**Epidemiology of Endemic Bartonella bacilliformis: A Prospective Cohort Study in a Peruvian Mountain Valley Community**

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*Bartonella bacilliformis* has caused debilitating illness since pre-Incan times, but relatively little is known about its epidemiology. A population-based, prospective cohort investigation was conducted in a Peruvian community with endemic bartonellosis. By use of house-to-house and hospital surveillance methods, cohort participants were monitored for evidence of bartonellosis. Of 690 participants, 0.5% had asymptomatic bacteremia at study initiation. After 2 years of follow-up, the incidence of infection was 12.7/100 person-years. The highest rates were in children <5 years old, and there was a linear decrease in incidence with increasing age. Seventy percent of cases were clustered in 18% of households. Age and bartonellosis in a family member were the best predictors of *B. bacilliformis* infection. There were multiple clinical presentations and significant subclinical infection. A cost-effective control strategy should include vector control and surveillance efforts focused on children and clusters of households with highest endemicity.

Bartonellosis is a potentially fatal, vectorborne, emerging infectious disease found throughout the medically underserved communities of the Andes Mountains of South America. In Peru, bartonellosis affects inhabitants of mountainous river valleys, where ecologic conditions allow the principal suspected vector, *Lutzomyia verrucarum*, to thrive [1, 2]. Recently, epidemics of bartonellosis were reported for the first time in the Urubamba River Valley (Cusco) and in areas of the high Amazonas jungle [3–5]. Along with this emergence in new locations is the recognition of a growing number of syndromes associated with *Bartonella* species [6–12]. Consequently, there has been a renewal of medical interest in South American bartonellosis. However, despite evidence that the disease has existed in Peru since pre-Incan times [13–15], there are many unanswered questions concerning its epidemiology and especially its transmission dynamics.

The causative agent of bartonellosis is the bacterium *Bartonella bacilliformis*, and the clinical symptoms are well described [16–18]. The genus *Bartonella* is the only bacterial pathogen known to invade the human red blood cell [19, 20]. After an estimated incubation period of 7–100 days, bartonellosis presents in multiple stages. Bacteremia and red blood cell invasion characterize the first stage, known as acute hematic bartonellosis (also known as Oroya fever or Carrion disease), with resulting fever, headache, bone and muscle pain, malaise, and variable degrees of anemia. The severity of the first stage ranges from subclinical infection to an acute hemolytic crisis, when *B. bacilliformis* organisms invade up to 100% of a patient’s red blood cells [21]. When acute hematic bartonellosis occurs in epidemic form or when rapid antibiotic treatment is not available, case-fatality rates can be as high as 40%–90% [17, 22, 23]. After the first phase, the patient may experience recurring episodes of fever and transitory pain in the bones, joints, and muscles [16]. Then, within days to months of the acute hematic phase, the second phase of bartonellosis begins, which is characterized by hemangioma-like skin lesions known as “verruga peruana.” Persons with verruga peruana may have few other symptoms and frequently do not seek medical attention because of their geographic isolation and the expense of antibiotics [20].

Humans are reported to be the reservoir for *B. bacilliformis*. In an early study [24], 5 of 53 asymptomatic subjects from an area of endemcity had cultures positive for *B. bacilliformis*. However, the current prevalence of culture-proven, asymptomatic bacteremia and the incidence of clinical and subclinical...
infections are unknown in communities in which *B. bacilliformis* is endemic.

Most studies of bartonellosis have used cross-sectional or casecontrol designs to examine risk factors associated with infection [3, 21, 23, 25, 26]. Several potential risk factors have been hypothesized: sand fly exposure, domiciliary rodents, occupations other than farming, keeping animals inside the home, age, migration to an area of endemicity by immunologically naive persons, and pregnancy. The absence of population-based, prospective epidemiologic data has impeded the identification and verification of these and other possible risk factors. Until the mechanisms of bartonellosis transmission are identified, the development of a rational control program remains difficult.

Here, we present the results of a prospective, population-based cohort study of bartonellosis in 4 villages of the Peruvian Andes Mountains. The purpose was to determine the prevalence of asymptomatic bacteremia and of *Bartonella*-specific IgG antibodies in the community, define incidences of symptomatic and asymptomatic infections, and identify associated risk factors.

