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Epidemiology of Spotted Fever Group and Typhus Group Rickettsial Infection in the Amazon Basin of Peru

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Abstract. A seroprevalence study for IgG antibodies against spotted fever group (SFGR) and typhus group (TGR) *Rickettsia* among humans and domestic pets was conducted in the city of Iquitos, located in the Amazon basin of Peru. Of 1,195 human sera analyzed, 521 (43.6%) and 123 (10.3%) were positive for SFGR and TGR antibodies, respectively. District of residence and participant age were associated with antibody positivity for both groups, whereas rodent sightings in the home were associated with TGR antibody positivity. Of the 71 canines tested, 42 (59.2%) were positive for SFGR antibodies, and two (2.8%) were positive for TGR antibodies; one active SFGR infection was detected by polymerase chain reaction. An uncharacterized SFGR species was detected in 95.9% (71/74) of *Ctenocephalides felis* pools collected from domestic pets. These data suggest that rickettsial transmission is widespread in Iquitos. *Rickettsia* species should be further explored as potential causes of acute febrile illnesses in the region.

INTRODUCTION

Rickettsioses are caused by obligate intracellular Gram-negative bacteria of the order *Rickettsiales* that are transmitted to vertebrate hosts by arthropod vectors, including ticks, mites, lice, and fleas. *Rickettsiae* are typically divided into two antigenic and genetic groups, the spotted fever group (SFGR) and the typhus group (TGR). Both groups have a global distribution, although individual species may be associated with more defined geographic foci because of the ecological restrictions of their reservoirs or vectors.[1,2] Over the past decade there has been a greater recognition of rickettsial diversity and disease around the world,[3] leading to an ever-expanding list of recognized human pathogens with a concomitant range of disease spectra, ranging from mild or asymptomatic infection to severe disease leading to death. To date, at least 13 SFGR, including *Reckettsia rickettsii*, *Reckettsia honei*, *Reckettsia conorii*, *Reckettsia africae*, *Reckettsia parkeri*, and *Reckettsia felis*, and two TGR (*Reckettsia typhi* and *Reckettsia prowazekii*) have been associated with human disease.

Little is known about rickettsial disease burden and transmission cycles in many tropical regions of the world,[2] particularly in South America.[4] The severity of illness is likely to be greater among inhabitants of these regions because of limited access to adequate health care infrastructure, including diagnostic laboratories and antibiotic treatment. Furthermore, similar disease presentations and overlapping pathogen distribution may lead to misdiagnosis of rickettsial infections as dengue virus or leptospirosis,[5] leading to chronic underreporting. Thus far, reports of rickettsial-associated human illness in South America have been mostly limited to sporadic cases of severe disease (Rocky Mountain spotted fever and Brazilian spotted fever) resulting from tick-transmitted *R. rickettsii* infection in Brazil,[6–12] Colombia,[13] and Argentina.[14] Other data suggest that rickettsial transmission is more prevalent than reported. A wide variety of rickettsial species, such as *R. rickettsii*, *Reckettsia belli*, *Reckettsia amblyommii*, and *R. felis*, have been isolated from fleas and ticks across the continent, including in Uruguay,[15,16] Argentina,[17] Brazil,[18] Chile, and Colombia. In addition, data from serological surveys conducted in the region[8,10,19] suggest that both SFGR and TGR infection are more common than would be suspected from case reports.

In Peru, there have been few epidemiological studies to determine the extent of rickettsial transmission and disease. Tropical rainforest regions in particular have been neglected. Serological and molecular data suggest that in certain Andean regions SFGR may contribute to greater than 20% of febrile cases.[20,21] A preliminary study of febrile patients conducted by Sihuincha and others[22] and Ramal and others[23] suggested that approximately 4% of acute febrile episodes detected in Iquitos, a city located in the Amazon region of northeastern Peru, could be attributed to SFGR infection. To begin to characterize rickettsial transmission cycles in Iquitos, we conducted a cross-sectional serosurvey of healthy participants recruited from four districts of the city. Demographic data were collected and serum samples were screened by enzyme-linked immunosorbent assay (ELISA) for SFGR and TGR antibodies. To incriminate potential vectors and reservoirs, we collected and analyzed serum samples and ectoparasites from peridomestic pets in the same neighborhoods as the human participants. Herein, we report the results of the serological surveys and associated risk factors, and the preliminary molecular characterization of an SFGR species detected in *Ctenocephalides felis*.

