INTRODUCTION

Toxocariasis is caused by roundworms commonly found in the intestine of dogs (*Toxocara canis*) and cats (*Toxocara catti*). Although infection with these parasites have been described in their usual hosts for more than two centuries, only in the 1950s were they recognized as important human pathogens.\(^1,2\) When embryonated *Toxocara* eggs are accidentally ingested by humans, second-stage (L\(_2\)) larvae hatch in the small intestine and wander through the body, but fail to develop to mature adult worms within this aberrant host. The clinical spectrum of human toxocariasis, which varies from asymptomatic infections to severe organ injury, is determined by the parasite load, the sites of larval migration, and the host’s inflammatory response.\(^3\) Two main clinical syndromes are classically recognized: visceral larva migrans (systemic disease caused by larval migration through major organs) and ocular larva migrans (disease limited to the eye and optic nerve).\(^4\) Most human infections are diagnosed serologically; enzyme-linked immunosorbent assay (ELISA) for detection of IgG antibodies to antigens secreted by L\(_2\) larvae of *T. canis* has sufficient sensitivity and specificity for use as a screening test.\(^4\)

The proportion of subjects with detectable IgG antibodies to *Toxocara* varies between 2 and 93% in different regions of the world, with the highest seroprevalence rates found among children living in rural areas of tropical countries.\(^5\) Environmental risk factors for *Toxocara* infection have been often investigated in urban areas, and studies consistently show heavy soil contamination with *Toxocara* eggs in public parks of cities worldwide.\(^5,10\) However, few comparable data are currently available for rural areas, where human infections are usually most prevalent.\(^11\)

Seropositivity rates between 8.7% and 39.0% have been found in different samples of Brazilian children (sample size, 100–483) examined over the past decade,\(^12\)–\(^18\) but exposure to *Toxocara* has been rarely investigated in other age groups and no data are available for rural populations of this country.\(^19\) Here we describe the epidemiology of human toxocariasis in one of the largest agricultural settlements in the Amazon Basin of Brazil, the Pedro Peixoto settlement in the state of Acre. We analyzed individual and household-level risk factors for the presence of IgG antibodies to larval antigens of *Toxocara canis*, examined the spatial distribution of seropositive subjects, and discussed the prospects for controlling human toxocariasis in this and other similar rural settings.

SUBJECTS, MATERIALS, AND METHODS

Study area. The state of Acre is located in the Western Amazon Basin of Brazil, bordering with Peru, Bolivia, and the Brazilian states of Amazonas and Rondônia. The study site, Ramal do Granada (9°41’S–9°49’S, 67°05’W–67°07’W), was a sparsely peopled rubber tapper settlement in the eastern corner of Acre that became part of the Pedro Peixoto Agricultural Settlement Project in 1982. The area is characterized by a humid equatorial climate and receives most rainfall (annual average, 2198.5 mm) between December and March. The mean annual temperature is 24.5°C. Subsistence agriculture and cattle ranching are currently the main economic activities, with coffee, banana, and rice as the main cash crops.

Study population. Recruitment strategies have been described elsewhere.\(^20\) Briefly, all households enumerated during a census performed by our field team in Ramal do Granada were visited between March and April 2004, and 466 dwellers aged < 1–90 years (98.5% of the 473 permanent residents in the area found at the time of the census) were enrolled. An additional 43 individuals (mostly newcomers to the area) were enrolled between September and October 2004. The 425 study participants aged 5 years or older who were enrolled either in March–April or September–October 2004 were invited to contribute a 5-mL venous blood sample for serum separation; 403 subjects (94.8% of the eligible; age range, 5–90 years; median, 24 years), living in 122 households, had their sera tested for IgG antibodies to larval antigens of *Toxocara canis* and constituted the population sample analyzed in this survey. A single stool sample preserved with 10% formalin, which was provided by 382 (94.8%) study subjects, was examined for intestinal parasites. The location of all...
households was determined using a hand-held, 12-channel global positioning system receiver (eTrex Personal Navigator, Garmin, Olathe, KS), which gives a positional accuracy within 15 m.

