

EVIDENCE OF RICKETTSIAL AND LEPTOSPIRA INFECTIONS IN ANDEAN NORTHERN PERU

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Abstract. Between May and October 2002, a cluster of acute febrile illnesses occurred in the subtropical Andean foothills of Peru. Serologic evidence in villages where disease had been documented showed that the prevalence of IgM antibody to *Leptospira* ranged from 6% to 52%, that of IgM antibody to spotted fever group (SFG) rickettsia ranged from 10% to 19%, and that of IgM antibody to *Coxiella burnetii* from 1% to 15%. Measurement of IgG antibodies for SFG rickettsiae suggested that this disease was endemic. In contrast, IgG antibodies against *C. burnetii* were largely absent. In humans, microagglutination tests identified pathogenic variants of *Leptospira*. The presence of an SFG rickettsial infection was confirmed in four febrile patients following polymerase chain reaction and sequencing of the conserved 17-kD common antigen gene (*htrA*). Collectively, these analyses indicated that *Rickettsia* sp., *C. burnetii*, and *Leptospira* sp. were circulating in the region during the time of disease outbreak and implicate the involvement of an as yet undetermined SFG rickettsia in northwestern Peru.

INTRODUCTION

Morbidity and mortality in South America are often a consequence of infectious pathogens, and in the last century, outbreaks of dengue fever, cholera, leptospirosis, bartonella, malaria, shigellosis, and infection with *Brucella* have afflicted both urban and rural areas.^{1,2} In many instances, patients present with undifferentiated febrile illnesses characterized by fever, headache, and focalized pain. The etiologic agents responsible for disease are often unknown, although outbreaks occasionally are associated with enzootic vectors, such as mosquitoes, fleas, and ticks.

Between May and October 2002, a number of febrile cases, including two deaths, were reported in northwestern Peru in the area around Sapolilca. Under a collaborative protocol with the Peruvian Ministry of Health to determine the causes of acute febrile illness in subjects in Peru, samples were initially screened for viral infection at the Naval Medical Research Center Detachment in Lima Peru and found to be negative. However, analysis by indirect immunofluorescence assay (IFA) conducted by the Peruvian National Institutes of Health suggested that bacteria of the genera *Rickettsia* and *Leptospira* were possibly infecting people in the area (Moron C, Cespedes M, unpublished data).

Members of the genus *Rickettsia* can be segregated based upon antigenic and genetic differences into the spotted fever and typhus groups.^{3,4} Serosurveys conducted throughout South America have documented evidence of human infection with rickettsial agents in Brazil,^{5,6} Colombia,⁷ and Argentina,⁸ but to date no data have been reported from Peru. Q Fever, an acute rickettsial disease, is caused by the bacterium *Coxiella burnetii*. A number of birds, mammals, and ticks serve as natural reservoirs for this disease.⁹ The mode of transmission to humans is generally through inhalation of infectious aerosols generated during the slaughter of domestic animals, or less frequently, from drinking non-pasteurized milk.⁹ While Q fever is a worldwide zoonosis, it has not often been attributed as a common cause of fever in tropical regions, although outbreaks have been documented in French Guiana.¹⁰ The bacterial disease leptospirosis is an emerging

infectious disease that commonly infects both humans and animals in temperate or tropical climates throughout the world.¹¹ Severe forms of leptospirosis, referred to as Weil's disease, can result in a degenerating disease that afflicts the kidneys, liver, lungs, central nervous system, and heart. Its primary hosts are sylvatic and domestic mammals, and transmission to humans occurs through the contamination of food and water with infectious urine and feces from infected animals. In rural areas, likely reservoirs for disease are often domesticated pigs, bovines, sheep, and goats.

In October 2002, a joint investigation was undertaken to determine the etiologic agents present in the afflicted region of northwestern Peru. The subsequent investigation documented a high prevalence of three agents, spotted fever group (SFG) rickettsiae, *C. burnetii*, and *Leptospira* species, and the molecular identification of a perhaps novel SFG member.