MATERIALS AND METHODS

Study site. Iquitos (73.2°W, 3.7°S; 120 m above sea level) is a city of approximately 350,000 people, situated between the Amazon, Nanay, and Itaya Rivers in the Department of Loreto in northeastern Peru. The climate is tropical, with a mean annual temperature of 27.5°C and a mean annual precipitation 2.7 m.[24–27]

Study cohort. The initial serological surveys were carried out in the context of an investigation of a Venezuelan equine encephalitis virus (VEEV) outbreak detected during the first half of 2006.[24] The study protocol was reviewed and approved by the Naval Medical Research Center Detachment (NMRCD) Institutional Review Board (PJT.NMRCD.014). The study was conducted between September 2006 through

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December 2006 in four districts of Iquitos, including Belen, a district located along the Itaya River in the eastern ridge of the city; Bellavista Nanay, a district located along the Nanay River in the northern point of the city; three neighborhoods (Las Mercedes, San Pablo, and 26 de Febrero) located along Avenida Participación in the district of San Juan; and 22 blocks spread across the central part of Iquitos.[24] Participation was limited to residents ≥ 5 years of age. Written consent was obtained from all adults; for participants younger than 18 years of age, parental consent was required for participation. If participants were unable to read and sign the consent form, oral consent was obtained and documented in the presence of a witness. Participants were asked a series of questions about their homes, travel histories, presence of animals, and illnesses they had experienced in the previous year. Parents responded for children who were unable to answer for themselves (typically those younger than 14 years of age). During the consent process of the initial VEEV outbreak, study participants were given the option to permit future analysis of their serum samples for other pathogen- and group-specific antibodies. Of the 1,327 participants of the initial survey, 1,195 (90.1%) consented to future analysis of their serum samples and were included in the current study.

Domestic animal serosurvey and ectoparasite collection. The protocol to test canine and feline serum samples for prior rickettsial exposure was reviewed and approved by the NMRCD Institutional Animal Care and Use Committee (NMRCD07-07). Permission was obtained from the owners to survey dogs ($N = 79$) and cats ($N = 19$) in the same four districts of Iquitos described previously. Before blood withdrawal, animals were muzzled and manually restrained by trained personnel. The blood withdrawal site was wiped with 70% isopropyl alcohol, and up to 5 mL of blood was collected using syringes or vacutainer tubes from either the cephalic, saphenous, or jugular veins in dogs and the jugular, cephalic, or femoral veins in cats. Additionally, whole blood samples (~50 μL) were spotted onto filter paper at the time of collection. Serum samples were separated by centrifugation (1,500 × *g* for 10 minutes) and stored at −20°C. Ectoparasites were collected by combing the restrained animals and were placed into cryovials, which were stored on ice until taxonomically identified and separated. Ectoparasites were subsequently pooled (by species, sex, and the individual animal from which they were collected) for trituration and polymerase chain reaction (PCR) analysis.

Laboratory analyses. *ELISA.* Human, canine, and feline sera were tested by ELISA for IgG antibodies against SFGR and TGR. Microtiter plates were coated at 4°C for 2 days with appropriate antigen diluted in 1X phosphate buffered saline (PBS) (for SFGR 0.15 μg fragment X and 0.3 μg fragment Y from the OmpA gene product of *R. rickettsii*; for TGR 0.15 μg fragment AN and 0.15 μg fragment K from the Omp B gene product of *R. typhi*[28]). Following coating, plates were blocked in 10% milk and 0.1% Tween 20 in 1 X PBS for 1 hour at room temperature. Serum samples were diluted 1:100 in 5% milk in 0.1% Tween20 in 1X PBS and were applied to coated plates for 1 hr at room temperature, followed by washing and the addition of peroxidase-conjugated goat anti-human IgG (1:4000), anti-feline IgG (1:4000), or anti-canine IgG (1:4000) for an additional hour. Following the addition of peroxidase substrate, plates were read at 405 nm. Positive cut-off optical density values were calculated as the mean of negative controls plus five standard deviations. Anti-SFGR and anti-TGR antibody ELISAs were validated with sera previously classified as positive and negative for *Rickettsia* antibody by immunofluorescence assay. For SFGR, 22 of 24 (91.7%) indirect immunofluorescence assay (IFA)-positive sera (*R. rickettsii, R. akari, R. honei, R. conorii,* and *R. australis*) were also positive by ELISA, whereas none (0/10) of *R. typhi* IFA-positive samples were positive. For the anti-TGR antibody ELISA, 21 of 25 (84.0%) of *R. typhi* IFA-positive sera were also positive by ELISA, and all *Orientia tsutsugamushi* (scrub typhus) IFA-positive samples tested were negative (Chen HW and others, unpublished data).

