Am. J. Trop. Med. Hyg., 100(6), 2019, pp. 1391–1400
doi:10.4269/ajtmh.18-0029

Seroprevalence and Risk Factors for *Rickettsia* and *Leptospira* Infection in Four Ecologically Distinct Regions of Peru

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Abstract. *Rickettsia* and *Leptospira* spp. are under-recognized causes of acute febrile disease worldwide. *Rickettsia* species are often placed into the spotted fever group rickettsiae (SFGR) and typhus group rickettsiae (TGR). We explored the antibody prevalence among humans for these two groups of rickettsiae in four regions of Peru (Lima, Cusco, Puerto Maldonado, and Tumbes) and for *Leptospira* spp. in Puerto Maldonado and Tumbes. We also assessed risk factors for seropositivity and collected serum samples and ectoparasites from peri-domestic animals from households in sites with high human seroprevalence. In total, we tested 2,165 human sera for antibodies (IgG) against SFGR and TGR by ELISA and for antibodies against *Leptospira* by a microscopic agglutination test. Overall, human antibody prevalence across the four sites was 10.6% for SFGR (ranging from 6.2% to 14.0%, highest in Tumbes) and 3.3% for TGR (ranging from 2.6% to 6.4%, highest in Puerto Maldonado). Factors associated with seroreactivity against SFGR were male gender, older age, contact with backyard birds, and working in agriculture or with livestock. However, exposure to any kind of animal within the household decreased the odds ratio by half. Age was the only variable associated with higher TGR seroprevalence. The prevalence of *Leptospira* was 11.3% in Puerto Maldonado and 5.8% in Tumbes, with a borderline association with keeping animals in the household. We tested animal sera for *Leptospira* and conducted polymerase chain reaction (PCR) to detect *Rickettsia* species among ectoparasites collected from domestic animals in 63 households of seropositive participants and controls. We did not find any association between animal infection and human serostatus.

INTRODUCTION

Rickettsial diseases are caused by infection with one of several obligate intracellular bacteria from the genus, *Rickettsia*. The *Rickettsia* species are arranged into two disease-associated groups, the spotted fever group *Rickettsia* (SFGR) and typhus fever group,[1] one non–disease-associated group that includes the ancestral group of rickettsiae which includes *Rickettsia belli* and *Rickettsia canadensis*,[2] and last, a transitional group which includes *Rickettsia felis* and *R. felis*–like organisms (RFLOs).[3,4] The geographical distribution of the agents is tightly related to the distribution of its arthropod hosts, which can be ticks, mites, fleas, and lice.[3,5]

Rickettsioses usually develop between 1 and 2 weeks after exposures to the agent. The most common symptoms are fever, malaise, headache, rash, nausea, and vomiting. In some cases, there may be an eschar at the site of the vector bite.[1] However, their clinical features are related to their geographic location.[2]

In South America, several species of the SFGR have been identified in recent decades besides *Rickettsia rickettsii*, such as *Rickettsia parkeri*, *R. felis*, *Rickettsia massiliae*,[2,6–8] and a new *Rickettsia* agent that has been identified in northern Peru and proposed as *Candidatus* Rickettsia andeanae.[9,10] The typhus group rickettsiae (TGR) including *Rickettsia prowazekii* and *Rickettsia typhi* has also been identified in South America, the former being responsible for epidemic typhus in Cusco, Peru,[8] and the latter responsible for murine typhus in the Amazon Basin.[4,11,12] Likewise, the presence of antibodies to SFGR has been confirmed in different human populations in Peru[13–16] and in pets, establishing its endemicity.[17] Several of these studies have shown the presence of rickettsial infections in the region, ranging from ~1% to more than 40% seroprevalence. However, there are still gaps in the knowledge of rickettsial diseases in the Americas, such as evaluating the role of amplifier hosts and their specific influence in the ecology of *Rickettsia* species or targeted studies oriented to certain species, such as *R. typhi*.[6]

Leptospira spp. are a ubiquitous group of spirochetes, with a worldwide distribution and a wide variety of vertebrate hosts. These agents are taxonomically organized by genetic determinants[18,19]; however, classically they are organized in serogroups and serovars according to antigenic determinants. The genus *Leptospira* is harbored in a wide variety of mammals, including domestic species and wildlife.[19,20] Likewise, different species may maintain several serovars depending on their geographic location.[21]

The zoonosis caused by *Leptospira* spp., leptospirosis, can manifest with a multitude of flu-like symptoms including fever, headache, and malaise, with more severe disease involving the organs, particularly the liver and kidney, as well as potential pulmonary involvement and hemorrhage.[19,22] Leptospirosis used to be considered an occupational disease, especially among military populations, who are exposed through contact with contaminated water in their working environments.[20,21] However, with the increase in the use of personal protective equipment, the burden of disease has shifted to the general population, especially in tropical areas where seroprevalence can sometimes be up to 30%.[23–25] Recent decades have not only seen a rise in cases in industrialized countries, mainly tourists returning from endemic areas,[25] but also from local transmission in some urban centers.[23,24,26]

Leptospirosis is also endemic in Peru.[27,28] Studies have shown that cities in the Amazon Basin have relatively high seroprevalence and periodic outbreaks of symptomatic