**Subjects and Methods**

**Study population.** The study was initiated in January 1997 in a community of ~1600 inhabitants (J.C., unpublished 1997 population data) who resided in 4 contiguous villages of the Andes Mountains. The study ended in February 1999. The study site was selected by Peruvian Ministry of Health (MOH) officials as being representative of areas with long-established endemic bartonellosis. The villages are located in a 4.8-km-wide by 8-km-long semiarid valley at an elevation of 2300 m, 475 km northeast of Lima in Huaylas Province, Caraz District, Peru (figure 1). All inhabitants are of Quechua-Spanish descent. Several generations of a family typically live in a 2–4-room mud-brick house without plumbing. Community livelihood comes largely from agricultural activities and raising animals. Community members seek medical care at a single 32-bed MOH Regional Hospital in Caraz, the closest town. Few inhabitants travel outside the local area for evaluation of their illnesses.

Community members were informed about the study in several ways. First, we held community meetings to acquaint the residents with the study’s specific aims. Then, in January 1997, a house-tohouse census of the 4 study communities was conducted, and all permanent residents were invited to participate. Work-site visits also were made to contact village residents working away from home. Persons with *B. bacilliformis* bacteremia or verrucaous skin lesions on initial contact were excluded.

**Evaluation.** In January 1997, volunteers completed a baseline, standardized, interviewer-administered questionnaire on demographic characteristics, self-assessed health status, history of bartonellosis, and environmental exposures. Clinicians collected blood for determining the January 1997 point prevalence of asymptomatic bacteremia and for serologic testing. Volunteers ≥5 years old contributed blood for peripheral blood smear, bacterial culture, and serologic testing. Children <5 years old contributed blood from a lancet fingerstick for the detection of bacteremia by peripheral blood smear and by polymerase chain reaction (PCR) from samples collected on dried-blood spot cards (IsoCode cards; Schleicher & Schuell). The volume of serum collected from young children was not adequate for culture for bacteria or for serologic testing.

Cohort members were monitored for 25 months for signs and symptoms of bartonellosis. Laboratory and patient log books from the MOH Regional Hospital in Caraz were monitored weekly to determine whether study participants were evaluated for bartonellosis. A trained study investigator conducted weekly site surveillance, and research teams performed biannual house-to-house health surveys. During February 1998 and February 1999, all willing cohort members contributed blood for serial serologic testing and completed a standardized, interviewer-administered questionnaire on change in health status.

A case patient in this study was a participant with laboratory-confirmed bartonellosis, determined on the basis of seroconversion during the study period (>4-fold serial change in titer of antibody to *B. bacilliformis* in paired serum samples by indirect fluorescent antibody [IFA] test, previously shown to be 93% sensitive and 86% specific [27]) and/or verruga peruana with a confirmatory IFA test result. The results of tests of blood collected from symptomatic patients for isolation and identification of *B. bacilliformis* by peripheral blood smear, bacterial culture, and PCR also were used as confirmatory tests. All symptomatic patients were referred to the MOH for treatment.

Details of the laboratory methods for the isolation and identification of *B. bacilliformis* by Giemsa-stained peripheral blood thin smear, blood culture, PCR, and serologic testing were presented elsewhere [27]. In brief, peripheral blood thin smears were prepared at the MOH Hospital and later confirmed at the reference laboratory in Lima. Blood samples for culture were collected in sodium-citrate tubes and transported to Lima, where they were cultured in sealed flasks, using a modified F-1 medium with liquid overlay of RPMI 1640 medium with 10% fetal bovine serum, and then were observed for 8 weeks at 28°C without additional CO₂. Confirmation of bacterial isolates as *B. bacilliformis* was done by PCR. DNA from blood and bacterial cultures examined by PCR was obtained with a commercial extraction kit (Qiagen). DNA from samples collected on dried-blood spot cards was extracted according to the manufacturer’s protocol (Schleicher & Schuell). We used a PCR Master kit (Boheringer Mannheim) for all assays. The PCR conditions and primers used to examine all samples were those described by Norman et al. [28]. These primers were designed to amplify a portion of the citrate synthase gene of *B. henselae*. The assay is not specific for *B. bacilliformis* and detects most members of the genus *Bartonella*. Positive samples required sequencing to confirm the identity of *Bartonella* species detected. DNA extracted from cultures of an American Type Culture Collection strain of *B. bacilliformis* (ATCC 35685; strain KCS83) was used as a positive control. PCR products from positive samples were purified by use of a commercial kit (QIAquick; Qiagen) and then directly sequenced by means of a cycle sequencing protocol (PerkinElmer). *Bartonella* sequences obtained from patients with positive culture results were submitted to GenBank (accession nos. AY114111–AY114119). Serum samples were separated and frozen for transport to the Uniformed Services University of the Health Sciences, Bethesda, Maryland, and stored at −70°C until testing for *Bartonella*-specific IgG antibodies by IFA assay.
Statistical analysis. The primary statistical analysis was based on incidence, with person-years of follow-up used as the denominator. Person-years of follow-up began at the time of study enrollment and ended when bartonellosis was documented, when the participant died or was lost to follow-up, or when the study ended in February 1999. When the exact time of disease onset was unknown (e.g., participants with asymptomatic infections diagnosed by IgG antibody seroconversion), disease onset was assigned as the halfway point between relevant follow-up visits. We computed age-specific incidences by 5-year categories for participants <11 years old and by 10-year categories otherwise.

