with conventional knowledge of the parasite. The rate of infection based on our serologic assay was very high; 85% of adults ≥ 18 years old and 61% of children, showed seroreactivity for *S. stercoralis*. Published data show that ELISA optical density values decrease after successful anthelmintic treatment,^{22–24} but in one study, optical density values remained above the diagnostic cutoff in 32% of the cases.²² Thus, a person with past infection that cleared spontaneously or after treatment may remain seropositive, and positive serologic results in our data likely represent a mixture of past and current infections.

Given the persistent nature of infection and the mortality associated with the hyperinfection syndrome, we recommend that residents of the Amazon region be routinely screened for *S. stercoralis* if a diagnosis of HIV or another immunosuppressive condition is made, or prior to the institution of immunosuppressive therapy. Since the ELISA has a negative predictive value of 98%, this assay is a highly appropriate screening tool, as long as possible co-endemicity with cross-reacting filarial species is taken into consideration.

Acknowledgements

Acknowledgments: We thank Hermman Silva for his interest and collaboration in the current project, Franklin Neva for the provision of L3 extract and protocol for its use, Patrick J. Lammie for advice in the interpretation of serologic testing, M. A. Single for providing input and support on early versions of the work, and S. T. Unt for a critical revision of the final manuscript.

REFERENCES

1. Dreyfuss ML, Msamanga GI, Spiegelman D, Hunter DJ, Urassa EJ, Hertzmark E, Fawzi WW. Determinants of low birth weight among HIV-infected pregnant women in Tanzania. *Am J Clin Nutr* 2001;74:814–826. [PubMed: 11722965]
2. Arenas-Pinto A, Certad G, Ferrara G, Castro J, Bello MA, Nunez LT. Association between parasitic intestinal infections and acute or chronic diarrhoea in HIV-infected patients in Caracas, Venezuela. *Int J STD AIDS* 2003;14:487–492. [PubMed: 12869231]
3. Gendrel D, Treluyer JM, Richard-Lenoble D. Parasitic diarrhea in normal and malnourished children. *Fundam Clin Pharmacol* 2003;17:189–197. [PubMed: 12667229]
4. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. *Parasitology* 2000;121(Suppl):S23–S38. [PubMed: 11386688]
5. Jongwutiwes S, Charoenkorn M, Sitthichareonchai P, Akaraborvorn P, Putaporntip C. Increased sensitivity of routine laboratory detection of *Strongyloides stercoralis* and hookworm by agar-plate culture. *Trans R Soc Trop Med Hyg* 1999;93:398–400. [PubMed: 10674087]
6. Salazar SA, Gutierrez C, Berk SL. Value of the agar plate method for the diagnosis of intestinal strongyloidiasis. *Diagn Microbiol Infect Dis* 1995;23:141–145. [PubMed: 9407219]
7. Muck AE, Pires ML, Lammie PJ. Influence of infection with non-filarial helminths on the specificity of serological assays for antifilarial immunoglobulin G4. *Trans R Soc Trop Med Hyg* 2003;97:88–90. [PubMed: 12886811]
8. Schaffel R, Nucci M, Carvalho E, Braga M, Almeida L, Portugal R, Pulcheri W. The value of an immunoenzymatic test (enzyme-linked immunosorbent assay) for the diagnosis of strongyloidiasis in patients immunosuppressed by hematologic malignancies. *Am J Trop Med Hyg* 2001;65:346–350. [PubMed: 11693882]