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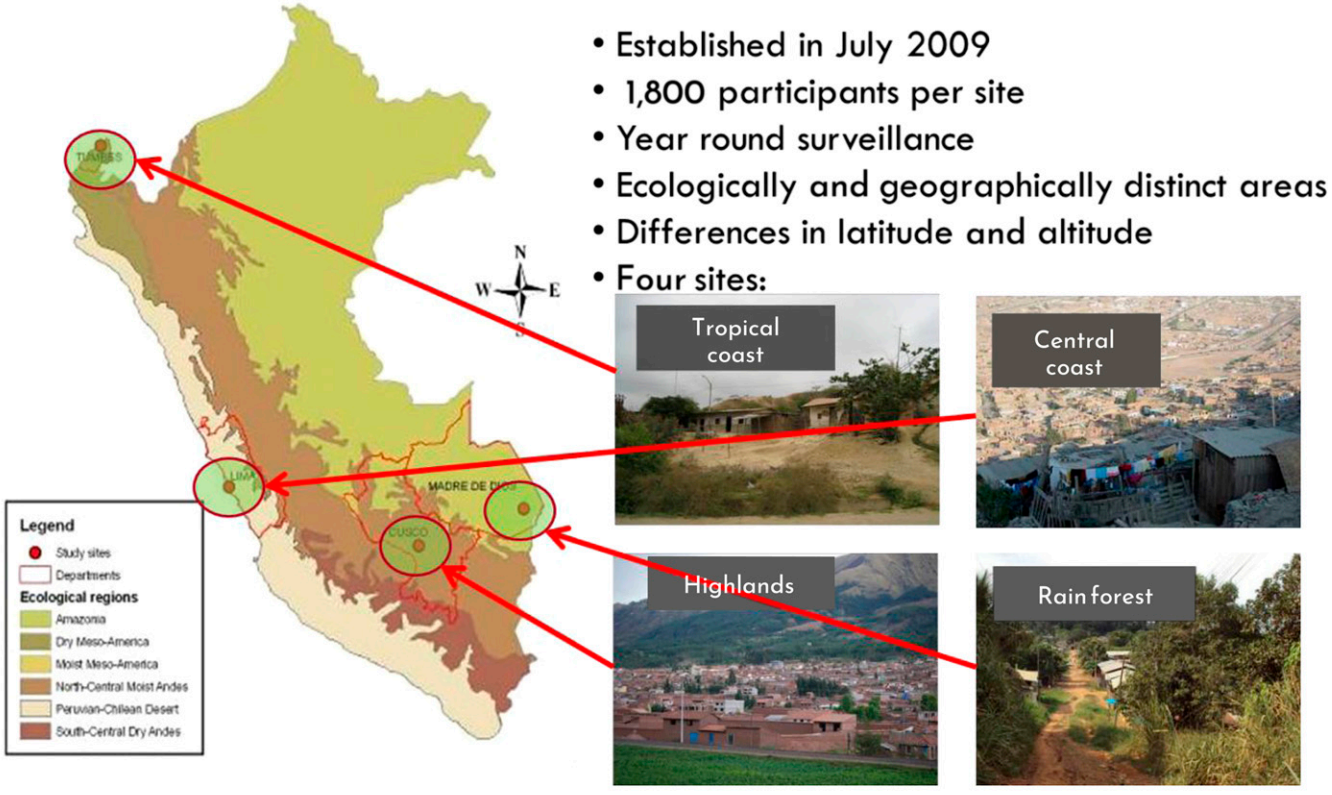
cases.[29] Likewise, the disease is also found in other areas of Peru,[9] with more than a third of febrile cases in endemic areas potentially due to *Leptospira* spp.[30]

Rickettsia and *Leptospira* infections are relatively common, but often under-recognized causes of acute febrile disease in tropical areas.[14,31] Despite the growing body of evidence regarding the impact of rickettisae and *Leptospira* among human populations, there is still scarce information about the burden of the disease at the household and community levels, and little is known regarding the changing epidemiological patterns for *Leptospira*.[20,31] Previously well-described risk factors of exposure may not be clearly defined in current changing environments[26] and are needed to target effective prevention strategies.[22,27,32] Therefore, we explored the prevalence of exposure and risk factors associated with human rickettsial infections in four ecologically distinct regions of Peru (Lima, Cusco, Puerto Maldonado, and Tumbes) and for *Leptospira* in the wet regions (Puerto Maldonado and Tumbes).

METHODS

Study sites. The four study sites where the human samples were collected in the first tier of this study have very distinct climatic and ecological characteristics. Therefore, Tumbes, located in the northern coast of Peru, is semiarid and has warm weather with an average temperature of 25°C. The rainy season extends from December through April (average precipitation: 250 mm) and is located at 36 m above sea level (masl).[33,34] Puerto Maldonado also has a similar rainy season, but extending from October, it is located in the tropical rainforest. The average temperature is 30°C, precipitation ranges from 1,500 to 2,860 mm, and the altitude is 139 masl.[34,35] The Cusco site was located in the Andean forest—moist Andes. It has an average temperature of 13°C, average rainfall of 800 mm, and at 3,244 masl.[34] Finally, Lima, the capital city, is located in the coastal desert with an average temperature of 19°C, rainfall of 15 mm, and at 141 masl.[34]

Study design. This study was a cross-sectional design nested within an ongoing influenza longitudinal cohort study, conducted by researchers from the Naval Medical Research Unit No. 6 (NAMRU-6), Lima, Peru. The influenza cohort study enrolled a total of 8,000 individuals of all ages, 2,000 individuals in each of four sites. The sites were located in San Jacinto, Tumbes; Puerto Maldonado, Madre de Dios; Pampas de San Juan de Lurigancho, Lima; and San Jeronimo, Cusco[36] (Figure 1). All enrolled participants were healthy and asked to provide a blood sample after giving their consent to evaluate influenza and other diseases' seroprevalence. Paired serum samples were obtained from participants in July 2011 and



Tropical coast: Tumbes; central coast: Lima; highlands: Cusco; rainforest: Puerto Maldonado

FIGURE 1. Location of sites and brief summary of influenza cohort study where the present study was nested. This figure appears in color at www.ajtmh.org.

