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A Community-based Survey of Human Toxoplasmosis in Rural Amazonia: Seroprevalence, Seroconversion Rate, and Associated Risk Factors

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Abstract. IgG antibodies to *Toxoplasma gondii* were detected in, March–April 2004, in 65.8% (95% confidence interval, 60.8–70.8%) of 342 systematically sampled subjects 5–90 years of age (87.5% of the eligible) living in a rural settlement in Amazonia, with a seroconversion rate of 9% over 1 year of follow-up of 99 seronegative subjects. Multiple logistic regression analysis identified age as the only significant independent predictor of seropositivity at the baseline. Each additional year of age increases the odds of being seropositive by 6%, and 76.8% of the subjects are expected to be seropositive at 30 years of age. A single high-prevalence spatial cluster, comprising 11.9% of the seropositive subjects, was detected in the area; households in the cluster were less likely to have dogs as pets and their heads had a lower education level, when compared with households located outside the cluster. The challenges for preventing human toxoplasmosis in tropical rural settings are discussed.

INTRODUCTION

Toxoplasma gondii can infect virtually all nucleated cells of a wide range of warm-blooded animals and causes one of the most common parasitic zoonoses worldwide. This coccidian protozoon has three infectious stages: tachyzoites, which multiply quickly during acute infections; bradyzoites, which multiply slowly inside tissue cysts during chronic infections; and sporozoites, which are found in mature oocysts.[1] Humans usually acquire toxoplasmosis 1) by ingesting or handling undercooked or raw meat, mainly pork or lamb, containing tissue cysts with viable bradyzoites; 2) by ingesting water or vegetables containing oocysts shed by cats and other felids (waterborne transmission); or 3) congenitally, by transplacental transfer of tachyzoites.[2] The proportion of adults with detectable IgG antibodies to *T. gondii* ranges between 6% and 80% in different populations, with remarkably high seroprevalence rates in some temperate and tropical countries in Europe, Africa, and Latin America.[3–5] The wide geographic variation in toxoplasmosis prevalence has been attributed to differences in eating habits and environmental factors, such as the preference for eating raw or undercooked meat in some temperate countries and the abundance of stray cats and climate conditions favoring oocyst survival in the tropics.[2,4–6]

Seroprevalence rates between 70% and 80% have been found in adults, mostly pregnant women attending antenatal care clinics,[7–9] living in large cities of Brazil. Nevertheless, exposure to *Toxoplasma* has been less extensively studied in other age groups[10,11] and few data are available for rural populations of this country.[12,13] Although some of the highest age-adjusted seroprevalence rates of toxoplasmosis in Brazil have been observed across the Amazon Basin, both in native Amerindian populations[14–17] and in agricultural settlements inhabited mostly by migrants,[13] the factors that favor *Toxoplasma* transmission in Amazonian communities are poorly understood.

Here, we describe the epidemiology of human toxoplasmosis 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 environmental risk factors for the presence of IgG antibodies to *T. gondii* among subjects 5–90 years of age, estimated the IgG seroconversion rate over nearly 1 year of follow-up, and examined the spatial distribution of seropositive subjects in the study area. We briefly discuss some challenges for the primary prevention of human toxoplasmosis in this and rural settings in the tropical world.

SUBJECTS, MATERIALS, AND METHODS

Study area. The state of Acre, in the western Amazon Basin of Brazil, borders with Peru, Bolivia, and the Brazilian states of Amazonas and Rondônia. The study site, Granada (9°41′S–9°49′S, 67°05′W–67°07′W), was a sparsely peopled rubber taper 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.

Baseline seroprevalence study. Recruitment strategies have been described elsewhere.[18] The households enumerated during a census performed by our field team in Granada were visited between March and April 2004. All households in the area were visited and 466 dwellers < 1–90 years of age (those who gave informed consent, representing 98.5% of the 473 area's permanent residents) were enrolled. The 399 study participants ≥ 5 years of age were invited to contribute a 5-mL venous blood sample for serum separation. Of these, 342 subjects (85.7% of the eligible; age range, 5–90 years), living in 107 households, gave informed consent for blood sample collection and had their sera tested for IgG antibodies to *T. gondii*. These subjects constituted the population sample analyzed in the baseline seroprevalence survey. The location of all households was determined using a hand-held, 12-chanel 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

