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HUMAN *TOXOPLASMA* INFECTION IN KUNA AND EMBERA CHILDREN IN THE BAYANO AND SAN BLAS, EASTERN PANAMA

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Abstract. We conducted a survey of 760 Amerindian children 2—12 years of age in the Bayano and San Blas areas of Panama in 1991 to determine the prevalence of serum antibodies to *Toxoplasma gondii* and the importance of hypothesized risk factors in human-induced native and sylvatic conditions, which have had few environmental changes, as opposed to rural and urban areas in Panama previously studied. The overall prevalence of infection ranged between 0% and 42.5%. No age curve was detected, indicative of nonconstant transmission. Only two hypothesized risk factors, floor type and having cats inside the house, were significantly associated with the presence of antibodies in some of the communities. Antibody prevalence appeared to be associated more with the community of residence than with any specific behavior. The risk factor of importance may be the level of oocyst contamination, since infection by tissue cysts in meat was excluded. On three of the nine islands studied, no antibody was detected in the children or the cats. It would appear that *T. gondii* is not present on these islands. Although the data did not support the importance of many of the hypothesized risk factors, the study is consistent with the theory of transmission by oocysts and the importance of cats in transmission.

Toxoplasmosis was studied by Wallace and others in the Pacific,[1-3] and they implicated the role of cats shedding oocysts in transmission. In South and Central America, high prevalence rates were noted among populations in Chile,[3] Brazil,[4] Ecuador,[5-6] Colombia,[8] (Gajdusek DC, unpublished data), Panama,[9-11] and Costa Rica.[10, 12, 13] Seroprevalence studies in Latin America have shown marked differences in antibody prevalence rates dependent upon ethnic group,[2] age,[8-10] sex,[11] social class[2, 8, 9] residence,[8, 9, 13, 14] cat contact,[5, 8, 9, 13-16] and soil contact.[9, 15]

Infection with *Toxoplasma* is widespread in Latin America, particularly in neotropical areas, with *Toxoplasma* antibody detectable in as many as 65% of the population over the age of 60.[9] Detection of immunoglobulin G (IgG) antibodies, as in this study, measures past infection. Since meat is generally well-cooked in Panama, transmission by tissue cysts can be generally excluded, which is not the case in North America, Europe, and southern South America. In Latin America, toxoplasmosis is primarily due to infection with oocysts from infective cat feces.

Because we already had data on urban and rural antibody prevalence for Panama,[9] we wanted to explore transmission to humans in the rain forest that involved two different ethnic groups from the eastern areas of Colon and Panama provinces, and the difference between mainland and island transmission. Prevalence data only was available from previous studies in the rain forest in Brazil,[4] Ecuador,[6] and Colombia (Gajdusek DC, unpublished data), and from animals in the Bayano area from Panama.[17]

Previous studies in humans lacked details regarding the environment. This investigation attempted to analyze how transmission takes place, particularly how prevalent the infection is in the forested and semiforested area, and how infection prevalences changed following the initiation of agricultural practices and increased urbanization. Although the study area used to be relatively inaccessible, colonization along the Pan American Highway and intensive logging during the last five years is rapidly transforming the area directly adjacent to the highway. Although the general climate is still typical rain forest, secondary growth near human habitation mixed with small agricultural plots, low vegetation, secondary shrub, and erosion are evident along certain portions of the highway. As a result of deforestation, the microclimate is hotter and drier where the sun reaches the soil.[18] Study sites on the mainland differed in the amount of forest surrounding them, allowing an opportunity to study *Toxoplasma* transmission in as close to an original state as possible, and in gradations.

Since previous studies showed that the prevalence of antibodies to *Toxoplasma* increased with age,[9] we chose to study children 2–12 years of age for whom travel had been minimal and residence history could be better defined than at later ages to explore differences in transmission patterns and to examine the relationship between antibody status and various hypothesized risk factors.

MATERIALS AND METHODS

Study populations. The two areas chosen for study were the Cuenca Alta del Bayano (Bayano River Basin) and the Islas de San Blas (San Blas Islands), Panama. The Bayano Basin, which drains approximately 4,273.5 km^2,[19] is located east of Panama City and the Panama Canal, along the Pan American Highway, between longitudes 78°70′ and 79°15′W and latitudes 8°45′ and 9°20′N.[20] The San Blas islands comprise more than 300 distinct islands of various sizes located off the Caribbean coast, running from north of Panama City to the Colombian border, between longitudes 77°20′ and 79°05′W and latitudes 8°35′ and 9°38′N (Figure 1). Both the Bayano and San Blas have a similarly wet tropical climate, with the rainy season usually extending from May to December. Only occasional light showers occur in San Blas during the dry season. The mean annual temperature near Panama City is 26.7°C, with an average annual rainfall of 254 cm. The average annual rainfall for the San Blas islands is 219 cm (Smithsonian Tropical Research Institute, Marine Environmental Science Program, Panama City, Panama, unpublished data).