January 2012, separated by 6 months. Participants were asked if their samples could be stored for future use, specifically for testing for other relevant infectious diseases.

The activities for this cross-sectional study were arranged in two components: a human serosurvey analysis and an animal sample collection. These components were tiered, so the results of the human serosurvey would guide the animal data collection. In the site where we found the highest seroprevalence to the diseases under study, we returned to collect additional samples and data from animals in these areas. This component was designed using case and control households, according to the serological findings of the human component. This design was adopted to contrast potential risk factors between these households.

Data collection. *Human serosurvey.* Within the influenza cohort study, human blood specimens were collected following standard venipuncture techniques. Samples were taken at the home of the participants and kept in portable ice chests at 4°C until the end of the visits (within 4 hours) when they were taken to the laboratory for centrifugation and storage. Centrifugation was performed at 3,000 rpm, 4°C for 10 minutes and storage was in an ultralow (–70°C) freezer. Samples were shipped to NAMRU-6's central laboratory in Lima, where they were stored in ultralow freezers until testing.

We selected a random sample of sera specimens from the last serum collection (January 2012) from each site of the cohort, whose participants had agreed for future use of their samples. These samples were tested for antibodies (IgG) against TGR and SFGR. Only the samples from Tumbes and Madre de Dios were tested for *Leptospira*, as these are the wet areas of Peru where leptospirosis is known to be endemic, but there is very little documented transmission in the dry areas of Cusco and Lima.

We aimed to test at least 630 human sera samples from each of the sites to estimate the prevalence with a precision of 4%, 80% power, and 95% confidence. These samples were selected randomly from the stored sera of the influenza cohort study.

Testing was performed at the NAMRU-6 laboratory in Callao, where samples were stored. The results from serology were analyzed to guide the subsequent animal and ectoparasite collections. The population with more antibodies was considered for site selection for further animal and vector studies.

Household information. Household information was gathered through the influenza cohort study. These data included household facilities such as sewage, running water, waste collection practices, age, gender, and occupation of all members of the household. Data about the household members and characteristics of the housing facilities were updated yearly and were used for analysis.

Animal sample selection. Based on the highest human seroprevalence evaluation, one site was selected to work with each pathogen in domestic and peri-domestic animals of positive households along with a number of control households from seronegative participants: *Rickettsia*, *Leptospira*, or both. A positive household was defined as having at least one of the members' seropositive for either of the pathogens. Hence, we had households that were positive for *Leptospira* only, positive for *Rickettsia* only, or positive for both pathogens. Negative households were defined as having all members who tested seronegative for all agents. With this design, a household positive for *Leptospira* could act as a control for a household positive for *Rickettsia*, and vice versa; and households negative for both pathogens were controls for either. We aimed to assign a control household for each household that was considered positive for any of the agents under study. Hence, we selected a total of 65 households for each agent under study: 47 control households and 18 case households for *Leptospira*, and 32 control households and 33 case households for *Rickettsia* (Table 1).

Animal sera and ectoparasite collection. In August 2014, we invited members from selected households to allow the participation with their domestic or backyard animals in this component. Samples obtained consisted of 1) blood, 2) urine when available, 3) ectoparasites from backyard and domestic animals (i.e., dogs, cats, guinea pigs, backyard birds, and pigs), and 4) rodents captured in and around the household. To collect rodents, we set up and baited five live traps (one Tomahawk and four Sherman) inside the house and five (same ratio) in the outdoor areas of the premises for five consecutive nights, aiming for a total of 50 trap nights per house. To sample domestic animals, we worked to obtain samples from all individuals if the total population was 10 or less. If not, a random sample of four individuals from each species was chosen.

Two trained veterinarians and experienced field-workers organized in two teams to enroll the households and collect the specimens. Standard venipuncture procedures were used to obtain blood samples. Domestic animals were properly restrained with a muzzle and the help of the owner to maintain the animal as calm as possible and released after the procedure was completed. Blood samples were kept in portable ice chests at 4°C until the end of the visits (within 4 hours) when they were taken to the laboratory for centrifugation and storage. While restrained, domestic animals were also combed for ectoparasites.

Live traps were checked each morning and closed during the day until these were baited at sundown and activated. The teams collected trapped rodents and took them to the laboratory facilities. The animals were sedated and humanely euthanized for blood draw, to obtain morphological measurements and ectoparasites, and to preserve the carcasses for further identification performed by the Natural History Museum (NHM) of the Universidad Nacional Mayor de San Marcos. Carcasses were kept in formalin and transported to the NHM for identification confirmation.

Blood samples were centrifuged at 3,000 rpm, 4°C, for 10 minutes and sera were stored in an ultralow (–70°C) freezer. All collected fleas, mites, lice, and ticks were also stored at –70°C for later identification using entomological keys.[37,38] All samples were shipped to NAMRU-6's central laboratory in Lima in liquid nitrogen, where they were processed.

TABLE 1
Case and control households for animal sampling

	*Leptospira**		
Rickettsia	Case household	Control household	Total
Case household	10	23	33
Control household	8	24	32
Total	18	47	65

*Serovars tested for microscopic agglutination tests: australis, autumnalis, ballum, bataviae, bratislava, canicola, celledoni, copenhageni, cynopteri, djaseman, georgia, grippotyphosa, icterohaemorrhagiae, javanica, panama, tarassovi, wolffi, mankarso, pomona, harjdo, pyrogenes, varillal, and shermani.