A baseline questionnaire was applied to study participants to obtain demographic, clinical, and socioeconomic information. The number of years of schooling of the household head, the number of persons per room, the source of water used for cooking and bathing, and the presence of dogs and cats as pets in the household were recorded. To derive a wealth index, we also obtained information on: (a) the ownership of six household assets (gas stove, coach, bicycle, motor vehicle, and cattle), (b) land tenure (yes or no), (c) the type of housing material (brick walls versus others), and (d) the number of inhabitants per room (≤ 1 per room or > 1 per room). Principal component analysis was used to define weights for each variable. The first principal component explained 25.6% of the variability and gave greatest weight to ownership of a sofa set (0.670), a motor vehicle (car or motorcycle) (0.641), and lower number of inhabitants per room (0.574). Principal component analysis was carried out using the XLSTAT software, version 7.5.2 (Addinsoft, New York, NY). After the standardized variables were weighted, the highest scores were given to the ownership of a brick house (2.262), a sofa set (1.040), and a motor vehicle (0.742). Lowest scores were given to households lacking gas stove (−1.237), with no land tenure (−1.054), with > 1 inhabitant per room (−0.619) and without cattle (−0.614). The scores were summed to a wealth index for each household (range, −4.871 to 5.409).

Antigen preparation. Excretory-secretory larval antigen for ELISA was prepared as described by Elefant and colleagues. Briefly, T. canis eggs collected from the uterus of female worms were embryonated after incubation in 2% formalin for approximately 1 month at 28°C and artificially hatched in serum-free Eagle medium. L2 larvae were recovered and incubated at 37°C. At weekly intervals, the culture supernatant was removed, treated with 5 μM/mL of the protease inhibitor phenyl-methyl-sulfonyl fluoride (200 mM), concentrated with Amicon Ultrafiltration units (Millipore, Danvers, MA), dialyzed against distilled water, centrifuged (18,500 g for 60 min at 4°C), and filtered in 0.22 μM Millipore membranes. The excretory-secretary larval antigen prepared with T. canis is likely to contain both species-specific epitopes and common epitopes that are shared by T. cati, but no attempt was made to determine the levels of between-species cross-reactivity observed in standard diagnostic ELISA.

Preincubation of sera with Ascaris suum adult worm extract (AWE). To remove antibodies elicited by exposure to Ascaris, which could cross-react with Toxocara antigens, test samples were preincubated with an adult worm extract (AWE) of Ascaris suum. Briefly, adult worms recovered from porcine intestine were macerated in distilled water, and 1.5 M NaOH was added to a final concentration of 0.15 M. After 2-hr incubation at room temperature, this mixture had its pH neutralized with 6M HCl and was centrifuged (18,500 g for 20 min at 4°C). After having its lipids removed with ether, the supernatant was filtered in 0.22 μM Millipore membranes. All test sera were preincubated for 30 min at 37°C with a solution (25 μg/mL) of AWE in 0.01 M phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 (PBS-T) before use in ELISA.

Antibody detection. Serum samples were tested for IgG antibodies to T. canis excreted-secreted larval antigens by ELISA at a dilution of 1:320 essentially as described. Poly- styrene 96-well microplates (Corning, Costar, New York, NY) were coated for 1 hr at 37°C followed by 18 hr at 4°C with 1.9 μg/mL of solid-phase antigen dissolved in 0.06 M carbonate-bicarbonate buffer, pH 9.6 (100 μL/well) and subsequently blocked for 2 hr at 37°C with PBS-T containing 2.5% bovine serum albumin (Sigma, St. Louis, MO). After a 40-min incubation at 37°C, serum samples were removed and horseradish peroxidase-conjugated goat anti-human IgG (Sigma) was added at a 1:10,000 dilution (40 min at 37°C), followed by the o-phenylenediamine substrate (0.4 mg/mL, Sigma). Absorbance readings were made at 492 nm; a cut-off absorbance value was defined as the mean absorbance reading for 96 negative control sera plus three standard deviations. Antibody levels were expressed as reactivity indices (RIs), which were calculated as the ratio between the absorbance values of each test sample and the cut-off value; positive samples had RIs greater than 1.