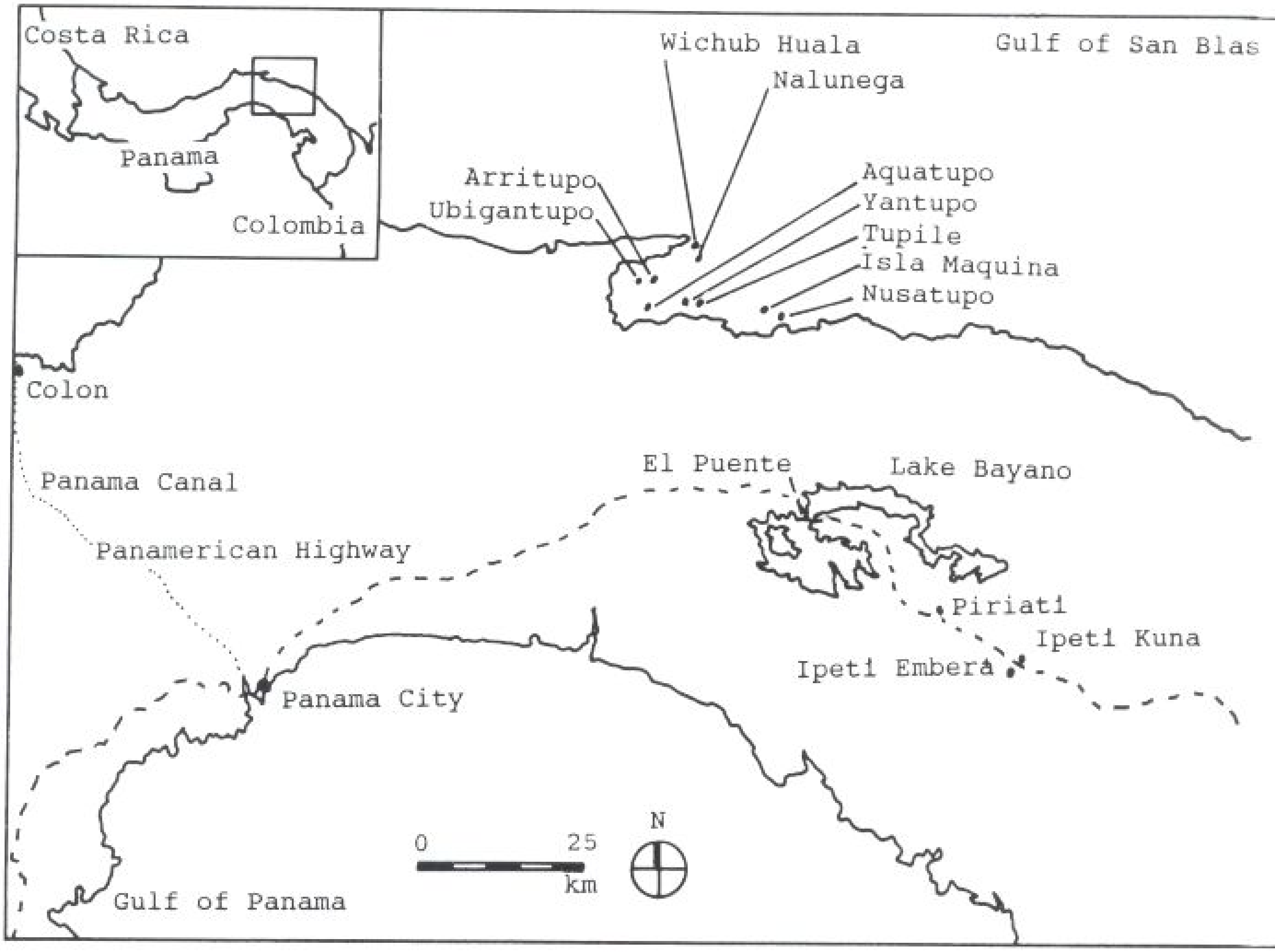

FIGURE 1. Map of the Bayano and San Blas areas of Panama, east of the Panama Canal, indicating the Panamerican Highway and location of the mainland and island communities studied.

The two ethnic groups in these areas, the Kuna and the Embera, live in villages that are culturally relatively homogeneous. The Kuna live in the Northern Bayano Basin and the San Blas Islands, whereas the Embera live in the Bayano and farther east towards Colombia. The majority of the Kuna and Embera practice subsistence agriculture and use fish to supplement their diet.

The villages were grouped by mainland or island and stratified by size, after which 13 villages (four mainland and nine island) were selected at random for inclusion in the study (Figure 1). All island villages were Kuna and were located 2–4 km from shore.

Permission to work in both the San Blas and Bayano Kuna areas was granted after visits with the most important caciques (highest leaders) in the Kuna hierarchical governing structure.

Data collection methods. An interview was conducted and serologic samples were collected in 1991 by 10 Panamanian medical students. Kuna interpreters from each village were trained by one of the authors (GDE) in their respective villages to work with the medical student interviewers. These interpreters included the health assistant from the village if there was one. The other interpreters were chosen by the community at the the nightly community meeting attended by the research team on the eve of starting the study in each community. The interpreters first aided in the construction of a map for their community and in the identification of the study population (children between the ages of 2 and 12) within each community.