Laboratory analysis. Human and animal samples were screened by microscopic agglutination test (MAT) for *Leptospira* using present guidelines from the WHO and the International Leptospirosis Society.[22] Samples were tested against 24 serovars for the MATs, listed in Table 1. Samples were considered positive if they reacted at a dilution of 1:100. Human sera were screened for IgG antibodies against TGR and SFGR using ELISA to distinguish between the two groups. The test hast been published elsewhere and validated in different contexts.[17,39–41]

Ectoparasites were identified by morphological keys,[37,38] and washed and pooled by individual animal hosts, species of ectoparasite, and life stage of ectoparasite. Pools consisted of only two to five ectoparasites. Individual ectoparasites were divided in half for DNA extraction and molecular analysis and to attempt culture in the future if required. Individual ectoparasite halves were disrupted mechanically using Kontes Pellet Pestle (Thermo Fisher Scientific, Waltham, MA) in 100 μL of PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Waltham, MA) as previously described.[17] The triturated halve samples were subsequently pooled as described earlier. Pooled ectoparasite DNA preparations were subsequently screened for the presence of *Rickettsia* species DNA by the quantitative real-time PCR (qPCR) assay, Rick17b, that targets the conserved *Rickettsia* 17-kDa antigen genus-specific gene.[39] Because of a previous *Rickettsia* study in which it was found that a high-prevalence *Rickettsia asembonensis* (Rasem) existed within the ectoparasites,[42] our downstream workflow consisted of testing the individual tick (or flea)-half DNA preparations from the Rick17b-positive pools with the species-specific qPCR for Rasem that targets a portion of the variable *ompB* gene.[42,43] *Rickettsia asembonensis*-negative samples were subsequently screened by two additional methods: 1) the RfelG qPCR assay that targets a sequence fragment of the *ompB* gene which is shared by multiple *Rickettsia* strains grouped into the *R. felis* and RFLO genetic group[6,42] and 2) a nested PCR that can differentiate between SFGR and TGR using the 17-kD gene.[6,7] Sera from domestic animals could not be tested for *Rickettsia* antibodies because of lack of resources. Therefore, we grouped the host animals as positive (at least one ectoparasite positive) or negative (no detection of the pathogen in the ectoparasites) based on the status of the ectoparasites for data analysis.

Data analysis. Antibody prevalence against *Rickettsia* and *Leptospira* species among human participants was estimated using binomial exact methods. We used χ^2 test with the Bonferroni correction to compare the seroprevalence of antibodies against *Rickettsia* and *Leptospira* infections among study sites. Disease risk modeling was performed with a multilevel logistic regression, accounting for correlation among participants within households and for clustering within the sites. The outcome variables of antibodies reactive against *Leptospira* or *Rickettsia* species were assessed for exposure variables such as location and occupation, controlling for age and gender. The variables included in the initial multivariate model were at least marginally significant ($P < 0.10$) in the bivariate analysis and those maintained in the final multivariate model were site, gender, age, occupation, and other significant variables ($P < 0.05$).

Among the study animals, we estimated the prevalence of pathogen-positive ectoparasites for each species that provided a sample with the binomial exact methods. We compared the mean number of animals, ectoparasites, and the proportion of pathogen-positive ectoparasites between case and control households using *t*-test or χ^2, respectively. All analysis was performed using Stata 14 (StataCorp, College Station, TX) using a statistical significance cutoff of $P < 0.05$.

RESULTS

Human seroprevalence to *Rickettsia* and *Leptospira* among study sites. We randomly collected 2,165 serum samples from participants of the ongoing influenza cohort study at each of the four study sites in Peru. Our goal was to obtain at least 600 samples from each study site, but several participants had not provided consent for future use testing of their samples and, therefore, we had different sample sizes in each site: 538 for Cusco, 583 in Lima, 353 in Puerto Maldonado, and 691 in Tumbes. A larger proportion of participants (58.2%) across all sites were female; participants were 28 years old on average and were mostly students (Table 2). Overall antibody prevalence across the four sites was 10.6% for SFGR and 3.3% for TGR (Table 3). The antibody prevalence to *Leptospira* was 11.3% in Puerto Maldonado and 5.8% in Tumbes (Table 3). A few participants (0.7%) had antibody evidence of previous infection with SFGR and TGR. Similarly, only 1.8% of participants were seropositive for SFGR and *Leptospira* and 0.3% had evidence of past infection with TGR and *Leptospira*.

TABLE 2
General demographic information by site

	Site	Lima		Tumbes		Cusco		Puerto Maldonado		All sites	
Population	*N* (%)	583	26.9	691	31.9	538	24.8	353	16.3	2,165	100.0
Gender	Female	359	61.6	365	52.8	333	61.9	204	57.8	1,261	58.2
	Male	224	38.4	326	47.2	205	38.1	149	42.2	904	41.8
Age (years)	Mean	28.2	NA	29.6	NA	27.2	NA	29.0	NA	28.5	NA
	SD	17.9	NA	20.6	NA	17.3	NA	19.1	NA	18.9	NA
Occupation	Unemployed*	28	4.8	36	5.2	30	5.6	18	5.1	112	5.2
	White collar†	117	20.1	40	5.8	121	22.5	72	20.4	350	16.2
	Blue collar‡	109	18.7	77	11.2	53	9.9	42	11.9	281	13.0
	Housekeeping	126	21.6	200	29.0	96	17.9	73	20.7	495	22.9
	Agriculture and farming	4	0.7	99	14.4	4	0.7	6	1.7	113	5.2
	Student	199	34.1	237	34.4	232	43.2	142	40.2	810	37.5
Have contact with animals		431	73.9	582	84.2	454	84.4	337	95.5	1804	83.3

NA = not available.
* Retired, younger than 3 years and currently unemployed.
† Health, education, and commerce.
‡ Cleaning, factory employees, and transport.