Stool sample examination. To determine whether seropositivity to Toxocara was associated with current exposure to common intestinal nematodes of humans, we examined stool samples from 382 study participants for parasite eggs, cysts, and larvae according to a standard sedimentation-concentration method. Logistic limitations prevented the collection of more than one stool sample from each subject. Although parasite prevalence rates derived from the examination of a single stool sample are likely to be underestimated, this bias is considered to be relatively small for most common intestinal nematodes of humans, except for Strongyloides stercoralis.

Data analysis. A database was created with SPSS 13.0 (SPSS Inc., Chicago, IL). Prevalence rates are given with exact binomial 95% confidence intervals (95% CI) and compared with χ² or Fisher exact tests, while continuous variables were compared with nonparametric Mann-Whitney U tests; unadjusted odds ratios were also calculated for potential risk factors. Multiple logistic regression models with stepwise backward deletion were built to describe independent associations between potential risk factors (independent variables) and a positive serology to T. canis. Variables associated with P values < 0.20 in unadjusted analysis were included into logistic regression models. Because the data have a nested structure, where individuals are nested within households, the assumption of independence of observations underlying standard logistic regression analysis is violated. We therefore used two-level logistic models with individual-level covariates (age, gender, and current infection with intestinal nematodes) and household-level risk covariates (sector of residence, education of the household head, wealth index, source of water for cooking and bathing, and presence of dogs and cats as pets in the household). To account for differences in the time and pattern of land occupation across the Ramal do Granada, we divided the study area into four sectors: the first area to be colonized was sector A (32 households), followed by sectors B (31 households), C (45 households), and D (14 households). Average wealth indices vary widely across the sectors, ranging between 1.34 (standard deviation [SD], 2.07) in sector A and −1.61 (SD, 2.04) in sector D. The HML software package (version 6.03, Scientific Software International, Lincolnwood,
RESULTS

Prevalence of antibodies and associated risk factors. IgG antibodies to *T. canis* larval antigens were detected in 108 subjects aged 5–90 years (median, 17 years), with an overall seroprevalence rate of 26.8% (95% CI, 22.5–31.4%). The seroprevalence rate was substantially higher among preschool and school children aged 5–14 years (36.6%; 95% CI, 28.1–45.7%; N = 123) than in older subjects (22.5%; 95% CI, 17.7–27.8%; N = 280) (P = 0.005, χ² test with Yates correction; see Figure 1). High-titer antibodies (corresponding to the upper quartile of reactivity indices) were most prevalent highest among children aged 5–14 years (Figure 1), suggesting that recent or current exposure to *Toxocara* is most common in this age group. Seropositivity was slightly more prevalent in males (29.9%) than in females (23.4%), but without statistical significance (P = 0.180, χ² test with Yates correction).

The most prevalent intestinal nematodes found in the study population (382 stool samples examined) were hookworm (prevalence, 6.5%), *Ascaris lumbricoides* (3.9%), *Strongyloides stercoralis* (2.9%), and *Trichuris trichiura* (2.1%); a detailed account of these findings is presented elsewhere. Overall, the seropositivity rates were significantly higher among subjects harboring intestinal nematodes (20 of 41, 48.8%) than in those free of infection (87 of 341, 25.5%) (P = 0.003, χ² test with Yates correction; 382 subjects examined for both antibodies and intestinal helminths). Antibodies to *Toxocara* were more frequently detected in subjects carrying hookworm (seroprevalence, 52.0%) and *Strongyloides* (seroprevalence, 63.9%), when compared with subjects who are not infected with these nematodes (seroprevalence, 26.3% and 27.0%, respectively), with P values of 0.011 and 0.020, respectively (χ² test with Yates correction); no significant association was found between *Toxocara* seropositivity and carriage of *Ascaris* or *Trichuris*.