The interview questions were first translated into Spanish and then pretested with native Kuna speakers to assess the comprehension of the questions. The interview was preceded by an explanation of the study by the data collector and by obtaining the informed consent of the mother. Few mothers refused to participate. Random spot checks of the interviewers were conducted to ensure that the interview protocol was being followed. The questionnaires were reviewed for completeness of answers by one of the research team at the end of each day.

At the end of the interview with the mother, capillary blood samples were collected on filter paper from each study-eligible child using the techniques described by Wallace,[21] air-dried, and stored at ambient temperature until sent to Panama City weekly, whereupon they were frozen at −20°C. Pretesting of this method indicated that storage of dried filter paper samples at ambient temperature for one

TABLE 1
Distribution of the study population by community

Community	Community size*	Study No. (1991)	Study %	Ethnic group	No. of houses with children 2–12 years of age
Ipeti Embera	208	67	9	Embera	30
Piriati	168	76	10	Embera	23
Ipeti Kuna	345	80	11	Kuna	34
El Puente	118	55	7	Kuna	25
Tupile/island	560	50	7	Kuna	23
Yantupo/island	407	48	6	Kuna	20
Aquatupo/island	310	63	8	Kuna	31
Ubigantupo/island	258	65	9	Kuna	27
Arritupo/island	279	46	6	Kuna	23
Nalunega/island	218	55	7	Kuna	20
Wichub Huala/island	302	45	6	Kuna	18
Nusatupo/island	325	77	10	Kuna	31
Isla Maquina/island	245	33	4	Kuna	18
Total		760	100		

* Figures are from the 1990 national census.

week did not lead to deterioration of samples when compared with refrigerated storage.

Laboratory methods. The serologic assays were performed at the Gorgas Laboratory (University of Kansas Toxoplasmosis Laboratory) once the fieldwork was completed. A 7-mm disk was punched from each filter paper blood sample. The sera were eluted from the filter papers and tested for IgG antibodies using the direct *Toxoplasma* agglutination method with antigen of formalin-fixed suspensions of *Toxoplasma* tachyzoites.[22] Undiluted sera were screened first. If positive, sera were serially diluted beginning at 1:40 to determine the specific antibody titer.[23] Antigen was provided courtesy of Dr. Philippe Thulliez (Institut de Puericulture, Paris, France). The lowest dilution tested was 1:40 and specimens positive at this dilution or higher dilutions were considered positive for this study.

Statistical methods. Data from interview schedules and serologic examinations were recorded using DBASE III (Ashton Tate, Torrance, CA). The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) was used for both bivariate and multivariate analyses.[24] Antibody status was examined in relation to the hypothesized or postulated risk factors using the chi-square test. Contingent upon the findings from bivariate analyses, logistic regression was performed to determine the explanatory power of each independent variable taken in combination with the other independent variables. The method used was forward stepwise, with no limit on the number of times a variable could be entered or removed. Because of confounding by community, locality was always entered into the model.

We considered antibody status the outcome (dependent) variable for purposes of multivariate analyses; this assumes that the independent variables affected the status of the dependent variable because a prevalence study does not indicate whether the independent variables actually preceded the dependent variable in time.

RESULTS

Complete interview and serologic data were collected for 760 children from the 13 localities, representing 89% of the eligible children.

Study populations and their habits. There was some variability in the number of children from each town (Table 1) due to the various sizes of the towns. Participation rates were high, varying from 82% to 100%. The sex distribution was approximately equal, with 337 (44%) males and 407 (56%) females. Similarly, there was a relatively even distribution of ages.

The number of children reportedly eating different kinds of meat are listed in Table 2. All children ate fish and this factor was therefore not included in Table 2.

Most (95.5%) of the study population's families practiced agriculture, usually on the mainland at some distance from the village; 193 (25.4%) of the children went to the farming site and 386 (50.8%) of the children gardened near the house. Most (87.5%) of the children played in the dirt. The distribution of types of flooring was as follows: loose soil (59.9%), wood (18.6%), compact soil (16.4%), compact and loose soil (2.2%), and other (3%). About one-fifth (20.4%) of the population lived in raised houses a minimum of four feet above ground for flood protection.

All communities had cats. Cats were reported around or near the house by 654 interviewees (86.1%) and inside the house by 255 interviewees (33.6%). The interviewers actually saw at least one cat in the house during 127 (16.7%) interviews. Cat feces in the house were noted in three (0.4%) instances. In approximately half (53.7%) of the study population, mothers or children fed cats.

In an attempt to determine the relationship between *Toxoplasma* infection and what cats are fed or eat, various variables were examined. Approximately one-third of the study population (282 subjects [37.1%]) gave their cats viscera of animals they consumed as opposed to not giving their cats viscera. Since roaches can be transport hosts of *Toxoplasma*,[25] the subjects were asked if cats had been observed eating cockroaches. One-third (32.9%) answered that they had seen cats eating roaches. When asked if they had seen cats eating birds, 306 (40.3%) answered that they had.