PCR. Arthropod pools and animal blood spots were initially screened using a nested PCR protocol targeting the 17kd gene as previously described.[29] Briefly, pan-rickettsial primers R17-122 and R17-500 were used in the initial PCR, followed by the nested primers specific for SFGR (TZ15 and TZ16) and TGR (RP2 and RPID). For PCR-positive pools, the 17kd region was further amplified and analyzed using primers Rr1175F and Rr2608R to generate a 434-bp amplicon. In addition, partial citrate synthase (*gltA*) and outer membrane protein A (*ompA*) gene sequences were amplified and analyzed, using previously described primers and conditions: RpCS.877p and RpCS.1258n for *gltA*, and RR190-70 and RR190-701 (first round) and 190-FN1 and 190-RN1 (nested) for *OmpA*.[29] The DNA extracted *R. Rickettsia* strain 364D was used as a positive control in all assays. The DNA sequences were submitted to GenBank under the accession nos. GU117904 (17 kD antigen gene sequence), GU117905 (*ompA* gene sequence), and GU117906 (*gltA* gene sequence).

Statistical analysis. Proportions were compared using a χ^2 test using the FREQ procedure in SAS (version 8, 1999, SAS Institute Inc., Cary, NC). Risk factors for infection with SFGR and TGR were evaluated by logistic regression using the GENMOD procedure in SAS with adjustment for household clustering. Multivariate models were constructed with the dichotomous dependent variable: SFGR or TGR IgG ELISA positive and the following independent variables: age (adult, child [< 18 years of age]); district, housing construction materials (concrete and/or brick, only wood); occupation (student, home-based, away from home, and rural); travel history (report of multiple day trips outside Iquitos); cats within the home, pet birds (primarily macaws and parakeets) within the home; and rodent sightings in or around the home using backward selection. The four different districts of Iquitos were collapsed into two groups: those with higher antibody levels for SFGR and lower antibody levels for TGR (Belen and Bellavista Nanay), and those with lower antibody levels for SFGR and higher antibody levels for TGR (central neighborhoods and San Juan) for overall models. It became clear, however that many of our potential risk factors were confounded by district, therefore a set of district-stratified models was also constructed.

RESULTS

Antibody prevalence survey and risk factors for infection. To identify potential risk factors for rickettsial transmission in urban Iquitos, we screened 1,195 human serum samples for the presence of both SFGR and TGR IgG antibodies. As previously described,[24] participants were recruited from four general districts of urban Iquitos (Table 1): Belen (318; 26.6%

TABLE 1
Distribution of selected household characteristics among different districts of Iquitos

Characteristic	Total no. (%)	Central no. (%)	Belen no. (%)	Bellavista Nanay no. (%)	San Juan no. (%)	*P* value
Participants	1195 (100)	290 (24.3)	318 (26.6)	283 (23.6)	304 (25.4)	0.7523
House construction						
Wood/mud	892 (74.6)	98 (33.8)	292 (91.8)	259 (91.2)	243 (80.2)	< 0.0001
Concrete/brick	303 (26.4)	192 (66.2)	26 (8.2)	25 (8.8)	60 (19.8)	
Recent day trips	298 (24.9)	62 (21.4)	72 (22.6)	108 (38.2)	56 (18.4)	< 0.0001
Animals on property						
Dogs	424 (35.5)	115 (39.7)	100 (31.4)	85 (30.0)	124 (40.8)	0.0078
Cats	294 (32.5)	143 (49.3)	81 (25.5)	70 (24.7)	89 (29.3)	< 0.0001
Chickens	366 (30.6)	106 (36.6)	76 (23.9)	67 (23.7)	117 (38.5)	< 0.0001
Pet birds	148 (12.4)	53 (18.3)	22 (6.9)	27 (9.5)	46 (15.1)	< 0.0001
Rodent sightings	1042 (87.2)	232 (80.0)	303 (95.3)	254 (89.8)	253 (83.2)	< 0.0001

of total participants), Bellavista Nanay (283; 23.6%), San Juan (304; 25.4%), and 22 neighborhoods spread across the central part of the city (290; 24.3%). The median age of the participants was 27 (range 5–88); 66.8% of participants were female. There were no significant differences in sex or age distribution among the neighborhoods.[24] Demographics variables varied significantly among the four zones (Table 1) for factors including primary house construction materials, recent travel history, rodent sightings in the home, and peridomestic animal ownership, including dogs, cats, chickens, and pet birds (primarily macaws and parakeets).