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number of years of schooling of the household head, the source of water used for cooking and bathing, the type of treatment of drinking water, and the presence of dogs and cats as pets in the household were recorded; no information, however, was obtained about the ingestion of raw or undercooked meat. To derive a wealth index, we also obtained information on 1) the ownership of six household assets (gas stove, coach, bicycle, motor vehicle, and cattle); 2) land tenure (yes or no); 3) the type of housing material (brick walls versus others), and 4) the number of inhabitants per room (≤ 1 per room or > 1 per room). Principal component analysis was used to define weights for each variable.[19] The first principal component explained 25.6% of the variability and gave greatest weight to ownership of a couch (0.670), a motorized 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 Inc., NY). After the standardized variables were weighted,[19] 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 a 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).

Seroconversion study. All households were revisited in February–March 2005, and blood samples were collected for serology. Of 342 subjects originally enrolled at baseline, 228 had a second blood sample drawn and tested for IgG antibodies to *T. gondii.* Of these subjects with paired serum samples collected (66.7% of the original study population; age range, 5–79 years), 99 were seronegative at the baseline and comprised the population sample of the seroconversion study. To eliminate the effects of between-plate variation in enzyme-linked immunosorbent assay (ELISA) results on seroconversion data, all paired samples from the same subjects were tested on the same microplate.

Antigen preparation. *Toxoplasma gondii* antigen was prepared as described elsewhere.[20] Tachyzoites of the RH strain were harvested from mouse peritoneal cavity in phosphate buffered saline (PBS), recovered by centrifugation, washed, counted, and lysed by repeated cycles of sonication at 4°C. When most parasites were shown by phase-contrast microscopy to have been lysed, we added 0.3 M NaCl and centrifuged the suspension at 10,000 *g* for 3 min. Aliquots of the supernatant were maintained at –70°C until used as a solid-phase antigen for ELISA.

Antibody detection. Serum samples were tested for specific IgG antibodies using a standard ELISA protocol.[21] Polystyrene microplates (Multiwell, Sigma, St. Louis, MO) were incubated overnight, at 4°C, with 100 μL/well of a solution containing 1 mg/mL of the antigen extract diluted in 0.1 M carbonate buffer (pH 9.5). After antigen coating, microplates were then washed five times with PBS containing 0.03% Tween 20 (PBS-T), and blocked for 1 h at 37°C, with PBS containing 2% fat-free lyophilized milk, in a humid chamber. After this step, 100 μL of each serum sample, diluted 1:500 in PBS-T, were added to each well and the microplate was incubated for 1 h at 37°C. After additional washing with PBS-T, 100 μL of peroxidase-conjugated goat, anti-human IgG (Sigma) diluted 1:30.000 in PBS-T, was added to the wells. After an incubation at 37°C for 1 h, followed by five washings with PBS-T, the assay was developed with 100 μL per well of 1 mg/mL of ortho-phenylenediamine and 0.03% H_2O_2 (v/v) in 0.2 M phosphate-citrate buffer (pH 5.0). The reaction was stopped with 50 μL per well of 4N HCl and absorbance values were measured at 492 nm. The cut-off value was determined as the average absorbance reading obtained with 15 negative control sera plus 3 standard deviations.

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 χ^2, Mantel-Haenzel summary χ^2, or Fisher exact tests, whereas continuous variables were compared with nonparametric Mann-Whitney *U* tests; unadjusted odds ratios (ORs) were also calculated for potential risk factors. The seroconversion rate was estimated as the number of seroconverters per 100 person-years at risk, with time at risk defined as time interval between blood draws; this analysis was restricted to 99 subjects who were seronegative at the baseline and had a second blood sample drawn in February–March 2005. 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. gondii*. Variables selected for inclusion in the logistic regression models were those associated with *P* values < 0.20 in unadjusted analysis and those whose inclusion in the model resulted in a change in OR estimates of 10% or greater. 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 and gender) and household-level risk covariates (education of the household head, wealth index, source of water for cooking and bathing, type of treatment of drinking water, and presence of dogs and cats as pets in the household). To account for differences in the time and pattern of land occupation across Granada, we divided the study area into three sectors: the first area to be colonized was sector A (29 households), followed by sectors B (26 households), and C (52 households). The HML software package (version 6.03, Scientific Software International, Lincolnwood, IL) was used for multilevel analysis. Only variables associated with statistical significance at the 10% level and those whose inclusion resulted in a change in OR estimates for any other variable ≥ 10% were maintained in the final model.