Bivariate results. There was considerable variation in the prevalence of antibody to *Toxoplasma* in children by communities as shown in Table 3. There was a significant difference in antibody status when Kuna towns on the mainland

TABLE 2

Associations between antibody and hypothesized risk factors in different subgroups (crude and while controlling for the community)*

Factor	Crude			P value in group			
	n	(%)	P	All communities	Positive communities	Three communities with the highest prevalence	Mainland communities
Kuna	522	(80.2)	NS	NS	NS	NS	NS
Male sex	337	(44.3)	NS	0.0590†	0.0590†	0.0590†	NS
Age	760	(100)	NS	0.0337†	0.0337†	NS	NS
Drinks piped water	94	(12.4)	0.0000	NS	NS	NS	NS
Meat consumption							
Pork	681	(89.6)	NS	NS	NS	NS	NS
Chicken	657	(86.4)	NS	NS	NS	NS	NS
Iguana	596	(78.4)	NS	NS	NS	NS	NS
Peccary	529	(69.6)	NS	NS	NS	NS	NS
Deer	491	(64.6)	0.0433	NS	NS	NS	NS
Agouti	490	(64.5)	0.0062	NS	NS	NS	NS
Spotted cavie	437	(57.5)	0.0027	NS	NS	NS	NS
Tapir	356	(46.8)	NS	NS	NS	NS	NS
Beef	354	(46.6)	0.0024	0.0199†	0.0199†	0.0199†	NS
Rabbit	336	(44.2)	0.0041	NS	NS	NS	NS
Pigeons/other birds	331	(43.6)	NS	0.0437†	0.0437†	0.0437†	NS
Capybara	108	(14.2)	NS	0.0199†	0.0199†	0.0199†	NS
Family agriculturalists	726	(45.5)	NS	NS	NS	NS	NS
Child goes to farm	193	(32.0)	0.0006	NS	NS	NS	NS
Gardens near house	386	(50.8)	0.0315	0.0452†	0.0452†	NS	NS
Plays in dirt	665	(87.5)	NS	NS	NS	NS	NS
Living area on piers	155	(20.4)	NS	NS	NS	NS	NS
Type of floor‡ compact soil	125	(16.4)	0.0000	0.0092†	0.0001†	NS	0.0001†
Cats around house	654	(87.1)	NS	NS	NS	NS	NS
Cats inside house	255	(33.6)	0.0025	0.0397†	0.0397†	0.0397†	0.0397†
Interviewer sees cat	127	(16.8)	0.0082	NS	NS	NS	NS
Interviewer sees cat feces	3	(0.4)	NS	0.0638†	0.0638†	NS	NS
Feed cats	408	(53.8)	0.0082	NS	NS	NS	NS
Cats given viscera	282	(37.1)	0.0040	NS	NS	NS	NS
Cats observed eating roaches	250	(34.5)	NS	NS	NS	NS	NS
Cats observed eating birds	306	(42.2)	0.0229	NS	NS	NS	NS

* A crude association signifies all of the communities were analyzed at one time. n = number of subjects with factor; NS = not significant ($P > 0.10$). Gardens near house signifies that the child planted things near or around the house.
† Yates' correction for small cell size applied.

were compared with those on the the islands. On the mainland, 53 (19.1%) children were seropositive as compared with 41 children (8.5%) on the islands ($P < 0.0001$).

There were no significant differences in antibody status between the Kuna and the Embera. Not surprisingly, there was no significant difference in antibody status by sex. Unadjusted bivariate analysis showed no association between age and antibody status. Regrouping age by two-year intervals also showed no significant trend. Because age has been reported to be associated with increased antibody presence,[9-11] the relationship between children's age by year and antibody status was examined, using logistic regression, in four groups: the entire population, children from towns where antibody was detected, children from towns where antibody prevalence was 10% or greater, and children from families where at least one child had antibodies (Table 2). No relationship between age and antibody status was found in any of the four groups.

Variables significantly associated with the presence of antibodies included type of water consumed (prevalence of 26.6% in those with piped water versus 9.3% in those with river water and 12.2% in those with other water sources; $P < 0.0001$). Comparison of eating meat in general (14.3% in those eating meat versus 8.9% in those who do not eat meat; $P = 0.0433$) and specific meats showed the following differences: beef (16.4% in those eating beef versus 8.9% in those who do not eat beef; $P = 0.0024$), spotted cavie (15.6% versus 8.0%; $P = 0.0027$), rabbit (16.4% versus 9.2%; $P = 0.0041$), agouti (14.9% versus 7.8%; $P = 0.0062$), and deer (14.3% versus 8.9%; $P = 0.0433$). Soil and cat contact variables significantly associated with the presence of antibodies included floor type (30.4% in those with a compact soil floor versus 8.5% in those with wood floors, 7.9% in those with loose soil floors, and 20.5% in those with combination floors; $P < 0.0001$), going to the farm (19.7% among those children visiting the farming site versus 9.9% among those who do not; $P = 0.0006$), gardening near the house (15.0% among those children gardening versus 9.6% among those who do not; $P = 0.0315$), having a cat inside the house (17.6% versus 9.7%; $P = 0.0025$), and the interviewer seeing a cat in the house during the interview (18.9% versus 11.0%; $P = 0.0082$). Significant cat diet variables included feeding cats (15.4% among those who fed cats versus 8.8% among those who did not feed cats; $P = 0.0082$), cats given viscera to eat (17.0% among those who gave viscera to cats versus 8.8% among those who did not give viscera; $P = 0.0040$), and cats having been observed eating birds (14.7% among those who had seen cats eating birds versus 8.8% among those who had not seen cats eating birds; $P = 0.0229$).