BACKGROUND

Site description. Three sites that represented the foci of the original outbreak that began in June 2003 were surveyed to identify the cause of the outbreak, elucidate risk factors, and determine the prevalence of antibody to the etiologic agent(s). The district of Sapolilca is located approximately 250 miles northeast of the city of Sullana in the department of Piura (Figure 1). Many of the approximately 5,000 inhabitants reside in open dwellings in rural areas. The predominant occupation is agriculture, especially the cultivation of maize, yucca, bananas, cereal grains, and legumes. A large number of domestic animals (pigs, cows, sheep, goats) populate the area. At the time of this investigation, the towns of Naranjo and Coletas were accessible only across footpaths approximately 35 km from Sapolilca. Coletas was situated at the most distant location in the foothills of the Peruvian Andes, Naranjo roughly 15 km lower, and the semi-urban town of Sapolilca at the bottom of a narrow mountain trail.

Human epidemic study. The inclusion criteria for study participants included those more than five years old and less than 70 years old who had resided in the study area for at least two months. One hundred seventy-eight homes were visited

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FIGURE 1. Map of Peru. The inset on the lower right shows a detailed map of northwestern Peru with a focus on the town of Sapolilica, in the department of Piura.

(71 in Coletas, 28 in Naranjo, and 79 in Sapolilica). Study subjects were categorized as either febrile or afebrile. Febrile patients were defined as those that presented with an undifferentiated fever (oral) $\geq 38^{\circ}\text{C}$. Within each household, a single afebrile subject and all febrile subjects were bled. While the work described herein constitutes an outbreak investigation, both written and oral consent was obtained from all human adult participants and from parents or legal guardians of minors as prescribed by the ethical review board at the Institute of National Health (Peru). Prior to the drawing of a blood sample, patients were both questioned and examined by a physician and signs and symptoms were recorded. Those who declined to participate in the study or who showed signs of mental or psychological disorders were excluded. Blood samples were obtained from 169 subjects (152 afebrile and 17 febrile). Study participants provided a single 5-mL sample collected in vacutainer tubes without anti-coagulant (Becton Dickinson, Franklin Lakes, NJ). Blood spots were taken on FTA[®] filter paper (Life Technologies, Gaithersburg, MD) from those that had been categorized as febrile patients. Domestic animals, usually canines, were bled in each house. Sampling of houses was conducted with the goal of representing distinct geographic features in each study site (mountains, fields, stream beds). Samples were stored at room temperature for 1–2 days before they were transported to a central laboratory site in Sapolilica, where blood samples were centrifuged and sera were transferred into labeled tubes.

MATERIALS AND METHODS

IgM enzyme-linked immunosorbent assay (ELISA). Sera from febrile subjects were evaluated using a four-step indirect ELISA to detect IgM antibodies against SFG (SFM-200)

Rickettsia, *C. burnetii* (E-QFB01M), and *Leptospira* (LPM-200) as described by the manufacturer (PanBio, Brisbane, Queensland, Australia). Briefly, 1:100 dilutions of patient sera were tested in 96-microwell plates (Dynatech Laboratories, Inc., Chantilly, VA) containing passively adsorbed antigen. Rickettsial antigen preparations were prepared from *R. rickettsii*, *C. burnetii* antigens from the phase II antigen, and *Leptospira* antigens from a cocktail of *Leptospira interrogans* serovars. Samples were allowed to incubate for one hour at 37°C . The plates were then washed with phosphate-buffered saline (PBS), 0.1% Tween 20 (Sigma Chemical Company, St. Louis, MO), 100 μL of horseradish peroxidase (HRP)-conjugated goat anti-human IgM was added per well, and the plates were incubated for one hour at 37°C . The microwells were washed with PBS, 0.1% Tween 20 and paramethylbenzidine/hydrogen peroxide (TMB/ H_2O_2) was then added. After 30 minutes, absorbance was measured at 450 nm with a microplate reader (Dynex Laboratories, Inc.). A positive serum was one that exceeded the cut-off value, which was calculated as determined for each test as specified by the manufacturer.