The median age of seroconversion (i.e., the age at which 50% of the population had already seroconverted), along with its 95% CI, was estimated by fitting a weighted linear regression model to age-prevalence data. Prevalence data were transformed into probits and age values were log-transformed to fit the model.[22] This analysis is analogous to that commonly used in classic dose-response experiments; subjects in each age group are regarded as independent "batches" of subjects given the same "dose" (or length) of exposure to *T. gondii,* which corresponds to the mean age (in years) calculated for each group.[22] We distributed the baseline study population into six age groups with similar numbers of subjects each.

The Kulldorff spatial scan statistics was used to test whether *T. gondii* seropositivity was randomly distributed within the study area and, if not, to identify significant spatial clusters.[23] Analysis was made using the Bernoulli model implemented in the version 5.1 of the SaTScan software (available at: http://www.satscan.org), which creates and moves circular windows systematically throughout the geographic space to identify

significant clusters of infections. The windows are centered on each household; the largest possible cluster would encompass 30% of the households. For each location and size of the scanning window, SaTScan performs a likelihood ratio test to evaluate whether seropositivity is significantly more prevalent within than outside that given circular window. *P* values were determined by 10,000 Monte Carlo replications of the data set; a level of significance of 5% was adopted.

Ethical considerations. Approval of the study protocol was obtained from the Ethical Review Board of the Institute of Biomedical Sciences of the University of São Paulo, Brazil (318/2002, 538/2004). Written informed consent was obtained from all study participants or their parents/guardians.

RESULTS

Age-related prevalence of antibodies to *Toxoplasma gondii*. IgG antibodies to *T. gondii* were detected in 225 subjects 5–90 years of age (median, 30 years), with an overall seroprevalence rate of 65.8% (95% CI, 60.8–70.8%). The seroprevalence rate increased linearly with age (χ^2 for linear trend = 56.45, $P < 0.000001$), ranging from 35.5% (95% CI, 26.4–44.6%, $N = 107$) among children 5–14 years of age to 91.3% (95% CI, 79.8–100%; $N = 23$) among subjects > 60 years of age (Figure 1). The log-probit model fitted to age-prevalence data estimated the median age of seroconversion as 13.7 years (95% CI, 6.2–20.5 years). According to the log-probit model, the seroprevalence rate in the study population is expected to reach 76.8% (95% CI, 62.8–87.2%) at the age of 30 years and 87.9% (95% CI, 72.1–96.1%) at the age of 50 years (Figure 1). Seropositivity was slightly more prevalent in males (66.5%) than in females (65.1%), but without statistical significance ($P = 0.876$, χ^2 test with Yates correction). Because nearly two-thirds of the study population consists of natives of extra-Amazonian states who settled in Granada after 1982, many episodes of past exposure to *T. gondii* detected by serology at the baseline may have occurred before subjects migrated to the Granada area.

Risk factors for seropositivity. No household-level variable was significantly associated with the presence of IgG antibodies to *T. gondii* in unadjusted analysis (Table 1). Interestingly, the presence of cats in the household did not emerge as a significant risk factor in unadjusted analysis, although whether domestic cats are allowed to sleep in the houses had a small effect on the risk estimate. Most (73.8%) households in our study area have dogs, 45.9% have cats, and 36.4% have both dogs and cats. Of 49 households with cats as pets, 39 (79.6%) also had dogs but, conversely, of 79 households with dogs, only 39 (49.6%) also had cats.

Only age remained as a significant ($P < 0.05$) independent predictor of the presence of IgG antibodies to *T. gondii* after adjustment for confounding covariates by using two-level logistic regression analysis. The adjusted OR estimate was 1.06 (95% CI, 1.04–1.08, $P < 0.0001$), indicating that each additional year of age increases the odds of being seropositive by 6%. We observed a positive association between seropositivity and the presence of cats that are allowed to sleep in the house, of borderline statistical significance, with an adjusted OR estimate of 2.20 (95% CI, 0.90–5.41, $P = 0.084$). Interestingly, the presence of cats and dogs in the household have opposite effects on risk estimates (dogs allowed to sleep in the house appeared to be protective, but without statistical significance, $P = 0.471$), and the slightly increased risk of being seropositive associated with the presence of cats became evident only when the OR estimate was adjusted for the presence or not of dogs in the house.