Overall, of the 1,195 serum samples tested by ELISA, 521 (43.6%) were found to be positive for SFGR IgG antibodies and 123 (10.3%) were positive for TGR IgG antibodies (Table 2); a subset of the participants from these groups (69; 5.8%) had antibodies against both SFGR and TGR. Previous infection with SFGR and TGR was not independent: among those with SFGR antibodies, 13.2% were positive for TGR, compared with 8.0% for those negative for SFGR ($P < 0.005$).

There were no significant differences in antibody prevalence rates between males and females for either *Rickettsia* group (Table 3). An age-dependent increase for both SFGR and TGR IgG antibodies was observed overall and within each district (Table 2); seroprevalence among participants 18 years of age or older was significantly higher than for those younger than 18 for both rickettsial groups ($P < 0.005$ for SFG; $P < 0.0001$ for TG). Additionally, significant differences in SFGR and TGR seroprevalences were observed among the different districts ($P = < 0.0001$ and $P = 0.04785$, respectively; Table 2), with the highest prevalence of SFGR antibodies in Belen and Bellavista Nanay, and the highest prevalence of TGR antibody in the central neighborhoods and San Juan. Among neighborhoods within each district, no significant variability was observed for either SFGR or TGR, with the exception of SFGR antibody prevalence in San Juan. Within the San Pablo neighborhood of San Juan, 62.1% of participants were positive for SFGR antibodies, whereas in the Las Mercedes and 26 de Febrero neighborhoods SFGR antibody prevalence rates were 24.1% and 28.6%, respectively ($P < 0.001$).

For SFGR, in addition to age and district, house construction materials, pet bird and cat ownership were independently associated with serostatus in bivariate logistic regression analyses (Table 3). For TGR, in addition to age and district, house construction materials, report of recent day trips, and rodent sightings in the home were independently associated with antibody positivity. No association between dog ownership and antibody positivity was observed for either *Rickettsia* groups (Table 3). To identify potential risk factors based on daytime location, occupations were collapsed into the following four groups: students, those with work based in the home (housewives, home-based vendors, the retired, and the unemployed); those with work based outside the home but within the city (drivers, carpenters, mechanics, teachers, and other professionals); and those with rural occupations (agriculture, forestry, and fishing). The highest proportion of individuals positive for SFGR antibodies were observed for the rural occupations; for TGR antibodies, home-based, city-based, and rural occupation groups were similar, but all significantly higher than the student group, which is most likely because of age-dependent effects.

In the multivariate logistic regression model for SFGR, age, district, and pet bird ownership remained significantly

TABLE 2
Prevalence of spotted fever group (SFGR) and typhus group (TGR) antibodies by age group and district of Iquitos

		Age range			
		5–17 years	18–40 years	41 + years	Total
		No./total (%)	No./total (%)	No./total (%)	No./total (%)
Belen	SFGR	36/85 (42.4)	80/161 (49.7)	41/72 (56.9)	157/318 (49.4)
	TGR	3/85 (3.5)	16/161 (9.9)	12/72 (16.7)	31/318 (9.7)
Bellavista Nanay	SFGR	31/79 (39.2)	77/132 (58.3)	41/72 (57.0)	149/283 (52.7)
	TGR	2/79 (2.5)	8/132 (6.1)	8/72 (11.1)	18/283 (6.4)
Central	SFGR	35/101 (34.7)	35/118 (29.7)	31/71 (43.7)	101/290 (34.8)
	TGR	9/101 (8.9)	13/118 (11.0)	15/71 (21.1)	37/290 (12.8)
San Juan	SFGR	27/85 (31.8)	60/160 (37.5)	27/59 (45.8)	114/304 (37.5)
	TGR	4/85 (4.7)	21/160 (13.1)	12/59 (20.3)	37/304 (12.2)
Total	SFGR	129/350 (36.9)	252/571 (44.1)	140/274 (51.1)	521/1195 (43.6)*
	TGR	18/350 (5.1)	58/571 (10.2)	47/274 (17.2)	123/1195 (10.3)†

* $P < 0.0001$ for differences in overall SFGR antibody positivity among the four districts.
† $P = 0.048$ for differences in overall TGR antibody positivity among the four districts.