IgG ELISA. Testing for IgG antibodies against *C. burnetii* was conducted on 1:100 diluted human serum reacted with *C. burnetii* phase II antigen as recommended by the manufacturer (PanBio). Samples were washed with PBS, 0.1% Tween 20, peroxidase-conjugated anti-human IgG was added, the plates were incubated for 30 minutes at 37°C , the microwells were washed with PBS, 0.1% Tween 20, and TMB/ H_2O_2 was then added. After 30 minutes, the plates were read at 450 nm. To detect IgG antibodies against SFG rickettsia, *R. rickettsii* antigen (1:3,000 dilution) was passively adsorbed on the plates for 24 hours at 4°C . The plates were then blocked with 5% skim milk plus 0.1% Tween 20 for one hour at room temperature. Serially diluted serum samples were allowed to incubate on the plates for at least one hour at room temperature. The plates were then washed with PBS, 0.1% Tween 20, HRP-conjugated anti-human IgG and anti-canine IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added at a 1:2,000 dilution, after which the plates were incubated for one hour at room temperature. Following final washes with PBS, 0.1% Tween 20 and the addition of an equal volume of 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) and hydrogen peroxide (Kirkegaard and Perry Laboratories) for 15–30 minutes, the plates were read at 405 nm.

***Leptospira* microscopic agglutination test.** The Martin-Pettit micro-agglutination test (MAT) was conducted as previously described¹² following determination of end point titer dilutions for agglutination using live reference stains. Optimal concentrations for reference antigen were extrapolated following dark field microscopy. The MAT was performed in a 96-well format starting at dilutions of 1:50. Serial dilutions of serum were then mixed 1:1 with suspensions of *Leptospira* antigen. After a two-hour incubation at room temperature, reactions were read at a magnification of $\times 100$ using a dark-field microscope. End points were gauged when agglutination reached 50% or more of the leptospires relative to the dilution of the buffer control.

Indirect immunofluorescence assay. Antibodies reactive with *R. rickettsii*, *R. prowazekii*, and *C. burnetii* antigens (kindly provided by Dr. H. Thompson, Centers for Disease Control and Prevention, Atlanta, GA) were tested by IFA following standard procedures. Two-fold serial dilutions of

patient serum beginning at 1:32 were incubated on the antigen slides for 30 minutes at 37°C, and then washed three times in phosphate-buffered saline (PBS). A 1:30 dilution of fluorescein isothiocyanate-conjugated goat anti-human IgG antibody (γ chain heavy and light chains) was applied to each well and the slides were incubated and washed with PBS as described. Evan's blue (Sigma Chemical Company) was added as a counter stain. Cover slips were mounted in glycerol-PBS (9:1) buffer, pH 9.0, and slides were examined with an ultraviolet epifluorescence microscope. End point titers were recorded as the reciprocal of the last dilution exhibiting specific fluorescence. Titers ≥ 64 were considered positive.

Extraction of DNA and *Rickettsia* PCR. DNA was extracted from blood spots on FTAR paper per the manufacturer's instructions (Life Technologies) and the PCR for the *Rickettsia* genus-specific 17-kD protein gene (*htrA*) was conducted based upon previously defined sequences as previously described.^{13,14} Briefly, the broad range primers R17-122 5'-CAGAGTGCTATGAACAAACAAGG-3' and R17-500 5'-CTTGCCATTGCCATCAGGTT-3' were used in first round amplification on 2 μ L of product under the following conditions: 95°C for five minutes, 40 cycles at 95°C for seconds, 55°C for 30 seconds, and 72°C for 60 seconds, followed by an extension at 72°C for five minutes.

Nested reactions were conducted on 5 μ L of first-round product using genus-specific primers (spotted fever) TZ15 5'-TTC TCA ATT CGG TAA GGG C-3' and TZ16 5'-ATA-TTGACGATCGCTATTTTC-3' or (typhus group) RP2 5'-TTCACGGCAATATTGACCTGTACTGTTCC-3' and RP1D 5'-CGGTACACTTCTTGGTGGCGCAGGAGGT-3' under the following conditions: 95°C for five minutes, 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds, followed by an extension at 72°C for five minutes. Following electrophoresis, proper-size PCR products were extracted from 1.5% agarose gels and sequenced using an ABI 373A gene sequencer (Applied Biosystems, Inc., Foster City, CA).