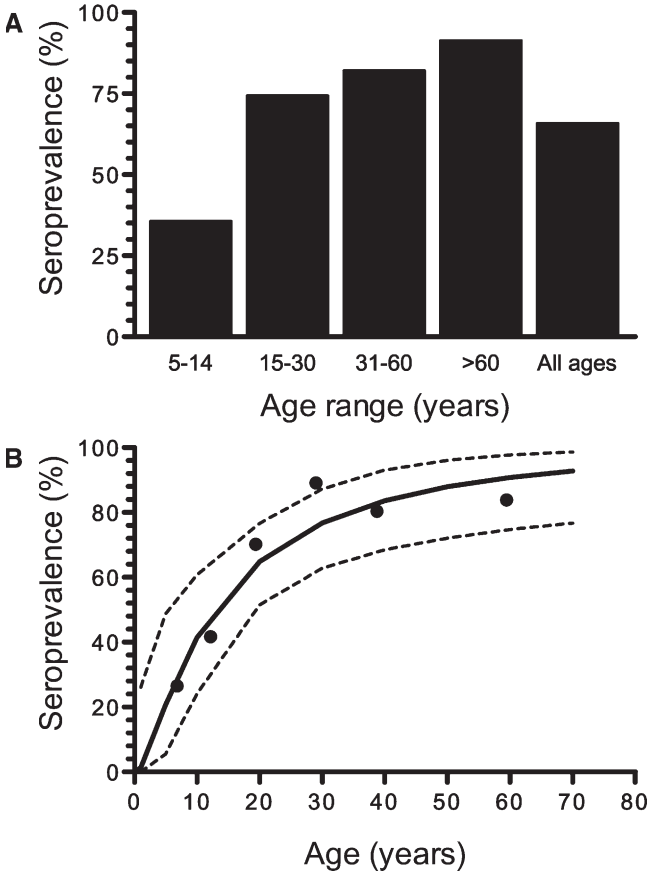

FIGURE 1. Age-related prevalence of IgG antibodies to *Toxoplasma gondii* in Granada, Brazil, 2004. The upper panel (**A**) shows the proportion (%) of subjects with IgG antibodies in children 5–14 years of age ($N = 107$), adolescents and young adults 15–30 years of age ($N = 101$), adults 31–60 years of age ($N = 111$), and elderly subjects > 60 years of age ($N = 23$). In the lower panel (**B**), the study population was distributed into six age groups with similar sizes ($N = 49$–60). Black circles show the proportion (%) of seropositive subjects in each of these groups (values on *x* axis are within-group age averages), whereas the lines show the expected proportions of seropositive subjects according to the log-probit model fitted to the age-prevalence data (continuous line) and the 95% confidence intervals of the estimate (dotted lines). The coefficient of determination (r^2) of the model was 0.848.

***Toxoplasma gondii* seroconversion during the follow-up.** Of 228 subjects who contributed paired serum samples (collected at the baseline and in February–March 2005), 129 (56.6%) had IgG antibodies detected in the first serum sample. Ninety-nine subjects who were seronegative at the baseline were considered at risk of *T. gondii* infection during the follow-up between March and April 2004 and February and March 2005, contributing 88.6 person-years at risk of follow-up. Eight of them (8.1%) had IgG antibodies detected in the second serum sample, indicating that they have been exposed to *T. gondii* between the blood draws. The seroconverters (5 females, 3 males) were aged between 8 and 58 years (median, 22); most (7 of 8) had cats as pets. The seroconversion rate was therefore estimated as 9.0 episodes/100 person-years at risk (95% CI, 3.9–17.8 episodes/100 person-years at risk).