TABLE 3
Human seroprevalence of *Rickettsia* and *Leptospira* antibodies

		SFGR		TGR		*Leptospira*	
Site	*N*	Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI
Overall*	2,165	10.6	9.3; 11.9	3.2	2.5; 4.1	7.6	6.1; 9.4
Cusco	538	8.7	6.5; 11.4	3.1	1.8; 5.0	NA	NA
Lima	583	6.2	4.4; 8.4	2.6	1.4; 4.2	NA	NA
Puerto Maldonado	353	13.9	10.4; 17.9	6.2	3.9; 9.3	11.3	8.2; 15.1
Tumbes	691	14.0	11.5; 16.8	2.3	1.3; 3.7	5.8	4.2; 7.8

SFGR = spotted fever group rickettsiae; TGR = typhus group rickettsiae.
* Microscopic agglutination test for leptospirosis was only performed for two sites (*N* = 1,044).

Leptospira. We evaluated separately each of the following variables: site, gender, age, occupation, and contact with animals. In bivariate analysis, the prevalence of *Leptospira* infection in Puerto Maldonado was significantly higher than that in Tumbes ($P = 0.001$). Consequently, OR of infection was 2.1 (95% CI: 1.3; 3.4) for this site, in contrast to Tumbes as the reference. Overall seroprevalence in men was 9.3%, compared with women who had 6.3%. Nonetheless, the difference was not statistically significant ($P = 0.076$). Bivariate analysis did not show an association with a specific occupation ($P = 0.201$) at either site. Likewise, seropositivity was not associated to contact with dogs (0.996), guinea pigs (0.070), pigs (0.328), or any kind of animal without distinction (0.355). By contrast, contact with cats ($P = 0.023$) and animals such as donkeys, sheep, cows, and rabbits was statistically associated with seropositivity ($P = 0.015$).

We examined the presence of antibodies to *Leptospira* with the multivariate logistic regression. The positive MAT test in study subjects was associated only with the study site location (2.4; CI 2.2; 2.7), and the strength of association was similar to the bivariate analysis. Puerto Maldonado residents had a higher risk of having positive MAT results (2.3, 95% CI: 1.3; 4.3) than residents at Tumbes in this multivariate analysis (Table 4). The most common serovars from the human sera participants were *icterohemorrhagiae*, *bratislava*, and *mankarso*.

Rickettsia. Bivariate analysis for both groups of *Rickettsia* species showed a significant difference in the prevalence among study sites (Table 3). Gender was initially related to the presence of SFGR antibodies, with males doubling the prevalence of females (14.6% and 7.7%, $P < 0.001$) in the bivariate analysis. This was not the case for TGR ($P = 0.297$). Farming and agriculture activities were clearly associated with SFGR antibody prevalence, with more than 40% of participants in this occupation with antibodies ($P < 0.001$). The presence of antibodies for TGR also showed a difference in prevalence among occupations ($P = 0.007$). Contact with animals was not significant for the presence of antibodies to SFGR and TGR, except for having contact with birds and SFGR seropositive outcome. Likewise, the presence of antibodies for both

TABLE 4
Multilevel logistic model for spotted fever group rickettsiae (SFGR), typhus group rickettsiae (TGR), and *Leptospira* exposure risk factors

	SFGR		TGR		*Leptospira*	
	OR	95% CI	OR	95% CI	OR	95% CI
Region						
Cusco	Ref		Ref		–	–
Lima	0.7^{+++}	0.6; 0.8	0.7^{+++}	0.7; 0.8	–	–
Puerto Maldonado	1.5^{+++}	1.4; 1.6	2.0^{+++}	1.9; 2.2	2.4^{+++}	2.2; 2.7
Tumbes	1.0	0.7; 1.3	0.6^{+++}	0.5; 0.7	Ref	
Gender						
Female	Ref		Ref		Ref	
Male	2.0*	1.0; 4.0	0.7*	0.4; 1.0	1.6	0.7; 3.6
Age						
Newborn to 9 years old	Ref		Ref		Ref	
10–19 years old	3.5^{+++}	2.2; 5.5	2.5	0.6; 9.9	2.1^{+}	1.1; 4.2
20–31 years old	4.0^{++}	1.3; 11.9	3.5*	0.9; 14.1	2.5*	1.0; 6.3
32–48 years old	8.9^{++}	1.5; 10.3	5.1^{+}	1.2; 22.5	2.0	0.6; 7.1
48–85 years old	11.4^{+++}	3.9; 33.4	11.3^{++}	2.5; 50.3	1.7	0.7; 4.2
Occupation						
Stay at home†	Ref		Ref		Ref	
White collar	1.2	0.5; 2.9	0.4	0.05; 2.8	1.9	0.3; 11.1
Blue collar	1.5	0.7; 2.9	0.8	0.1; 4.8	2.5	0.2; 35.3
Housewife	1.2	0.4; 3.6	0.5	0.1; 3.2	1.8	0.1; 22.2
Agriculture/farming	3.1^{+}	1.1; 8.9	0.8	0.2; 3.5	2.8	0.2; 44.2
Student	0.7	0.3; 1.5	0.5	0.1; 3.1	1.6	0.3; 7.2
Have birds						
No	Ref		–	–	–	–
Yes	2.1^{+}	1.0; 4.2	–	–	–	
Have any animal						
No	Ref		–	–	–	–
Yes	0.5^{+++}	0.3; 0.7	–	–	–	

$^{+++}P < 0.001$; $^{++}P < 0.01$; $^{+}P < 0.05$ and $^{*}P < 0.10$.
† Unemployed, retired, and infants.

rickettsia groups showed an increasing trend with age ($P < 0.001$, for both outcomes).