TABLE 3

Association of community and antibody status and the probability of being seropositive in the final forced model using logistic regression*

Community	No.	Mean age (years)	Child's antibody status		Probability of being seropositive
			No. positive	(%) positive	
Ipeti Embera	67	6.03	9	(13.4)	0.1167
Piriati	76	6.61	7	(9.2)	0.0441
Ipeti Kuna	80	6.10	34	(42.5)	0.4359
El Puente	55	6.69	3	(5.5)	0.0652
Tupile	50	7.05	7	(14.0)	0.1489
Yantupo	48	6.92	3	(6.3)	0.0652
Aquatupo	63	6.98	7	(11.1)	0.1167
Ubigantupo	65	6.92	17	(26.2)	0.2273
Arritupo	46	6.98	0	(0.0)	0.0000
Nalunega	55	6.55	0	(0.0)	0.0000
Wichub Huala	45	6.56	0	(0.0)	0.0000
Nusatupo	77	6.81	2	(2.6)	0.0113
Isla Maquina	33	7.52	5	(15.2)	0.1666
Total positive			94	(12.4)	
Total negative			666	(87.6)	

* Community was forced into the model (multivariate analysis). Bivariate analysis of community and antibody: $\chi^2 = 111.10$, $P < 0.0001$; mainland versus island: $\chi^2 = 17.17$, $P < 0.0001$; Kuna mainland versus Kuna island: $\chi^2 = 32.42$, $P < 0.0001$.

Higher antibody titers were noted in towns with higher prevalence rates of infection in children (Table 4), although statistical analysis did not show this to be significant.

The initial survey did not include a complete cat census, which was deemed impossible because of the secretive habits of feral cats that usually live outside of the mainland village, coming inside only under the cover of darkness.

An attempt was made to bleed cats in all of the communities postsurvey. In Ipeti Embera, where 13.4% of the children surveyed were seropositive, two (50%) of the four cats that were bled were seropositive at a dilution of 1:40. In Piriati, where 9.2% of the children were seropositive, the one cat that was available for bleeding was seronegative at a dilution of 1:40; no other cats could be found in town between 1:00 PM and 3:30 PM. In Ipeti Kuna, where 42.5% of the children were seropositive, six cats were bled. All four adult cats were seropositive (one at a dilution of 1:40 and three at a dilution of 1:4,000). Two kittens, one approximately six months of age and the other four months of age, were both seronegative at a dilution of 1:40. In El Puente, where 5.5% of the children were seropositive, of the three cats bled postsurvey, all were seropositive. Of the 14 cats available for bleeding in the Bayano, nine (64%) were seropositive, which is higher than the antibody prevalence of cats in Panama City (51%).[26]

An attempt was also made to bleed cats on all of the islands postsurvey. On Ubigantupo, the community with the highest prevalence of antibodies among the islands (26.2% among the children), 50% (4 of 8) of the cats tested had antibody at a dilution of 1:40. Of special interest is the finding that on the three islands, Arritupo, Nalunega, and Wichub Huala, no antibodies to *Toxoplasma* were found in the children tested, and all nine cats bled lacked antibody. Table 5 shows that the percentage of cats with antibody is greater on the mainland (64%) than on the islands (46%), as it is for humans.

The number of cats in a community will determine the proliferation of cats and thus sustainability of *Toxoplasma* infection if there are sufficient intermediate hosts. The absolute number of cats in a community was not determined in this survey. However, by asking whether a cat(s) entered the house, a relative measure of cat density in each com-

TABLE 4

Distribution of antibody titers by community and rank of community based on percentage with cats entering the house

Community	Rank*	Antibody status	Reciprocal antibody titer									
		No. (%) positive	40	60	180	540	1,620	4,000	6,000	18,000	54,000	162,000
Ipeti Kuna	1	34 (42.5)						29	2	3		
Ubigantupo†	8	17 (26.2)						7	4	3	2	1
Isla Maquina†	2	5 (15.2)				1		4				
Tupile†	3	7 (14.0)	2	1		1		3				
Ipeti Embera	7	9 (13.4)						9				
Aquatupo†	13	7 (11.1)		3	2			1	1			
Piriati	10	7 (9.2)						6	1			
Yantupo†	4	3 (6.3)			3							
El Puente	5	3 (5.5)						3				
Nusatupo†	11	2 (2.6)						1	1			
Arritupo†	9	0 (0.0)										
Nalunega†	12	0 (0.0)										
Wichub Huala†	6	0 (0.0)										

* Communities were ranked by the percentage of houses reported having cats entering the houses.
† Island communities.