Spatial analysis. The Kulldorf spatial scan statistic revealed a single significant high-prevalence cluster of households in

Table 1
Prevalence of IgG antibodies to *Toxoplasma gondii* according to household-level risk factors in Granada, Brazil, 2004*

Variable	No. of subjects†	Prevalence of IgG antibodies	Odds ratio (95% CI)	*P*
Sector of residence				
A	91	67.0%	1.00	
B	88	63.6%	0.86 (0.44–1.67)	0.749
C	163	66.2%	0.97 (0.54–1.71)	0.989
Education of household head (years of schooling)				
0	64	68.8%	1.94 (0.83–4.54)	0.116‡
1–4	161	67.7%	1.84 (0.90–3.76)	
5–8	70	67.1%	1.80 (0.78–4.11)	
> 8	47	53.2%	1.00	
Wealth index (quartiles)§				
1 (poorest)	56	55.4%	0.64 (0.31–1.34)	0.657‡
2	87	74.7%	1.53 (0.76–3.09)	
3	108	63.9%	0.91 (0.49–1.71)	
4 (least poor)	91	65.9%	1.00	
Water source				
Well	323	65.9%	1.00	1.000
River or stream	19	63.2%	0.89 (0.31–2.73)	
Drinking water filtrated or chlorinated?				
Yes	276	64.5%	1.00	0.374
No	66	71.2%	1.36 (0.73–2.60)	
Dogs in the household?				
Yes	280	64.3%	1.00	0.272
No	62	72.6%	1.47 (0.78–2.89)	
Dogs allowed to sleep in the house?				
Yes	25	76.0%	1.00	0.369
No	317	65.0%	0.59 (0.19–1.58)	
Cats in the household?				
Yes	171	63.7%	0.83 (0.52–1.34)	0.830
No	171	67.8%	1.00	
Cats allowed to sleep in the house?				
Yes	20	80.0%	2.16 (0.67–9.08)	0.255
No	322	64.9%	1.00	

* CI = confidence interval.
† Number of individuals differs for some variables, because of missing values.
‡ *P* values of χ^2 tests for linear trend; all other *P* values are for standard χ^2 or Fisher exact tests.
§ Wealth index derived from information on household assets and other socioeconomic data; see the Subjects, Materials, and Methods section.

the study area. The cluster comprises 23 seropositive subjects (versus 14.98 expected, $P = 0.025$) distributed into 11 dwellings within a radius of 100 m in sector C. The high-prevalence cluster includes 11.9% of the seropositive subjects living in 10.3% of the households. The comparison of characteristics of households within and outside the high-prevalence cluster might provide further insights into environmental risk factors for *T. gondii* seropositivity. The 11 households in the cluster were less likely to have dogs as pets than the 96 households outside the cluster (45.4% versus 77.1%, $P = 0.034$, Fisher's exact test), and their household heads had fewer years of formal schooling ($P = 0.037$, Mann-Whitney *U* test). No significant difference between households within and outside the high-prevalence cluster was found for average wealth index ($P = 0.511$), the source ($P = 1.000$) and type of treatment of potable water ($P = 0.464$), and the presence of cats as pets in the households ($P = 0.731$). Interestingly, cats were not allowed to sleep in any of the dwellings included in the high-prevalence cluster.

DISCUSSION

This community-based study in rural Brazil found a high baseline prevalence of IgG antibodies to *T. gondii*, with an annual seroconversion rate of 9%. As in most populations studied,[2,5] seroprevalence increased with age and did not vary between sexes. Half of the children ≤ 14 years of age had already been exposed to the parasite and nearly 77% of the study subjects are expected to be seropositive at 30 years of age. The overall seroprevalence rate in our site is comparable to those for other rural Amazonian populations, including native Amerindians (55.6–87.6%)[14–16,24] and migrants living in frontier agricultural settlements (73.3%).[13] Because most of our study subjects are migrants, primary exposure to *T. gondii* may have occurred either in Granada or in their regions of origin, mostly in extra-Amazonian states.