Several household-level variables (sector of residence, education of the household head, wealth index, and presence of cats in the household) were significantly associated with the presence of *Toxocara* antibodies in unadjusted analysis (Table 1). Interestingly, the presence of cats in the household emerged as a putative protection (rather than risk) factor. Most (70.5%) households in our study area have dogs, 42.6%...
have cats, and 33.6% have both dogs and cats. Of 52 households with cats as pets, 41 (78.8%) also had dogs but, conversely, of 86 households with dogs, only 41 (47.7%) also had cats. Whether domestic dogs and cats are allowed to sleep in the houses does not change the risk estimates (data not shown).

Most of the putative risk factor associations shown in Table 1 became no longer significant after adjustment for confounding covariates by using two-level logistic regression analysis. Only young age, current infection with hookworm (but not with *Strongyloides*), and the sector of residence remained as significant (*P < 0.05*) independent predictors of the presence of IgG antibodies to *Toxocara*; the associations of seropositivity with the presence of cats in the household were of borderline statistical significance (Table 2).

**Spatial analysis.** The Kulldorf spatial scan statistic revealed two significant high-prevalence clusters and one significant low-prevalence cluster of households in the study area. The largest high-prevalence cluster comprises 35 seropositive subjects (versus 19.30 expected, *P = 0.006*) distributed in 21 households within a radius of 1.90 km in sector C, whereas the secondary high-prevalence cluster comprises 7 seropositive subjects (versus 1.88 expected, *P = 0.009*) distributed in three households within a radius of 0.27 km in sector D. The high-prevalence clusters together comprise 38.9% of the seropositive subjects living in 19.7% of the households. The low-prevalence cluster comprises 8 seropositive subjects (versus 22.24 expected, *P = 0.010*) distributed in 25 households within a radius of 0.79 km in sector A.

The comparison of characteristics of households within high-prevalence and low-prevalence clusters might provide further insights into environmental risk factors for *Toxocara* seropositivity. The 24 households within the high-prevalence clusters had slightly lower wealth indices (average, 0.14 versus 1.00, *P = 0.073*, Mann-Whitney *U* test) and were less likely to have cats as pets (25.0% versus 64.0%, *P = 0.014*, χ² test with Yates correction) than the 25 households within the low-prevalence cluster. No significant difference between households within the high- and low-prevalence clusters was found for the level of education of the household head (*P = 0.227*), the source and type of treatment of potable water (*P = 0.349*) or the presence of dogs as pets in the households (*P = 1.000*).

**DISCUSSION**

This population-based study found a high prevalence of seropositivity to *Toxocara* in a rural population of Brazil, specially among subjects aged 5–14 years (prevalence of IgG antibodies, 36.8%). In fact, age > 14 years appeared to be a protective factor and the proportion of subjects with higher titers was lowest in this age group. The seroprevalence in children in our site is comparable to recent estimates obtained for children aged 1–14 years living in urban areas of this country (8.7–39.0%); in the nearby town of Acrelândia, we recently found IgG antibodies to *Toxocara* in 21.5% of the 483 under-5 children examined. The only recent study of *Toxocara* seroprevalence across different age groups in Brazil, performed in a large city in the southeastern region, found IgG antibodies in 20.5% of subjects aged 15–80 years, compared with 27.7% in those aged < 15 years. No data for other rural communities of Brazil are available for comparison; the only other recent similar study in South America, carried out on a small sample (*N = 100*) of inhabitants in rural Argentina, found a similar overall seroprevalence rate (23.0%), with little variation according to age (23.6% in those aged 1–14 years and 21.4% in those aged 15 years or older). Several risk factors for toxocariasis have been identified in human populations, but inconsistent results are abundant. Male gender, for example, was suggested to be associated with both increased and decreased risk of infection, with some large studies showing no association between gender and risk. Young age, low socioeconomic status, low parental education, and poor sanitation are additional factors contributing to *Toxocara* exposure in some communities. Dog ownership has been identified as a risk factor in several studies of urban and rural populations. None of these known risk factors, except for young age, was significantly associated with *Toxocara* exposure in our population (*P* values between 0.338 and 0.921 in multivariate models). Since in our study area dogs often roam freely and, if infected, may spread eggs across large areas, the absence of significant association between the presence of dogs in the household and *Toxocara* seropositivity is not particularly surprising.