TABLE 3

Bivariate logistic regression analysis of spotted fever group (SFGR) and typhus group (TGR) serostatus and various demographic factors*

Characteristic	SFGR		TGR	
	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)
Age				
5–17 years	134/367 (36.5)	1.00	18/367 (4.9)	1.00
18–40 years	228/522 (43.7)	1.35 (1.05–1.73)	49/522 (9.4)	2.01 (1.15–3.50)
> 40 years	159/306 (52.0)	1.88 (1.38–2.57)	56/306 (18.3)	4.34 (2.55–7.40)
Sex				
Male	184/397 (46.4)	1.00	37/397 (9.3)	1.00
Female	337/798 (42.2)	0.85 (0.67–1.08)	86/798 (10.8)	1.24 (0.78–1.78)
Housing materials				
Concrete/brick	111/303 (36.6)	1.00	44/303 (14.5)	1.00
Wood	410/892 (46.0)	1.47 (1.09–1.98)	78/892 (8.9)	0.57 (0.36–0.91)
Occupation				
Student	142/394 (36.0)	1.00	22/394 (5.6)	1.00
Home-based	250/552 (45.3)	1.47 (1.14–1.89)	68/552 (12.3)	2.38 (1.47–3.83)
Away from the home	113/225 (50.2)	1.79 (1.30–2.47)	30/225 (13.3)	2.60 (1.45–4.67)
Rural occupation	16/24 (66.7)	3.55 (1.44–8.91)	3/24 (12.5)	1.95 (0.65–8.93)
Trips (> 1 day)				
No	422/991 (42.6)	1.00	100/991 (10.1)	1.00
Yes	99/204 (48.5)	1.27 (0.91–1.77)	23/204 (11.3)	0.88 (0.69–1.86)
Day trips (< 1 day)				
No	403/897 (44.9)	1.24 (0.93–1.67)	104/897 (11.6)	1.93 (1.14–3.25)
Yes	118/298 (39.6)	1.00	19/298 (6.4)	1.00
Cat ownership				
No	378/812 (46.6)	1.46 (1.12–1.91)	89/812 (11.0)	1.26 (0.79–2.01)
Yes	143/383 (37.3)	1.00	34/383 (8.9)	1.00
Dog ownership				
No	334/771 (43.3)	1.00	82/771 (10.6)	1.11 (0.71–1.75)
Yes	187/424 (44.1)	1.03 (0.79–1.34)	41/424 (9.7)	1.00
Chicken ownership				
No	364/829 (43.9)	1.04 (0.80–1.36)	78/829 (9.5)	1.00
Yes	157/366 (42.9)	1.00	44/366 (12.0)	1.30 (0.84–2.01)
Bird ownership				
No	472/1047 (45.1)	1.66 (1.15–2.39)	110/1047 (10.5)	1.22 (0.56–2.65)
Yes	49/148 (33.1)	1.00	13/148 (8.8)	1.00
Rodent sightings				
No	67/153 (43.8)	1.01 (0.69–1.47)	9/153 (5.9)	1.00
Yes	454/1042 (43.6)	1.00	114/1042 (10.9)	1.97 (1.01–3.84)
District				
Belen/Bellavista Nanay	306/601 (50.9)	1.83 (1.42–2.36)	49/601 (8.2)	1.00
Central/San Juan	215/594 (36.2)	1.00	74/594 (12.5)	1.60 (1.05–2.44)

*OR = odds ratio; CI = confidence interval.

associated with antibody positivity; whereas for TGR, age, district, and rodent sightings in the home remained significantly associated with antibody positivity (Table 4). To address potential confounding factors associated with district effects we examined potential interactions between housing materials, animal and rodent contact within different neighborhoods. Risk associated with travel, housing materials, and animal contact appeared to be different among districts. We tried to characterize these interactions by constructing district-stratified multivariate logistic regression models shown for SFGR in Table 5. These models are limited because of sample size problems, especially for TGR (data not shown), but provide some insight for interpreting the study-wide models. Final models for SFGR revealed that individuals whose occupation involved rural exposure were most at risk in Belen, whereas in Bellavista Nanay, age and trips outside of Iquitos remained in the model. Housing materials and age were significant in San Juan, whereas rodent exposure was the only variable that remained in the model in the central region of Iquitos.