RESULTS

Human serosurvey. Sera and or blood spots collected on filter paper were tested from 17 acutely ill febrile patients (eight from Coletas, four Naranjo and five from Sapillica) and the predominant signs and symptoms were recorded (Table 1). Samples were initially screened for viral infection by IgM ELISA. No sample was found to have IgM antibodies to dengue, yellow fever, Venezuelan equine encephalitis virus, oropoucho virus, or the alphavirus mayaro. However, a putative diagnosis was made in eight patients based either on positive IgM serology (*C. burnetii*), PCR and sequencing (SFG rickettsia), microagglutination (*Leptospira*), or a combination of these tests. Results from four febrile patients (24%) indicate that these individuals had been exposed to an SFG rickettsia, three (17%) to *Leptospira*, and one (5%) to *C. burnetii*. Etiologies were not determined in nine of the febrile patients (53%).

While the chief criterion for enrollment as a febrile patient was fever (100% of the cases), a majority of subjects also complained of headache, regardless of the diagnosis. Among those who were categorized as SFG, 75% exhibited malaise and 50% exhibited chills and/or joint pain, cough, or leg pain. A single patient showed both joint pain and a stiff neck.

TABLE 1

Signs and symptoms of diagnosed versus undiagnosed febrile illness, Sapillica, Peru, 2002*

	Undiagnosed (%)	SFG Rickettsia (%)	<i>Coxiella burnetii</i> (%)	<i>Leptospira</i> (%)
Number	9	4	1	3
Fever	9 (100)	4 (100)	1 (100)	3 (100)
Headache	6 (67)	4 (100)	1 (100)	3 (100)
Chills	3 (33)	2 (50)	0	1 (33)
Malaise	3 (33)	3 (75)	0	1 (33)
Joint pain	2 (22)	2 (50)	0	0
Leg pain	2 (22)	2 (50)	0	2 (66)
Stiff neck	1 (11)	1 (25)	0	0
Abdominal pain	1 (11)	1 (25)	0	1 (33)
Nausea	0	1 (25)	1 (100)	1 (33)
Vomiting	0	1 (25)	0	2 (66)
Diarrhea	0	1 (25)	0	2 (66)

* Diagnoses defined as those individuals determined to be positive based upon an IgM positive serology, microagglutination (*Leptospira*), or polymerase chain reaction (*Rickettsia*). SFG = spotted fever group.

These particular signs can suggest severe rickettsial disease.¹⁵ No patient had a noticeable rash, although the actual physical examination was limited and a rash could easily have been missed. Some patients mentioned receiving bites from ticks and fleas, but no ectoparasites were detected on patients upon examination. Clinical signs and symptoms of the single patient exposed to *C. burnetii* were vague. Symptoms of leptospirosis vary in severity from a mild flu-like illness to symptoms resulting from severe renal, hepatic, or meningeal involvement and are characterized by the aforementioned signs.¹⁶ Among the three patients diagnosed with *Leptospira*, two (66%) reported characteristic symptoms of leptospirosis that included bouts of diarrhea and vomiting in addition to leg pain.

We collected 152 sera from afebrile residents representing the same number of households. None of the 92 females or 53 males reported fever or illnesses at the time samples were provided. Men tended to be away from their homes during the day, when the study was conducted and this likely resulted in an observed disparity in the male-to-female ratio. However, it was noted that women in the study sites spent more time in the fields than their male counterparts and as such perhaps their risk for acquiring zoonotic diseases was as great than that of their male counterparts. Serum taken from subjects in the three study sites was diluted 1:100 and then assayed by IgM indirect immunoassay (Table 2). In Coletas, a small pueblo in the high jungle, roughly 10% of the sampled population (60 samples) had antibodies for SFG rickettsia, while 15% were positive for *C. burnetii*, and 32% positive for *Leptospira*. In Naranjo, located roughly 15 km from Coletas,

TABLE 2

Prevalence of IgM antibodies in afebrile human subjects sampled near Sapillica, Peru, 2002*

Location	Number tested	SFG Rickettsia		<i>Coxiella burnetii</i>		<i>Leptospira</i>	
		No. positive	%	No. positive	%	No. positive	%
Coletas	60	6	10	9	15	19	32
Naranjo	21	4	19	2	10	11	52
Sapillica	71	7	10	1	1	4	6
Total	152	17	11%	12	9%	34	22%

* SFG = spotted fever group.