Several lifestyle factors have been found to increase the risk of *T. gondii* infection in human populations, but contradictory results are abundant because of the different ways of acquiring the infection that prevail in different endemic settings. For example, most *T. gondii* infections in urban populations of Brazil are probably acquired through ingestion of oocysts contaminating drinking water or vegetables,[10,25] and similar findings were reported in other developing countries.[26,27] In contrast, infection through consumption of undercooked and cured meat products seems to predominate in Europe.[28]

The relative contribution of waterborne transmission to human *T. gondii* infection in rural Brazil remains little studied, although the consumption of homegrown vegetables and the source of drinking water (but not the consumption of undercooked meat) have been recently found to be risk factors for seropositivity in rural Amazonia.[13] No similar association between the source and type of treatment of drinking water and risk of infection, however, was found to be significant in Granada, and we have not studied patterns of consumption of raw or undercooked meat in our study population. However, the absence of teniasis in the Granada population[29] suggests that consumption of raw or undercooked beef or pork (the meat most commonly associated with foodborne human toxoplasmosis) plays a limited role in the acquisition of toxoplasmosis and other zoonoses with similar routes of transmission. Significantly, the Enawenê-Nawê Amerindians in Brazil, with one of the highest overall seroprevalence rates of *T. gondii* infection so far reported in this country (78.8%), do not consume any red meat and do not breed domestic animals such as chickens,[17] which may represent a significant source of human *Toxoplasma* infection in Brazil.[30]

Low socioeconomic status and residence in rural areas are additional factors contributing to *T. gondii* infection in Brazilian communities.[9,10] Although low socioeconomic status and lower education level failed to predict the risk of *T. gondii* infection in the multiple logistic regression analysis of data from Granada, spatial analysis provided some insights into putative household-level risk factors. The household heads living in the high-prevalence cluster identified by spatial analysis had significantly fewer years of formal schooling than those living outside the cluster. It remains to be studied whether lower education level reflects less strict adherence to a more hygienic lifestyle and therefore reduced risk of infection.

The association between cat ownership and risk of human infection with *T. gondii* is difficult to assess, because transmission depends on exposure to contaminated soil, rather than

direct exposure to cats.[31] Cat ownership has been identified as a risk factor in some,[32,33] but not all studies of urban and rural populations.[34–37] Because pet cats in Granada, as in other tropical settings,[38] are allowed to roam freely outdoors and are rarely trained to defecate in litter boxes, infected animals may potentially spread oocysts over relatively wide areas and place households without cats at similar risk of infection. Therefore, the absence of significant association between the presence of cats in the household and *T. gondii* seropositivity in its dwellers is not particularly surprising, although allowing cats to sleep in the house seemed to increase the risk of infection. Interestingly, high seroprevalence rates of toxoplasmosis have been found in native Amerindian communities where cats are not kept as companion animals, allowing for very limited contact (if any) between domestic cats and humans.[14,17]

The finding that only 45% of the households in the high-prevalence spatial cluster have dogs as pets, compared with 77% of the households outside the cluster, is of potential public health significance. The presence of dogs in the household may be a protective factor if they are able to repeal stray cats and feral felids, reducing the risk of environmental contamination with oocysts. Nevertheless, these results must be interpreted with caution, because multiple logistic regression analysis failed to detect a similar protective effect of dogs in the household. The presence of cats in the households has been recently suggested to reduce exposure to another zoonotic parasite, *Toxocara canis,* in Granada.[39] These findings, which require independent confirmation in other settings, may imply that the association between pet ownership and risk of a particular zoonotic infection is confounded by the complex patterns of interaction of different domestic animals found in the community.

This study confirms that inhabitants of rural communities of the Amazon Basin are heavily exposed to *T. gondii.* The presence of a large free-ranging cat population and environmental conditions favoring oocyst survival and maturation (sporogony), in the absence of effective primary prevention measures, contribute to the high seroprevalence rates observed. Locally, appropriate measures for toxoplasmosis prevention must be based on the identification of target populations; because of the high morbidity of congenital toxoplasmosis, health education campaigns usually focus on pregnant women,[5] but we show that nearly half of the primary infections in Granada are acquired at less than 14 years of age. Targeting schoolchildren, therefore, may represent a cost-effective approach to prevent a large proportion of human infections in this community. Changes in food habits may greatly reduce foodborne transmission of toxoplasmosis,[28] but the control of waterborne infection requires further individual and community-level interventions to improve overall hygienic standards and environmental sanitation. Finally, the subclinical nature of most *T. gondii* infections in immunocompetent humans and the low level of formal education of the population in Granada and other similar rural settings in the tropics are potentially major obstacles for the success of long-term policies for the primary prevention of human toxoplasmosis.

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