TABLE 4

Adjusted odds ratios of potential risk factors associated with spotted fever group (SFGR) and typhus group (TGR) antibody status, based on multivariate logistic regression models*

Rickettsia group	Variable	OR (95% CI)
SFGR	Age (referent < 18 years)	1.48 (1.14–1.90)
	District (referent Central/San Juan)	1.70 (1.31–2.20)
	Bird ownership (referent yes)	1.45 (1.00–2.11)
TGR	Age (referent < 18 years)	2.99 (1.79–5.00)
	District (referent Belen/Bellavista)	1.83 (1.18–2.82)
	Rodent sightings (referent no)	2.40 (1.16–4.95)

*OR = odds ratio; CI = confidence interval.

TABLE 5

Adjusted odds ratios of potential risk factors associated with spotted fever group (SFGR) antibody status from district-stratified multivariate logistic regression models

District	Variable	OR (95% CI)
Belen	Occupation (referent low risk)	5.99 (1.24–28.96)
Bellavista Nanay	Age (referent < 18 years)	1.87 (1.06–3.00)
	Trip (referent no)	2.12 (1.06–3.87)
San Juan	Age (referent < 18 years)	1.66 (1.04–2.64)
	Housing material (referent concrete/brick)	2.12 (1.06–3.87)
Central	Rodents (referent no)	2.22 (1.13–4.38)

*OR = odds ratio; CI = confidence interval.

Serosurvey among peridomestic pets. To further characterize rickettsial transmission cycles in Iquitos, a serosurvey was conducted among household pets in the same neighborhoods where the human surveys were conducted. Serum samples, blood spots, and ectoparasites were collected from both dogs and cats. Overall, 42 of 71 (59.2%) dogs tested were positive for SFGR antibodies, and 2 (2.8%) were positive for TGR antibodies (Table 6). The highest prevalence was found in the Central and San Juan neighborhoods, although the differences among the four districts were not statistically significant (Table 6; $P = 0.1211$). For the cats, only 1 of the 13 (7.7%) was positive for SFGR antibodies; none were positive for TGR antibodies. To determine the rate of active infection among the dogs and cats, whole blood specimens were screened by PCR using primers specific for the 17kd gene. In total, 98 animals were tested (79 dogs and 19 cats). Although none of the cats were found to be positive for rickettsial DNA, active infection was identified in one (1.3%) of the dogs by PCR. A partial sequence of the 17kd gene from the *Rickettsia* spp. detected in the dog was amplified and sequenced. The amplified sequence was indistinguishable from the published sequences of the SFGR members *R. rickettsii*, *R. parkeri*, and *R. peacockii*, but owing to the short sequence (248 bp) and the high conservation of the 17kd gene, the precise species could not be identified.

Detection of *Rickettsia* species in ectoparasites collected from cats and dogs. In addition to the serum samples, ectoparasites were collected from the cats and dogs. In total, 170 fleas (all *Ctenocephalides felis*), 43 lice (all *Menacanthus* species), and 2 ticks (both *Rhipicephalus sanguineus*) were collected. No statistically significant relationship was detected between the presence of ectoparasites and SFGR or TGR antibody positivity, or between the number of ectoparasites and SFGR or TGR antibody positivity (data not shown).

To incriminate potential vectors in the transmission of rickettsial pathogens in Iquitos, we screened the arthropods collected during the canine and feline serosurvey for the presence of rickettsial DNA. Ectoparasites were pooled by species, sex, and according to the animal from which they were collected, triturated, and analyzed by PCR using primers specific for 17kd gene. No rickettsial DNA was detected in the lice or ticks. However, 71 of 74 pools (range 1–11, median 2 fleas per pool) of *Ct. felis* fleas (95.9%) were found to be positive for SFGR DNA. For the dogs, there was no correlation between carrying SFGR-positive fleas and SFGR seropositivity ($P > 0.05$).