TABLE 5
Distribution of human and cat antibody status by locale

	Total no.	Antibody status No. (%) positive	χ^2	P
Human				
Mainland	278	53 (19.1)	17.17	0.0000
Island	482	41 (8.5)		
Kuna				
Mainland	135	37 (27.4)	32.42	0.0000
Island	482	41 (8.5)		
Cat				
Mainland	14	9 (64.3)	1.20	0.27
Island	26	12 (46.2)		

munity was determined. The communities can be ranked by the percentage of houses with cats entering the house per number of houses. The rank of the community correlates positively with higher prevalences of antibody among the children, with Ipeti Kuna notably having the highest antibody prevalence among children and the highest percentage of respondents having cats in the house. Isla Maquina and Tupile were third and fourth highest in antibody prevalence and ranked second and third highest in the percentage of having cats in the house (Table 4).

Confounding variables. To determine whether the hypothesized risk factors were similar or differed in the general communities, bivariate analyses were performed on those factors found to be significantly associated with the presence of *Toxoplasma* antibodies in the crude estimate for each community. These analyses revealed that most factors differed significantly between communities. Because of the wide range in prevalence of antibodies to *Toxoplasma* and because the strata-specific or community-specific prevalences of these factors varied, we decided to analyze the data while controlling for community. Once the communities had been controlled for, most of the significant differences found in the crude estimate of association did not show up. That is, within the communities themselves, there were no significant associations between hypothesized risk factors and antibody status with the exceptions listed in Table 6.

Multivariate analysis (logistic regression). This model allowed the selection of factors that predicted the presence of antibodies. Only the factors that were significant in the crude analysis were included in the modeling process. Because of confounding by community, community status was forced into the model. The model using all 13 communities adequately predicted antibody status ($P = 0.5473$).

The only variable that contributed to predicting antibody status, however, was community. The probability of being seropositive in each community using this model is illustrated in Table 3. Maps of the 13 towns with each house and each seropositive child marked suggested that infection tended to be clustered in certain areas in towns.

Bivariate and multivariate analyses were also performed using different subgroups of the total population of 760. Analyses were performed using only those communities in which antibody was found, the three communities with the highest prevalences, and the mainland communities. Table 6 shows the relationship between the hypothesized risk factors and antibody status while controlling for community (significant at the $P < 0.05$ level). In each instance, community confounded the relationship between the various risk factors and antibody status. In each multivariate analysis, community was the only factor that significantly predicted antibody status.

DISCUSSION

Several studies in Latin America suggest that human acquisition of *Toxoplasma* is primarily due to contact with soil that is contaminated with parasite oocysts from cats.[13,15] Ingestion of raw or undercooked meats did not play a role because they were not consumed in these communities.

Children 2–12 years of age were chosen for the study because an earlier investigation in Panama showed 50% of the children to have acquired antibody by the age of 10.[9] Because this age group was residentially the most stable, with the least amount of travel, it was considered to be the one best suited for studying differences in habits that may lead to acquiring the infection. This young age group would also not be subject to as great a recall bias by their mothers as would an older population. The overall antibody prevalence found in this study is lower than the overall prevalence found in Panamanian schoolchildren from three towns at different altitudes in western Panama,[11] and that found in children in rural central Panama and Panama City.[9]

Some of the hypothesized risk factors included in this study were significantly associated with increased antibody prevalence, at least in various crude estimates. Yet, the prevalence of hypothesized risk factors varied greatly by community. When confining analysis to individual communities, the associations between risk factors and antibody prevalence differed greatly from the combined or crude estimates. The crude estimates of association between postulated risk factors and antibody presence were confounded by strong community attributes.

Certain meats (beef, spotted cavie, rabbit, agouti, deer, and peccary) were associated with antibody presence in the crude estimates. However, further analysis revealed that this was a result of confounding by community attributes. Once each community was examined separately, only three associations were found between meat consumption and antibody status (beef, pigeons or birds, and capybara); all were found in the same community, Isla Maquina, suggesting that community attribute was important.

Wallace[1,27] and Munday[28] have shown in the Pacific Islands that in the absence of cats, antibody to *Toxoplasma* is absent, and that an increase in antibody prevalence is paralleled by an increased prevalence of cats.[3] Three factors, having a cat in the house, the interviewer seeing a cat during the interview, and reportedly feeding cats generally showed a statistically significant association with the presence of antibodies. However, for many reasons the magnitude of the cat population and whether they defecate in and around the house has been difficult to document.

Because of the few statistically significant associations found between postulated behavioral risk factors and antibody status once community was controlled for, the differences in antibody prevalence may have less to do with habits but more with the number of cats, the age and immune status

TABLE 6
Factors significantly associated with antibody status after controlling for community

Factor/community	Child's antibody status: With variable No. (%) positive	Child's antibody status: Without variable No. (%) positive	χ^2	P
Floor*/Piriati	4 (33.3)	3 (4.7)	6.78	0.0092†
Eats beef/Isla Maquina	4 (44.4)	1 (4.2)	5.42	0.0199†
Eats capybara/Isla Maquina	4 (44.4)	1 (4.2)	5.42	0.0199†
Age (years)/Aquatupo				
2	0 (0.0)		19.56	0.0337†
3	0 (0.0)			
4	0 (0.0)			
5	0 (0.0)			
6	3 (30.0)			
7	2 (66.7)			
8	1 (14.3)			
9	1 (33.3)			
10	0 (0.0)			
11	0 (0.0)			
12	0 (0.0)			
Cat inside house/Piriati	5 (21.7)	2 (3.8)	4.23	0.0397†
Eats pigeons or birds Isla Maquina	5 (31.3)	0 (0.0)	4.07	0.0437†
Gardens near house Isla Maquina	3 (50.0)	2 (7.4)	4.01	0.0452†

* Floor is designated as having part of the floor made of soil.
† Yates' correction for small cell size applied.

of the cats, the number of intermediate hosts, and oocyst survivability.