19% of the population of 21 afebrile subjects sampled was positive for SFG, 10% were positive for *C. burnetii*, and 52% were positive for *Leptospira*. In the third site in the small town of Sapollica, 10% of samples were positive for IgM antibodies against SFG, 1% were positive for *C. burnetii*, and 6% were positive for *Leptospira*. Some samples were positive for two or more agents. While the possibility exists that these were due to infection by more than one agent, it is also likely that there was cross-reactivity in these samples. In these instances, a determination was made based upon the test that gave the greatest optimal density value.

The MAT for *Leptospira* was performed on all sera obtained during the course of this investigation. In particular, efforts were focused on the identification of *L. interrogans*, a species that is pathogenic among various mammals, including humans. Results from these studies demonstrated that 120 (74%) of 163 samples were negative. Of the remaining samples, the majority (26 of 43) cross-reacted with more than one serovar. The greatest number of positive samples was seen in Coletas and Naranjo. The serovar most represented among the three sites was *Bratislava* ($n = 31$, 19%). Within the complete study population, other serovars in the *L. interrogans* species identified included *Australis* (2%), *Canicola* (2%), *Djasiman* (2%), and *Grippotyphosa* (2%).

The prevalence of IgG antibodies was determined by either ELISA or IFA. The frequencies of SFG IgG antibodies determined by the ELISA are shown in Figure 2. The sampled populations from each area were divided among age ranges defined in 15-year intervals. Data show the percentage of each group that was positive. The largest number of people sampled was between the ages of 16 and 30 years ($n = 47$) and 31 and 45 years ($n = 44$). The percentage of individuals less than 31 years of age that were positive for SFG IgG antibodies was less than 51% in all three areas. However, a dramatic increase was evident at all three sites as age increased, with levels increasing to 80% or higher in those more than 60 years old. In Naranjo, three (100%) of three subjects more than 60 years of age were IgG positive for SFG. These data suggest that SFG agents may have been endemic in the area in and around Sapollica for many years. In contrast, the prevalence of IgG antibodies against the phase II antigen of *C. burnetii* indicated sparse rates of infection in the three areas sampled, with only a single subject from Coletas found to be reactive. The results of the IFA analysis was also nega-

tive for *C. burnetii* antibodies among participants. These results suggest that *C. burnetii* has recently migrated into the region.

Determination of the Rickettsia group. Although serologic analysis indicated a moderate prevalence of rickettsial infections in both febrile and afebrile subjects, additional tests were conducted to isolate the etiologic agents in febrile patients and to definitively determine whether the agent present was a member of the typhus group or an SFG. First, sera from patients shown to be positive by ELISA were tested for antibodies reactive against *R. prowazekii*, a member of the typhus group that cross-reacts with other group members. A total of 17 afebrile and four febrile samples previously shown to be reactive to rickettsia by ELISA were selected. None were found to be positive for *R. prowazekii* (representative test, Figure 3C and D). However, 10 of 17 afebrile samples and all four febrile samples were found to be positive for antibodies to the SFG member *R. rickettsii* (Figure 3A and B). To identify the rickettsial agent in the four febrile samples, DNA was extracted from blood spots and a PCR was performed for detection of the 17-kD common antigen gene (*htrA*) found in both typhus and SFG members.¹⁷ In the initial reaction, broad range genus-specific primers were used followed by a nested PCR (Figure 4) to identify the typhus (286 basepairs, Figure 4A) or spotted fever (208 basepairs, Figure 4B) group member. A positive control sample for *R. typhi* (lane 1), *R. rickettsii* (lane 2), and *R. akari* (lane 3) were run for comparison. The four DNAs obtained from blood spots taken from febrile patients (lane 5, Coletas; lanes 6 and 7, Naranjo; lane 8, Sapollica) exhibited bands indicative of exposure to an SFG but not a typhus group member. Subsequent sequence analyses using a FASTA National Center for Biotechnology Information (Bethesda, MD)–Genbank search confirmed sequence homology (95%, 205 of 217) to known SFG members including *R. conorii* and *R. akari*. These samples were less similar to *R. felis* (88%, 162 of 184).