After introducing a multilevel logistic regression, some variables were still associated to the presence of SFGR antibodies (Table 4). These factors were: male gender, age, contact with backyard birds, working in agriculture or with livestock. However, exposure to any kind of animal within the household decreased the odds ratio of antibodies to SFGR by half (Table 4). By contrast, occupation was not associated with antibody positivity to TGR, but increasing age still was significant for the outcome (Table 4).

Animal testing *Rickettsia* and *Leptospira* among study sites. In 65 households we assessed 137 domestic animals (59 dogs, 37 chickens, 25 ducks, and 16 cats) for the presence of ectoparasites. We also captured 30 rodents (*Rattus rattus* and *Mus musculus*) during a total of 2,525 trap nights resulting in 130 sera samples and 432 ectoparasites (46% fleas, 36% ticks, 18% lice and 1% rodent mites) collected. The most common species in the ectoparasites were the cat flea, *Ctenocephalides felis* (34.0%), and the brown dog tick, *Rhipicephalus sanguineus* (32.5%) (Table 5). We examined ectoparasites from 68 individual hosts: 53 dogs, 11 chickens, two rodents, and two cats. The most parasitized hosts were dogs. Among all hosts there were approximately nine ectoparasites on average, ranging from 1 to 23 per animal.

Leptospira. The overall antibody prevalence to *Leptospira* among 130 sera samples from potential hosts was 60% (95% CI: 51.0; 68.5). We found the highest seroprevalence among dogs and birds (Table 6). When we examined the case or control status of the household with the findings from the animals, we determined that the number of domestic animals was higher among the case households (2.7 in contrast to 1.2, $P = 0.033$). However, we could not find any difference in the animal host seroprevalence and the household case or control status.

Among the domestic and wild animals, the most common serovars identified by MAT were *varillal* (reactive to 42 samples), *pyrogenes* (20), and *cynopteri* (20). We also assessed several samples with more than one infection, as was found before in the human samples.

Rickettsia. The overall prevalence of SFGR in the ectoparasites was 49.6% (95% CI: 44.8; 54.5). Fleas had the highest prevalence, followed by lice and mites and finally ticks (Table 7). Dogs also had the highest prevalence of infection (considering the host infected if at least one ectoparasite was positive to SFGR): 54.9%; 95% CI: 49.7; 60.0, in contrast to chickens that had 6.7% (95% CI: 1.4; 18.3). The species detected in almost all positive ectoparasite samples was Rasem. We also detected four strains characterized as *R. felis* or RFLOs that were Rick17b positive and RfelG positive but negative for the Rasem qPCR assay Rasem.

As the final step, we assessed whether the status of case or control household was related to the presence of positive ectoparasites or animal hosts to identify the link of animal or ectoparasite status and presence of *Rickettsia* and *Leptospira* to the status of human participants. Surprisingly, we found no association between case and control households with ectoparasite positivity.

DISCUSSION

Information regarding *Leptospira* and *Rickettsia* burden is limited in Peru. A few studies have assessed the seroprevalence of both pathogens in the past decades. However, there has been relatively more research conducted on *Leptospira*. Therefore, Céspedes et al.[27,28] had already reported a higher incidence of *Leptospira* infections from patients in Madre de Dios and a lower incidence in Tumbes. The prevalence of *Leptospira* antibodies has shown to vary significantly throughout Peru. Contrasting the findings of these studies with the seroprevalence we found in Puerto Maldonado and Tumbes places these results between what has been reported in Lima (0.7%) and the higher levels reported for Belen in Iquitos, Loreto (28.0%).[20] Our findings from Puerto Maldonado (11.3%) were more similar to those from areas near Iquitos (16.5%), but still significantly lower. The peri-urban areas in Iquitos, where the study was conducted, were located above the flood plain in contrast to Belen, which floods seasonally. This may explain the findings related to an analogous environment and water regime between the peri-urban sites and Puerto Maldonado and lower findings in sites such as Tumbes and Lima. In Tumbes, an old study performed on slaughterhouse workers reported 7.7% (95% CI: 0.9; 25.1) prevalence in a small sample of this population.[44] The most common serovars reported among slaughterhouse workers and animals in that study were not similar to our findings. By contrast, in the study conducted after an outbreak of febrile illness in Piura, northern coast of Peru, researchers found 26% (95% CI: 19.8; 33.8) human seroprevalence by MAT, significantly higher than that in Tumbes and in Puerto Maldonado. Nonetheless, the *bratislava* servovar was the most common in this site and one of the most common in Puerto Maldonado.[30] This serovar was also related to a human seroprevalence studies in the Manu Province in Madre de Dios, reported in 2003, and in 2001 in Coronel Portillo in Ucayali.[28,29]

The most common serovar among human samples from our study, *icteriohemorrhagiae*, was also the most frequent detected in a study among rice farmers in Alto Mayo, San Martín,