Logistic regression analysis using four different population groups (the entire population, the communities where antibody was detected, the three communities with the highest prevalences, and the four mainland communities) increases the likelihood of recognizing that oocyst availability is sufficient to explain the presence of antibodies. In all four analyses, community was the only factor predictive of antibody presence.

The age structure of cats visiting a community and their immune status will affect the availability of oocysts. Cats can shed hundreds of thousands to millions of oocysts in their feces during primary infection. Yet, a cat that has acquired the infection and has shed oocysts will generally not shed again when reinfected with tissue cysts.[29] Thus, a community inhabited only by immune cats poses fewer foci of contamination to humans than a community populated by a reproductive group of cats, which introduces young nonimmune kittens into the population.

Wild felids may also contaminate the environment. Ocelots and jaguars have been spotted in the Bayano, and both Kuna and Embera go into the mountains to hunt them. Wild cats have been shown to be capable of shedding oocysts after being fed tissue cysts.[30] Jaguars, in particular, splash in water as they hunt for fish, walk, and swim in water. It is also possible that jaguars defecate on the river banks or nearby slopes. Oocysts could be washed from the slopes or banks into the river during the rainy season.

Ipeti Kuna, where antibody prevalence was found to be 42.5%, and Piriati, where antibody prevalence was 9.2%, both used piped water as the main water source at the time of the survey. These two mainland communities each use a different river in the mountains above the communities, located in primary forest, for their aqueducts, which are located between a 1.25- and 2.5-hr walk from the communities. Residents in both areas have reported sightings of wild felids in the mountains where the aqueducts begin. Both dams were visited postsurvey; no cat tracks were found at either site. Cats may indeed drink from the dams, although because of the cement structure and the surrounding boulders and steep side slopes, no soft dirt was available for tracks to be visible at the Ipeti Kuna dam, and freshly fallen leaves, which may have also obscured tracks, covered the sand at the Piriati dam. Wild felids may have contaminated the aqueducts at the dams or upstream. It is also possible that the finding of no association of age and antibody presence in Ipeti Kuna or Piriati indicates intermittent aqueduct contamination, since those children drinking piped water at the time of contamination, regardless of age, would have been infected.

Ipeti Embera started receiving water from the same aqueduct as Ipeti Kuna in 1991, after the survey. Children in Ipeti Embera had an antibody prevalence of 13.4% compared with 42.3% in Ipeti Kuna. It would be of interest to sample the children of Ipeti Embera several years later to investigate whether their use of piped water increased the incidence of seroconversion to levels similar to those in Ipeti Kuna.

The source of water was found to be significantly associated with the presence of antibody ($P < 0.0001$), both in the crude estimates using the entire population and when using the three communities with the highest antibody prevalence compared with the three communities where antibody was not detected, and separately in the three communities with the highest antibody prevalence ($P = 0.0144$). Piped water was associated with higher antibody prevalences than lake or river water. Once again, the association with the water source disappeared once community was controlled for.

The lack of association between water source and antibody in Ipeti Kuna and Piriati may be attributed to the fact that 90% of the inhabitants used some piped water, resulting in small expected frequencies in the contingency table for those that did not used piped water.

Considering the report of an outbreak of toxoplasmosis in Panama during a U.S. Army exercise, where all food consumed was canned and where contaminated water appeared the only possibility for infection,[31] contamination of the water sources appeared plausible. The present study showed higher antibody prevalences associated with piped water that comes from streams in the mountains. Of the felines that shed oocysts, the jaguar appears noteworthy because of its riverine habits. Two Costa Rican jaguars captured had antibody to *Toxoplasma,* but shed no oocysts, probably because they were immune.[30]

If *Toxoplasma* infection had no relationship with the density of infected cat feces, one would not expect to see a pattern of clustering of infection evident in the maps of the 13 communities. The shape of the villages, the proximity of houses to each other, and the amount of open space varied from village to village.

One of the reasons that both island and mainland communities were selected for study was because we anticipated finding higher antibody prevalences in the densely populated Kuna island communities because there would be less soil in which cats could defecate so oocysts densities might be higher. In mainland communities, one would expect peripheral oocyst loss because of the cats living on the edge of the communities having the opportunity to defecate farther from the houses where they are fed.[15] The other reason was to determine if there were prevalence differences between the mainland and Kuna island based on their geographic location.