DISCUSSION

Our prospective surveillance of infectious disease agents in the coastal areas of northwestern Peru has shown that dengue fever is the most frequent cause of undifferentiated febrile illness, but has shed less light on the role of bacterial infec-

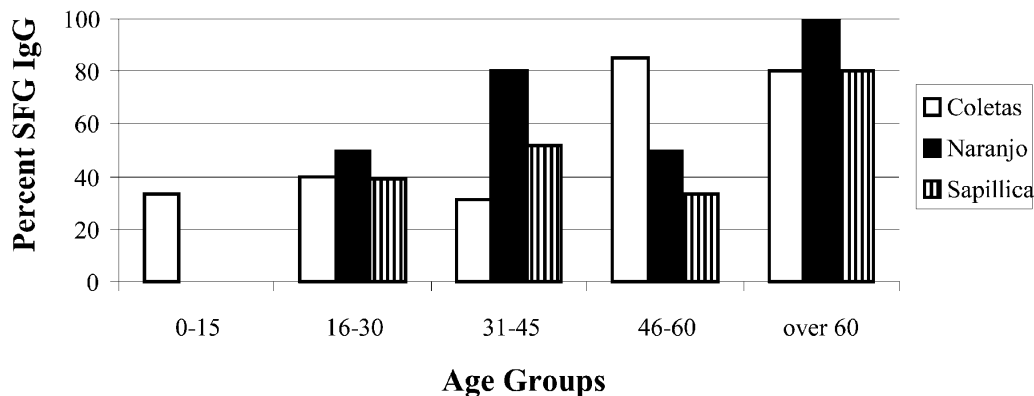


FIGURE 2. Percentages of populations by age group (years) in Coletas, Naranjo, or Sapollica, Peru who were positive for IgG antibodies against spotted fever group (SFG) rickettsiae.

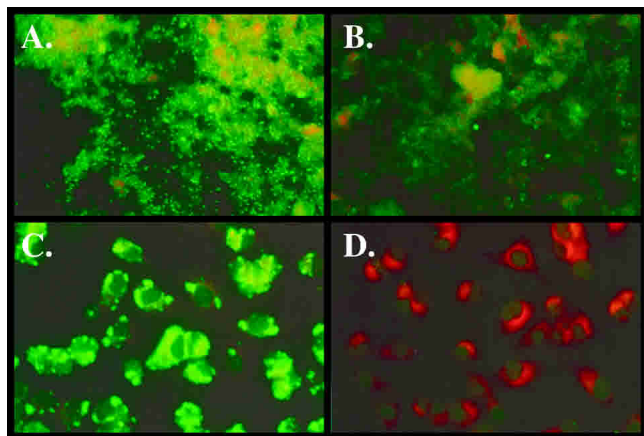


FIGURE 3. Representative indirect immunofluorescence assay staining of susceptible cells against either spotted fever group rickettsiae (A and B) or typhus group (C and D) members. A and C represent positive controls for *Rickettsia rickettsii* and *R. prowazekii*, respectively. Sera from study participants were tested in B and D. (Magnification $\times 400$.)

tions in the region (Blair PJ, Olson JG, unpublished data). During the aforementioned outbreak investigation east into the Andean foothills, viral agents were not isolated and IgM antibodies to these viruses were not detected among residents in the afflicted area. In contrast, we have documented the prevalence of three bacterial agents in the region and identified SFG in four febrile patients sampled during an outbreak investigation. Infection by *Leptospira* has previously been noted in Peru,^{18,19} although no data from northern Peru has