TABLE 5
Ectoparasites and hosts sampled for *Rickettsia*

Ectoparasite species	Host				Total (%)
	Dog (%)	Chicken (%)	Rodent (%)	Cat (%)	
Ctenophalides canis	50 (12)	0 (0)	0 (0)	1 (0)	51 (12)
Ctenocephalides felis	143 (33)	0 (0)	0 (0)	2 (0)	145 (34)
Lice	32 (7)	45 (11)	0 (0)	0 (0)	77 (18)
Mites	0 (0)	0 (0)	4 (1)	0 (0)	4 (1)
Ixodes sp.	11 (3)	0 (0)	0 (0)	0 (0)	11 (3)
Rhipicephalus sanguineus	139 (33)	0 (0)	0 (0)	0 (0)	139 (33)
Total	375 (88)	45 (11)	4 (1)	3 (1)	427

TABLE 6
Seroprevalence of *Leptospira* among different hosts

Host	*N*	Prevalence	95% CI
Rodent	30	6.7	0.8; 22.1
Bird	37	81.1	64.8; 92.0
Dog	53	83.0	70.2; 91.9
Cat	10	20.0	2.5; 55.6
Overall	130	60.0	51; 58.5

in 2010, although the seroprevalence among this population was higher.[32] In terms of risk factors, Johnson et al.[20] reported risk factors such as not wearing shoes and living in a flooding area related to *Leptospira* infection risk. Similarly, the lack of protective footwear and handling of rodents were also considered risk factors among rice farmers in Alto Mayo, San Martín.[32] Our study identified an increasing trend of seropositive participants with older age like Johnson et al.[20] Nonetheless, in the high group identified by Alarcón-Villaverde et al.,[32] age was not a significant risk factor. These differences are likely due to the populations represented in each of these studies. The population of rice farmers is by definition a high-risk group, whereas our study and the one carried out in Iquitos were conducted within the general population; therefore, the latter results likely portray the expected trend in areas with circulation of the bacteria but where the occupational exposure is not the main risk factor.

Contact with animals in the occupational setting and within the household is a known risk factor for *Leptospira* infection.[16,19] The animal hosts examined in the study had a high seroprevalence of *Leptospira*, even among birds. However, the latter group has not been linked to the transmission of infection to humans. The seroprevalence in dogs appears to be higher than that previously reported in other studies, such as Céspedes et al.[28,29] in Ucayali (48.4%; 95% CI: 43.2; 53.6) and in Manu (66.7%; 95% CI: 46.0; 83.5). In our study, the most common serovars identified among the hosts in the households were not the same as those harbored by human participants. A similar situation was reported by Cespedes in Coronel Portillo in Ucayali, but Liceras in Tumbes did find the same serovar among slaughterhouse workers and livestock under study, the same as Céspedes et al. in Manu, Madre de Dios.[28,29,44] The most common serovar identified in our animal studies was *varillal*, a serovar that has been associated with leptospirosis in the area of Iquitos, Loreto.[45] Serovar *pyrogenes* was reported among dogs by Céspedes et al. in Ucayali. Although the variety of results between human and animal samples may be due to different reasons, it is likely that in our study the difference in the periods of sample collection is the main motive for variation.

Unfortunately, Johnson et al.[20] did not report on rodent collections from the peri-urban sites of Iquitos. Nonetheless, the rodent point seroprevalence from Belen (10.6%), a flooding area in the city of Iquitos, was higher than the 6.7% we found in Puerto Maldonado, but still comparable with our findings (95% CI: 7.0; 15.3 and 0.8; 22.1, respectively). These outcomes underscore the risk for this infection in Puerto Maldonado, where prevalence seems to be more similar to rural areas around the city of Iquitos, probably because of similar living conditions and exposures. In addition, the evidence suggests that the risk of exposure in this population does not seem to be related to occupation, but likely takes place among adolescents and young adults. These exposures may be related to activities within these groups, such as bathing or playing barefoot. We should also consider contact with rodents, as exposed in other studies[32] because we are uncertain of the role rodents may have in maintaining the infection and potential exposure.

TABLE 7
Prevalence of *Rickettsia* species among different ectoparasites

Ectoparasites	*N*	Prevalence*	95% CI
Fleas	196	95.4	91.5; 97.9
Ticks	150	10.7	6.2; 16.7
Lice or mites	81	11.1	5.2; 20.0
Overall	427	49.6	44.8; 54.5

*This includes 212 Rick17b-positive samples (*Rickettsia asembonensis* + *Rickettsia felis*–like organisms).

Rickettsia surveillance studies are narrowly conducted in Peru and the region. Two studies conducted previously in Argentina and Colombia show the range of seroprevalence to SFGR found in South America. Ripoll et al.[46] reported a 4.8% (95% CI: 1.6; 10.8) seroprevalence to SFGR in Jujuy, Argentina, and Hidalgo et al.[47] reported 40.2% (95% CI: 35.1; 45.3) seroprevalence to SFGR in rural Colombia. Our findings from Cusco (8.7%) and Lima (6.2%) are somewhat higher to those from Jujuy, Argentina, but still comparable. However, the seroprevalence reported from Colombia and in Iquitos (43.6%), in the Amazon Basin, as reported in 2010 by Forshey et al.[14] is significantly higher than what we found in our four sites. The latter is closer to the range of the study conducted recently in Madagascar, where seroprevalence was more than 30% for both SFGR and TGR.[48] It is, however, still higher than what was reported previously by Schoeler et al.[49] in 2005, where they showed a prevalence to SFGR among fever patients from four locations in Peru to be 18%. Similarly, Blair et al.[9] reported human antibody levels to SFGR after an outbreak of febrile illness (11.2%, 95% CI: 6.5; 17.3) comparable to the findings from Tumbes and Puerto Maldonado. Following the seroprevalence study conducted herein, in 2016, Kocher et al.[17] reported an incidence of almost 2% of SFGR infections among febrile subjects (38/2,054) in Iquitos. Similar to Puerto Maldonado (13.9%) and Tumbes (14.0%) reported herein, 14.9% (305/2,054) of the febrile subjects from Iquitos were seroreactive with titers $\geq$ 400 for antibodies against SFGR (ALR, unpublished data). Our results confirmed the previous studies in Peru and South America of the presence of SFGR infections in multiple ecologically distinct locations throughout the region in such diverse areas as rural, suburban, and urban sites, in the semi-arid tropical coast, the central desert coast, the highlands, and the rainforest.