9. Whittington RJ. Evaluation of a simple method for improving the precision of an ELISA detecting antibody in serum. *J Immunol Methods* 1992;148:57–64. [PubMed: 1564335]
10. Kurstak E. Progress in enzyme immunoassays: production of reagents, experimental design, and interpretation. *Bull World Health Organ* 1985;63:793–811. [PubMed: 3910300]
11. McLaren ML, Lillywhite JE, Au AC. Indirect enzyme linked immunosorbent assay (ELISA): practical aspects of standardization and quality control. *Med Lab Sci* 1981;38:245–251. [PubMed: 7038368]
12. Wright PF, Kelly WA, Gall DE. Application of a timing protocol to the reduction of inter-plate variability in the indirect enzyme immunoassay for detection of anti-Brucella antibody. *J Immunoassay* 1985;6:189–205. [PubMed: 3930575]
13. Conway DJ, Bailey JW, Lindo JF, Robinson RD, Bundy DA, Bianco AE. Serum IgG reactivity with 41-, 31-, and 28- kDa larval proteins of *Strongyloides stercoralis* in individuals with strongyloidiasis. *J Infect Dis* 1993;168:784–787. [PubMed: 8354924]
14. Conway DJ, Atkins NS, Lillywhite JE, Bailey JW, Robinson RD, Lindo JF, Bundy DA, Bianco AE. Immunodiagnosis of *Strongyloides stercoralis* infection: a method for increasing the specificity of the indirect ELISA. *Trans R Soc Trop Med Hyg* 1993;87:173–176. [PubMed: 8337720]
15. Ravi V, Ramachandran S, Thompson RW, Andersen JF, Neva FA. Characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. *Mol Biochem Parasitol* 2002;125:73–81. [PubMed: 12467975]
16. Hall A, Conway DJ, Anwar KS, Rahman ML. *Strongyloides stercoralis* in an urban slum community in Bangladesh: factors independently associated with infection. *Trans R Soc Trop Med Hyg* 1994;88:527–530. [PubMed: 7992327]
17. Lindo JF, Robinson RD, Terry SI, Vogel P, Gam AA, Neva FA, Bundy DA. Age-prevalence and household clustering of *Strongyloides stercoralis* infection in Jamaica. *Parasitology* 1995;110:97–102. [PubMed: 7845718]
18. Atkins NS, Lindo JF, Lee MG, Conway DJ, Bailey JW, Robinson RD, Bundy DA. Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Trans R Soc Trop Med Hyg* 1997;91:609–613. [PubMed: 9463683]
19. Douce RW, Brown AE, Khamboonruang C, Walzer PD, Genta RM. Seroepidemiology of strongyloidiasis in a Thai village. *Int J Parasitol* 1987;17:1343–1348. [PubMed: 3429125]
20. Rodriguez J, Calderon J. Intestinal parasitosis in pre-school children from Tarapoto. *Rev Gastroenterol Peru* 1991;11:153–160. [PubMed: 1840844]
21. Egidio JM, De Diego JA, Penin P. The prevalence of enteropathy due to strongyloidiasis in Puerto Maldonado (Peruvian Amazon). *Braz J Infect Dis* 2001;5:119–123. [PubMed: 11506774]
22. Kobayashi J, Sato Y, Toma H, Takara M, Shiroma Y. Application of enzyme immunoassay for postchemotherapy evaluation of human strongyloidiasis. *Diagn Microbiol Infect Dis* 1994;18:19–23. [PubMed: 8026153]
23. Genta RM, Weil GJ. Antibodies to *Strongyloides stercoralis* larval surface antigens in chronic strongyloidiasis. *Lab Invest* 1982;47:87–90. [PubMed: 7045521]
24. Loutfy MR, Wilson M, Keystone JS, Kain KC. Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. *Am J Trop Med Hyg* 2002;66:749–752. [PubMed: 12224585]

FIGURE 1.
Study site in the Peruvian Amazon.

FIGURE 2.

Age-specific prevalence of *Strongyloides stercoralis* by stool examination and serologic results. ELISA= enzyme-linked immunosorbent assay.

TABLE 1

Intestinal parasites identified in single stool specimens from each of 792 residents of a community in the Peruvian Amazon

Parasite	N	%
<i>Ascaris lumbricoides</i>	332	41.9
<i>Entamoeba coli</i>	134	16.9
<i>Trichuris trichura</i>	134	16.9
<i>Strongyloides stercoralis</i> *	69	8.7
<i>Hymenolepis nana</i>	56	7.1
<i>Giardia intestinalis</i>	41	5.2
Hookworm	36	4.5
<i>Enterobius vermicularis</i>	6	0.8
<i>Endolimax nana</i>	3	0.4
<i>Chilimantix mesnilli</i>	1	0.1
<i>Entamoeba histolytica</i>	1	0.1

* Specimen was considered positive if *S. stercoralis* was detected by one or more of the three techniques performed (direct examination, modified Baermann method, and simple sedimentation).

TABLE 2Comparison of *Strongyloides stercoralis* serologic and stool examination results for 493 participants*

S. stercoralis ELISA	S. stercoralis in stool[†]		Total
	Present	Absent	
Positive	44	308	352
Negative	3	138	141
Total	47	446	493

* ELISA enzyme-linked immunosorbent assay.

[†] Stool specimen was considered positive if *S. stercoralis* was detected by one or more of the three techniques performed (direct examination, modified Baermann method, and simple sedimentation).

TABLE 3

Risk factors for *Strongyloides stercoralis* detected in stool and by ELISA in multivariable logistic regression models*

Model	Odds ratio	95% IC	P
<i>Strongyloides stercoralis</i> in stool [†]			
Age (each one-year increase)	1.015	1.001—1.029	0.04
Wears shoes occasionally or never	1.89	1.10—3.27	0.03
Wears shoes usually or always	Referent	—	—
Stool positive for hookworm	4.44	2.02—9.79	0.0002
Stool negative for hookworm	Referent	—	—
Strongyloides stercoralis positive by ELISA			
Age (each one—year increase)	1.04	1.02—1.06	0.0001
Wears shoes occasionally or never	1.54	1.01—2.37	0.04
Wears shoes usually or always	Referent	—	—

* ELISA enzyme-linked immunosorbent assay; CI confidence interval.

[†] Stool specimen was considered positive if *S. stercoralis* was detected by one or more of the three techniques performed (direct examination, modified Baermann method, and simple sedimentation).