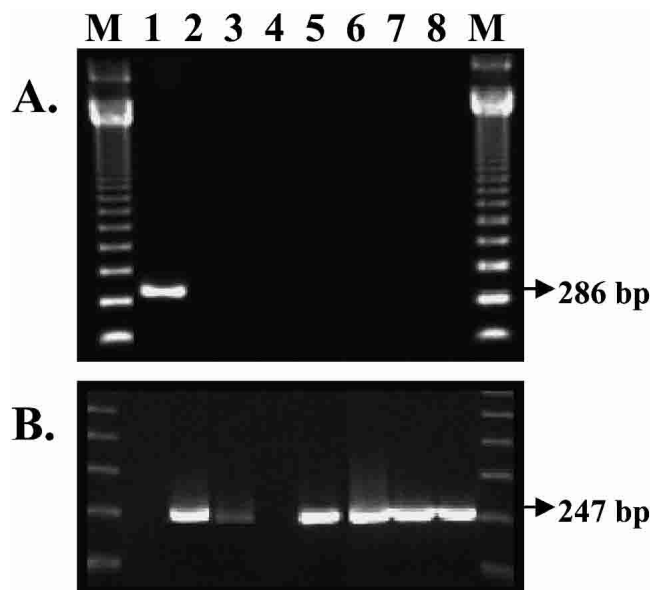


FIGURE 4. Polymerase chain reaction of the conserved 17-kD common rickettsial antigen gene (*htrA*). Shown is a 1.5% agarose gel stained with ethidium bromide of second-round (nested) reactions using primers specific for either the typhus (A) or spotted fever group (B). Lanes M, 123-basepair markers; lane 1, control for *Rickettsia typhi*; lane 2, positive control for *R. rickettsii*; lane 3, positive control for *R. akari*; lane 4, experimental sample that was positive by an IgG enzyme-linked immunosorbent assay but negative for IgM; lanes 5–8, DNAs from four acutely ill febrile patients.

been reported. To date, no account of rickettsial disease in Peru has been published. Worldwide, rickettsial diseases, (typhus, SFG rickettsioses, scrub typhus, and Q fever) pose a serious public health concern and account for considerable morbidity. Here we report for the first time evidence of the presence of SFG *Rickettsia* and the possible emergence of *C. burnetii* in northwestern Peru. We also document high IgM prevalence for pathogenic serovars of *Leptospira*.

The overall results of a serosurvey indicated that the prevalence of *Leptospira*, SFG *Rickettsia*, and *C. burnetii* were highest in Coletas and Naranjo. Indeed, within these small Andean towns, we identified three of the four positive samples for SFG *Rickettsia* and the greatest number and diversity of *Leptospira* serovars. Overall, the high prevalence of infection in these areas likely resulted from close exposure of the mostly agrarian population to animals, their parasites, and animal waste. Infection frequencies were generally lower in the town of Sapillica, 35 km down the mountain from Coletas, where paved streets and commerce apart from farming likely decreased the risk of encountering ectoparasites associated with animals, as well as animal waste products.

Q fever is endemic throughout most of the world, and it has been identified as an important cause of fever among those that frequently work closely with animals or animal waste products. The route of exposure is often via inhalation.^{9,20} Domestic cats have previously been implicated in a number of outbreaks of Q fever among humans,²¹ and a wild reservoir of Q fever has been reported in the South American country of French Guiana.²² The detection of IgM antibodies against *C. burnetii* in northwestern Peru is intriguing. However, since the organism was not isolated from or identified in samples obtained during this investigation, we cannot say conclusively that Q fever contributed to the febrile illnesses seen during this time. Still, the likelihood of *C. burnetii* infection in and around Sapillica is high, given the close association between the populace and domestic animals such as cattle, goats, and sheep. We are currently attempting to recover an isolate of *C. burnetii* in northwestern Peru.

Leptospirosis can be caused by any of the diverse antigenic (serovar) varieties of *L. interrogans*. The etiologic agents, which are gram-negative spirochetes, colonize in the renal tubules of reservoir animals and are often voided in urine. Advanced disease often presents as a severe hemorrhagic disorder, and leptospirosis is now considered a re-emerging diseases in many tropical and subtropical regions.¹¹ Outbreaks of *Leptospira* have been evident in Peru and throughout South America since 1917.¹⁹ These are usually caused by exposure to water, food, or soil contaminated by infected animals. The source of infection by leptospires is often unclear, although dogs have previously been shown to transmit disease in Argentina.²³ It is also quite likely that wild animals can serve as an important reservoir for disease. Previously new serovars of *L. interrogans* were isolated from opossums captured in Amazonian jungles of Peru.^{24,25} Marsupials and chiropterans have been found to be important reservoirs for agents of leptospirosis in the Amazon basin region of Peru.^{18,25}

The differential diagnosis of leptospirosis remains a challenge, and although the IgM ELISA is generally believed to demonstrate good sensitivity and specificity for the detection of positive sera,^{26,27} the often time-consuming and labor-intensive MAT remains the best assay for defining specific

serovars in the genus *Leptospira*. The species that is pathogenic to humans and animals, *L. interrogans*, is divided into more than 240 serovars and 23 serogroups based upon shared major agglutinins.²⁸ Reference samples were used in our analyses because the circulating serovars in northern Peru were not known at the time of this investigation.