To begin to characterize the rickettsial species detected in the fleas, the 17kd, *gltA*, and *OmpA* genes from 11 *Ct. felis* pools were amplified and sequenced. Sequences from the 11 pools were identical or nearly identical to each other in these genes, varying by no more than one nucleotide. Nucleotide identity analysis of the partial 17kd (376 bp; GenBank accession no. GU117904), *gltA* (324 bp; GU117906), and *OmpA* (558 bp; GU117905) genes support the inclusion of these flea-borne *Rickettsia* within the SFGR. Both 17kd and gltA amplicons display greater than 94% nucleotide identity with published sequences of SFGR members, but less than 91.1% identity with TGR, including *R. typhi* and *R. prowazekii*. Despite their clear inclusion within the SFGR, these amplicons shared less than 97% nucleotide identity with any of the currently well-characterized SFGR sp., including *R. felis*. Instead, the partial 17kd and *gltA* gene sequences showed the highest nucleotide identity with uncharacterized rickettsial species previously detected in fleas in South Carolina,[30] Cairo, Egypt,[31] and along the Thailand-Myanmar border.[32,33] The 17kd nucleotide sequences from Iquitos varied by less than 2% compared with the previously identified *Rickettsia* sp., whereas the aligned partial *gltA* sequences were identical.

DISCUSSION

On the basis of these data, it is clear that both SFGR and TGR are endemic to Iquitos and that transmission is widespread. Our finding that greater than 10% of Iquitos residents had prior infection with TGR is consistent with previous reports from the Peruvian sierra, where 20% of participants were found to have antibodies against TGR,[34] but is significantly higher than the 1.1% reported in southwestern Brazil.[35] Levels of SFGR antibodies comparable to those observed in Iquitos have been demonstrated elsewhere, most notably in rural sites in Colombia[19] (40.3%) and northwestern Peru (> 50%).[20] Typically, however, reported SFGR antibody prevalence levels are significantly lower in South America than the 43.6% found in Iquitos. For example, in several departments of Brazil[8,35–37] and in Jujuy, Argentina,[38] SFGR antibody prevalence was reported as lower than 10%. The reasons for such widely varying exposure to *Rickettsia* are unclear. Although methodological differences may explain some of the disparity, the climate and ecology of Iquitos may be conducive to reservoirs and vectors of rickettsial transmission cycles. Unfortunately, the broadly cross-reactive antigens used in this study preclude definitive identification of the *Rickettsia* species in circulation in the region.

Several risk factors were apparent for both SFGR and TGR antibody positivity in multivariate logistic regression models. Age was significantly associated with antibody positivity for both groups, suggesting that both groups of pathogens have been endemic in Iquitos for some time and that the SFGR and TGR antibodies are long-lived. For SFGR, pet bird (macaws and parakeets) ownership was associated with reduced antibody levels. The connection between these two variables is unclear. Although there may be some direct protective effect (e.g., zooprophalaxis), bird ownership likely serves as a surrogate

TABLE 6
Seroprevalence and distribution of spotted fever group (SFGR) and typhus group (TGR) antibodies among dogs and cats, by district in Iquitos

Neighborhood	Dogs		Cats	
	SFGR Pos./total (%)	TGR Pos./total (%)	SFGR Pos./total (%)	TGR Pos./total (%)
Central (Av. Salaverry)	11/18 (61.1)	0/18 (0.0)	–	–
Belen (Blasco Nunez)	5/13 (38.5)	0/13 (0.0)	0/2 (0.0)	0/2 (0.0)
Bellavista Nanay (Av. La Marina)	11/21 (52.4)	1/20 (5.0)	0/9 (0.0)	0/9 (0.0)
San Juan (San Luis)	15/19 (78.9)	1/18 (5.6)	1/1 (100)	0/2 (0.0)
Total	42/71 (59.2)	2/71 (2.8)	1/13 (7.7)	0/13 (0.0)

for increased socio-economic status or some other factor. For TGR, the existence of rodents in and around the home was associated with higher antibody prevalence. In this case a direct connection is easier to explain. Rats and other rodents may carry lice or fleas that are infected with TGR and may subsequently infect the residents of the house. On the basis of these results, domestic rodents should be surveyed for TGR antibodies and for *R. typhi*-infected ectoparasites to confirm their roles as reservoir hosts.