We found, however, a positive association between *Toxocara* seropositivity and current hookworm infection (Table 2). There has been concern about the cross-reactivity of antibodies to excretory-secretory *Toxocara* antigens with other closely related human nematodes, such as *Ascaris lumbricoides* and, for this reason, test sera were preincubated with an *Ascaris suum* extract. Because infection with other tissue nematodes was not investigated in our sample, and some luminal nematodes, particularly *Strongyloides stercoralis* larvae, may have been missed by the sedimentation technique used for stool examination, no conclusion can be drawn about the specificity of this association. Although it is possible that the association between infection with hookworm and other nematodes and *Toxocara* seropositivity results merely from antibody cross-reactivity, we believe that it may reflect the similar ways of acquisition of *Toxocara* and hookworm infections in this community, such as geophagia.

The finding that the presence of cats in the household may be a protective factor, which has not been previously reported, is particularly intriguing. The excretory-secretory larval *T. canis* antigen is known to contain both species-specific epitopes and epitopes that are shared between *T. cati* and *T. canis*. If species-specific epitopes predominate, ELISA would diagnose preferentially exposure to *T. canis* instead of *T. cati*. If cats are able to reapel stray dogs attempting to enter the households, their presence might reduce the contact of dwell-
ers with infected dogs and decrease the risk of T. canis infection, although it might increase the exposure to T. cati. The use of strictly species-specific recombinant antigens in ELISA may help to test this hypothesis in the near future. The significance (although not the magnitude) of the negative association between cat ownership and seropositivity attenuates after several covariates (including socioeconomic status) are controlled for in multivariate analysis (compare Tables 1 and 2), suggesting that socioeconomic status may be a confounder. In fact, households with cats have significantly higher wealth indexes (mean, 0.72) than those without cats (mean, –0.28) (P = 0.009, Mann-Whitney test). Although a similar trend is found for dog ownership, the difference in average wealth index (0.23 versus –0.05) did not reach statistical significance (P = 0.135, Mann-Whitney test).

Exposure to Toxocara is heterogeneously distributed in the study area, with seroprevalence rates ranging between 11.1% and 38.2% among inhabitants of different sectors (Table 1). The sectors with the highest infection rates are those more recently occupied, with poor-quality housing; two high-risk clusters, with less than 20% of the households in the study area, comprise 38.9% of the seropositive subjects. Although these findings may be useful to guide the spatial targeting of interventions for reducing the exposure of Toxocara in this population, they were little informative regarding potential environmental risk factors for infection; the only significant difference between high-risk and low-risk households is the proportion of them having cats as pets, again suggesting that the presence of cats in the household could provide some protection against seroconversion to excretory-secretory larval T. canis antigen or could be associated with some confounder that in fact is protective.

This study confirms that inhabitants of rural communities in the tropics, and particularly preschool and schoolchildren, may be heavily exposed to Toxocara. The presence of an unrestrained dog population and adequate climatic and environmental conditions for egg survival and larval hatching all contribute to the high seroprevalence rates observed. However, human toxocariasis remains relatively unknown by the public, including pet owners, limiting the adherence to simple preventive measures, such as restricting the access of dogs and cats to the households and periodically deworming the pets. Potential targets of health promotion efforts to increase the public understanding of toxocariasis in our rural community include preschool and schoolchildren, pet owners and inhabitants in the high-prevalence clusters. Targeting pets is further complicated by the limited access to veterinary care in the area (there are no veterinarians living in the nearby towns, within a radius of 50 km) and the fact that most animals are allowed to roam freely outside the properties, potentially spreading the environmental contamination and increasing the risks of getting infected.

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