In studying the risk factors associated with SFGR infections, it was striking that the antibody seroprevalence to SFGR was significantly higher across all age groups, suggesting widespread transmission. Other findings such as the apparent higher risk among males and those employed in agriculture have been reported before.[11,47,49] We also found an association with having backyard birds, in contrast to what was found previously by Forshey et al.[14] in Iquitos, Peru. Interestingly, we found a decrease by half of the OR of past infection with the presence of any kind of other animals within the household. We speculate that this may indicate that arthropod vectors on birds preferred nonhuman hosts when they were present, thus diminishing exposure to humans.

Forshey et al. had proposed zooprophylaxis before to explain their findings in Iquitos, which showed diminished risk among bird owners. In their study, they also purport that this variable could as well be representing higher socioeconomic status (ornamental birds), which would present a contrasting scenario with Puerto Maldonado, where most animals tested were from backyard farming.

We also found a high prevalence of SFGR among fleas of tested dogs. Although we were unable to test serological status of the animals for SFGR antibodies, household animals were severely parasitized. As has been reported in previous studies,[14,17] reservoir or ectoparasite status did not seem to be related to the human outcomes, despite the abundance of ectoparasites. In the case of this study, this was likely due to the difference in periods of data collection. Nonetheless, it should be noted the proposition stated earlier regarding vector preference, which would be in line with the findings by Kocher et al.[17] who did not find a relationship regarding domestic animal status and disease incidence, did indicate a high burden of disease among domestic animals. Hence, the links between *Rickettia* species, host transmission, and disease burden in humans remain to be determined.

With the exception of Puerto Maldonado, findings from TGR were lower than those previously reported for human seroprevalence in sites such as Iquitos, which was estimated at 10.3% (95% CI: 8.6; 12.2)[14] or from Andean communities in Cusco (20.1%; 95% CI: 14.7; 26.4).[8] Nonetheless, risk factors for both sites found an association with increasing age in all these populations, suggesting the circulation of the pathogens before. In contrast to the findings from Iquitos and more in line with the findings from Cusco,[8] we found a marginal association between gender and risk, placing women in a more vulnerable position. These findings, along with the increasing seropositive findings with older age, may be related to the origin of a large proportion of the population in Puerto Maldonado from areas from Cusco.[50] It is likely that the older population in this community have been exposed in the past and certain dressing customs are maintained by older women.[8] Likewise, the role of visiting family or to their native sites may also be a source of exposure.

An important limitation of the study, as has been mentioned already, is the lag in time of human sera collection and animal samples and arthropod collections. In addition, the selection of households with an arbitrary definition of case and control households, assuming all exposure takes place in the households, should also be considered with caution, especially because working in farming or agriculture seems to be related to a higher risk.

Despite the dearth of information and lack of ongoing prospective studies, exposure to *Rickettsia*, especially SFGR, and *Leptospira* appears to be frequent in Peru and has its unique dissemination patterns and risk factors, molded by community and population characteristics. For instance, the location of Lima or the living conditions in this city seem to be protective for *Rickettsia*, whereas Tumbes has lower risk for TGR, and Puerto Maldonado is a magnet of all diseases. The exposure to these diseases throughout the population groups appears to be well defined as well, posing higher risk for SFGR among males starting from their teen years. In comparison, *Leptospira* species seem to be in contact with the younger groups in Tumbes and Puerto Maldonado, in striking contrast to the presence of antibodies to TGR with increasing force of association with older age. Nonetheless, the finer links and networks of transmission including environmental and animal exposures remain to be explored, to devise the transmission patterns of disease in these sites. This would require more detailed prospective follow-up to inform patterns of transmission and disease risk.

Received January 14, 2018. Accepted for publication January 28, 2019.

Published online April 1, 2019.

Acknowledgments: We would like to acknowledge the invaluable help of Yeny Tinoco, Candice Romero, María Claudia Guezala, Bruno Ghersi, María Luisa Morales Fernández, Nelly Godoy Mendoza, and Elena Tapia for their help with the cohort data; Carlos Figueroa for laboratory support; Catherine Dupont and Tatiana Quevedo for untiring fieldwork during animal specimen collection; Gissella Vásquez for entomology support; and Olivia Melissa Del Alcázar for rodent identification.

Financial support: The study was funded by U.S. DoD Global Emerging Infections Surveillance and Response System, work unit 847705 82000 25GB B0016.

Disclosure: The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. government. Some authors are or were military service members and employees of the U.S. government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. §101 defines a U.S. government work as a work prepared by a military service member or employee of the U.S. government as part of that person's official duties. The study protocol was approved by the Naval Medical Research Unit-6 Institutional Review Board (Protocol No. NAMRU6.2013.0002) in compliance with all applicable Federal regulations governing the protection of human subjects. The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011. This study was approved via Resolucion Directoral No. 297-2015-SERFOR/DGGSPFFS by the Forestry and Wild Fauna Service, Peruvian Ministry of Agriculture.

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