High IgM levels in Coletas and Naranjo indicated that *Leptospira* infection is rampant in the area around Sapillica. The MAT results elucidated 12 specific serovars of the species *L. interrogans* from the sera of tested subjects. High titers of pathogenic serovars were also found in canines that resided in close proximity to human study subjects. Nineteen percent of the samples tested were positive for the serovar *Bratislava*. The serovars *Icterohaemorrhagiae* and *Canicola* have often been associated with canines, while *Bratislava* has been seen primarily in swine.

The spread of *R. felis* by fleas from either cats or dogs has previously been linked to disease in northwestern Peru (Moron C, unpublished data) and canines are a known reservoir for pathogenic *Leptospira* in South America.²³ In the course of this investigation, the seroprevalance of *Leptospira* infection in domestic dogs that resided in study homes was assessed to determine if there was an association between human disease and zoonotic reservoirs for disease (data not shown). High percentages were reported in all three towns, suggesting that the aforementioned bacterial agents could be harbored by resident canine populations in the region.

The finding of an SFG *Rickettsia* that demonstrated at least 5% divergence from known sequences is compelling evidence for a possible new species.²⁹ Rickettsial pathogens are highly specialized and thrive as obligatory intracellular parasites within both vertebrates and invertebrates hosts. Rickettsial parasites are often transmitted to humans via the bite of an infected arthropod. Other than *R. akari* (associated with mites) and *R. felis* (fleas), all other SFG rickettsiae are closely associated with ticks. In these instances, the rickettsiae are passed to subsequent generations or stages transovarially and transtadially.²⁴ During an initial survey, some subjects reported tick or other ectoparasitic bites, although most descriptions were vague. Many subjects with SFG IgM did not exhibit signs and symptoms of rickettsiosis, suggesting the possibility of mild or asymptomatic presentation. In the three study sites, both humans and domestic animals were bled. Wild-trapped rodents were also captured and their ectoparasites were collected and categorized. Initial findings indicate that at least one known and perhaps an unknown SFG member were present in samples taken from ectoparasites, rodents, and humans sampled (Blair PJ, unpublished data).

Serologic and molecular data suggest that bacterium of the genera *Leptospira* and *Rickettsia* likely contributed to an outbreak of undiagnosed febrile illnesses that occurred between June and October 2002 in northwestern Peru. Left untreated, both leptospirosis and rickettsiosis can evolve into serious if not fatal diseases. However, if clinically managed early, severe disease can be contained. Indeed, mild leptospirosis responds well to erythromycin or doxycycline,³⁰ while treatment with penicillin is efficacious in patients with late-stage disease.³¹ Recent trials have demonstrated that ceftriaxone, a third-generation cephalosporin, given at a dose of one gram a day for seven days was effective in treating patients with leptospirosis.³² Chemoprophylaxis seems to be helpful in persons exposed to *Leptospira*. In the same light, while many

factors such as advanced age, underlying chronic disease, and delayed treatment can complicate the course of disease induced by rickettsial agents,³³ empirical treatment with doxycycline, tetracycline, or chloramphenicol can shorten the duration of most symptoms and decrease the chance of severe disease. Once the results of the IgM ELISA suggested the involvement of the aforementioned bacterial agents in northwestern Peru, acutely ill patients were treated with 100-mg doses of doxycycline for five days. All patients recovered and appeared healthy upon follow up. This investigation emphasizes the importance of gaining an understanding of the agents of disease in northern Peru, their temporal and special distribution, and the potential vectors involved in their transmission. In the future, such knowledge will allow clinicians to effectively respond to and contain potential outbreaks.

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