Another variable that remained significantly associated with antibody positivity for both SFGR and TGR in the multivariate models was the district of residence of the participants, although with opposite trends for the two *Rickettsia* groups. Within Belen and Bellavista Nanay higher levels of SFGR antibody positivity and lower levels of TGR antibody positivity were observed when compared with San Juan and the central neighborhoods. As would be expected, these results point to distinct transmission cycles and risk factors for SFGR and TGR in Iquitos. In the VEEV study, antibody prevalence among the districts followed a pattern distinct from both SFGR and TGR, as Bellavista Nanay and San Juan had a significantly higher prevalence of VEEV neutralizing antibodies than in Belen or the central ("control") neighborhoods.[24] In that study, we had observed that higher VEEV antibody prevalence rates correlated with surrogates for mosquito exposure in the district of residence, including mosquito net use and mosquito abundance in household surveys and Centers for Disease Control and Prevention (CDC) light trap collections. As the initial study was established for a mosquito-borne arbovirus, we did not directly ask about participant exposure to ticks, fleas, or lice as would be more applicable for the current analysis.

A clear limitation of our study was the skewed distribution of potential risk factors within our individual study neighborhoods. For example, the Belen and Bellavista Nanay neighborhoods had predominantly wood housing, and nearly all houses reported rodents, indicating that for these variables exposure was nearly identical in these neighborhoods. These same variables were significantly associated with antibody positivity in models for San Juan and the central neighborhoods where there was sufficient variation in exposure to measure a difference. These observations reveal the potential importance of rodents and housing materials for exposure to SFGR infections and show that future study designs will need to ensure a more balanced design to evaluate these risks.

In this study, we observed a high prevalence of SFGR antibodies (59.2%) in dogs, similar to that found in dogs in the state of Sao Paulo, Brazil (69.6%).[10] Furthermore, we found that a high percentage of these dogs carried fleas that were positive for SFGR DNA, and an active infection with an SFGR species. Thus, it is surprising that we did not observe a relationship between dog ownership and SFGR antibody positivity. Current dog ownership may not be the best indicator for human exposure to infected arthropods for a number of reasons. Dogs may be better groomed in homes with higher socioeconomic status, and thus may be less likely to carry ectoparasites. In addition, we did not inquire as to past dog ownership, which may have been another source of bias. Furthermore, considering the ubiquity of street dogs in Iquitos and human and canine movement patterns, dog ownership may not be a suitable surrogate for regular exposure to dogs. Regardless, it is apparent that dogs can carry *Rickettsia* sp. that are pathogenic to humans,[18,39] and they can pass them on to their owners,[40,41] suggesting that further exploration of the role of dogs in rickettsial transmission cycles in Iquitos is justified.

In this study only a very small sample size of lice and ticks was obtained and analyzed, with all samples negative for *Rickettsia* species by PCR. More extensive surveying of such arthropods will be necessary to understand their role in SFGR and TGR epidemiology in Iquitos. For *Ct. felis* fleas, nearly 100% of the pools collected from cats and dogs were found to harbor DNA from an SFGR or SFGR-like organism. Similar infection rates have been seen elsewhere in fleas with *R. felis*[15,42] and *R. felis*-like organisms,[30,31] indicative of efficient vertical or horizontal transfer. To date, *R. felis* is the only well-characterized SFGR to be regularly isolated from fleas. The partial 17kd and *gltA* sequences from these Iquitos amplicons were identical or nearly identical to those of other uncharacterized *Rickettsia* spp. detected in fleas in other parts of the world, namely Egypt,[31] Thailand/Myanmar,[32] and the United States.[30] The relevance of these *Rickettsia* to the SFGR antibody prevalence observed in our study and to human health in general is unknown. Considering the prevalence in fleas, canine and human exposure to this species may be quite common. Further characterization of this species is warranted, as are further studies into potential vertebrate pathogenicity.

Overall, these results suggest a high level of transmission of both SFGR and TGR in Iquitos. Further studies are necessary to better identify the precise *Rickettsia* species in circulation and to quantify the associated disease burden. In studies of acute undifferentiated febrile illness conducted by our group in Iquitos since 2000, greater than 50% of all febrile cases remain undiagnosed (Kochel TJ and Forshey BM, unpublished data). Based on the data reported here, rickettsial infections are likely significant and underappreciated contributors to many of these febrile episodes, and the overall disease burden in Iquitos. Infection from a number of rickettsial species from both the SFGR and TGR, such as *R. rickettsii* and *R. prowazekii*, are associated with severe disease. If left untreated, these infections can at times lead to death. Based on the data presented here, greater awareness of potential rickettsial etiology on the part of clinicians in the region is warranted, as appropriate antibiotic therapy can stave off the more severe sequelae associated with rickettsial infection.

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