Tupile, Ubigantupo, and Nusatupo, all island communities, were those with the least amount of open space. Yet, only Ubigantupo had a notably higher antibody prevalence in children (26.2%), followed by Tupile (14.0%) and Nusatupo (2.6%). It is possible that the scarcity of available soil for cat defecation results in higher antibody densities, as suggested in a study in Costa Rica,[15] and is contributing to higher antibody prevalences in Ubigantupo and Tupile. The low antibody prevalence, in spite of high density of houses, on Nusatupo may be related to the low ranking number of cats, 11th of 13. House density in Ipeti Kuna does not explain the high seroprevalence (42.5%) because there is an abundance of open space on the mainland where cats may defecate. The correlation of village shape and soil available for cats to defecate has been cited in previous studies in Panama and Costa Rica.[9,15] We postulated that the configuration of a community would influence antibody prevalence for the community as a whole, and in the case of compact-shaped communities (square or circular), would increase antibody prevalence in the center of the community. In this scenario, linear communities, such as those with houses along a road, would have lower antibody prevalences. The data, however, did not support this hypothesis.

Higher antibody titers were noted in towns with higher prevalence rates of infection in children (Table 4), although statistical analysis did not show this to be significant. Although it is tempting to correlate these high antibody titers with a higher proportion of recent infections, this could be accounted for by reinfection. Similarly, low titers in communities with low prevalences may indicate that more of the infections in children dated from the past.

In contrast to the findings of Wallace and others[1,3] and Munday,[28] who found that in the absence of cats, antibody is absent, we have a situation in which even in the presence of cats, humans were seronegative. Findings of high antibody prevalences on islands in the Pacific,[2,27] in contrast with our results, may be partially explained by the greater abundance of trees and uncleared land, which provide both moisture and sustain intermediate hosts such as rodents and birds. The antibody prevalences on these islands with lush vegetation are similar to our mainland communities that have more space and flora. Low prevalences of antibody were found in New Guinea, despite lush vegetation, but there were few cats: the prevalence of *Toxoplasma* antibodies paralleled the population's contact with cats.[3]

As described earlier, the islands inhabited by the Kuna are very densely populated, with usually only narrow walkways between closely spaced houses, with the deep overhangs of the roofs often touching one another. The only open space we have seen is a basketball court and a small marshy pond; agricultural plots are maintained on the mainland. Birds on the north-south flyway migrate hugging the coast. It is doubtful that they would choose a densely populated island with few trees on which to spend time overnight,[32] although small passerine birds are commonly kept as pets. Likewise, the only mammals present appear to be rats, particularly Norway rats, unlike the diverse species encountered when the Bayano reservoir was created, with seropositivity encountered in opossums, armadillos, anteaters, pacas, agoutis, coatimundis, tayras, a peccary, and a deer.[17]

We examined the transmission of *Toxoplasma* in nearly natural rain forest settings and encountered an antibody prevalence much lower than in rural and urban settings in Panama.[9,11,26] As a consequence, childhood seroprevalence was spotty and the usual age correlation was inapparent, suggesting intermittent transmission. Unidentified community aspects were more predictive of antibody prevalence than postulated factors that we sought to investigate. Although we did not find differences in antibody rates that correlated with ethnicity, we did find differences in Kuna settlements. The mainland settlements had higher prevalences than the island villages, yet these sylvatic mainland prevalences were still lower than those found in rural Altos del Jobo,[9] and much lower than those found in Panama City.[26] The islands were without the rich mammalian fauna that might have perpetuated the infection and for which the Panama land bridge is famous.[33]

In addition to the factors examined in this study, various other factors may influence transmission: cats and intermediate hosts (terrestrial birds and mammals), as well as rate-modifying factors, such as number of nonimmune kittens, cat fecal density on the soil and human contact with it, drainage water, moisture, shade, source of water, community shape, type of house, and the proximity of cats to the houses. Among these, the cause of community differences must be sought.

Although the data did not support the importance of many of the hypothesized risk factors, which may merely be the result of low levels of antibody prevalence that we measured, the study is consistent with the theory of transmission by oocysts and the importance of cats in transmission. Transmission by meat was excluded and the rate of transplacental transmission is always too low to have epidemiologic importance. The single greatest problem encountered in this study was the lack of any measure of the amount of infective material in each town. No soil, cat, fecal, or piped water samples were taken to measure contamination. Even if these samples had been taken, it would have been unlikely that the samples would have been informative if not obtained in large numbers and over a lengthy period of time. This study looked at infection that may have predated the survey from one month to 12 years. It shows the infection prevalences in cleared primary rain forest and islands as close as possible to the aboriginal state. It also helps to elucidate the differences between transmission in urban, rural, and sylvatic settings. The sylvatic settings differ greatly from rural and urban areas.[9] It is likely that transmission is highest in urban areas because of the increased concentration of cats, particularly because of the multiple food sources and people feeding them, and the limited number of places for defecation, resulting in a concentration of possibly infected material in areas where children play. The lower seroprevalence in villages or rural areas may well be because of lower cat density and deposition of feces in peripheral areas not frequented by humans, complicated by the fact that most cats spend the day away from the villages and visit mainly at night.

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