We used relative risk (RR) as the measure of association to evaluate individual and environmental risk factors. To evaluate the risk to the individual of living in a household with a confirmed case of bartonellosis, 2 measures of risk were computed. First, a risk score was computed by assigning the numeric equivalent of
the number of incident cases in the household after controlling for
the number of household members sampled by use of a Cox propor-
tional hazards model and the Wald statistic as the test of sig-
nificance. Second, a risk score was computed by assigning the num-
eric equivalent of the number of incident cases in the household
divided by the number of household members sampled. Both risk
scores were highly significant, but because the first score is a more
conservative measure of the RR with a tighter confidence interval
(CI), it is the risk score (household case density) reported in these
analyses.

We calculated unadjusted RRs (with 95% CIs) with use of Cox
proportional hazards models. First, for descriptive purposes, a Cox
model was computed for each variable individually. Then, sequen-
tial models were analyzed: first, all variables were forced into the
model; next, all variables were entered in a stepwise fashion, both
forward and backward, based on the P value at each step (the P
value for entry was .05 and for removal was .10); and, finally, all
variables that had previously been significant in any of the models
were allowed to compete for entry. Interaction terms between sex
and other predictors of bartonellosis also were assessed. The vari-
ables that were significant, as well as those variables considered
to be potential confounders, were retained in the final model. Data
analyses were conducted with the SPSS statistical software package
(version 10.0).

Results

Study participants. Of nearly 1600 eligible community mem-
bers, 690 (43%) agreed to participate in the study. In January
1997, 574 persons were enrolled, representing >200 households.
In February 1998, 116 additional participants were added. The
690 participants were aged 1 month to 90 years, with a median
age of 14.2 years; 54% were preschool or school-aged children.
Participants tended to be somewhat younger (mean age, 21.7
years) than the general population (mean age, 25.8 years). A
slightly larger percentage of study participants were female
(58.7%) than seen in the general population (50%), reflecting
the frequent absence of the male head of household when the
survey team visited during the agricultural working day. The
mean family size was 5.7, and the heads of households had
completed an average of 4 years of formal education. Nearly
20% of families reported taking their drinking water from the
untreated irrigation streams running near their homes. Partic-
ipants typically did not seek medical care for their health-related
problems; <10% of the cohort members reported visiting a doc-
tor in the year before study enrollment, and only 18 participants
had been hospitalized. Only 2 (0.9%) of 209 households had
ever had a family member die of bartonellosis.

The cohort participants were well established in the com-
munity. More than 80% of families reported residing in the
valley for >10 years; the average length of residence was 32
years. Ninety-nine percent of participants worked or attended
school within 10 km of their homes. Seventy percent of families
reported that income from agricultural work provided the fam-
ily’s main financial support. Nine percent of the cohort reported
sleeping outdoors for agricultural or other reasons. All partic-
ipants who reported being bitten by sand flies indicated that
the bites occurred during the night, directly before and during
the hours the participants slept. No one reported being bitten
during the early predawn hours.

Prevalence of asymptomatic bacteremia. In January 1997,
clinicians collected blood from 555 asymptomatic volunteers
(97%) for isolation and identification of B. bacilliformis. Two of
352 blood culture specimens collected from participants ≥5 years
old and 1 of 203 specimens collected on dried-blood spot cards
from participants <5 years old were positive for B. bacilliformis
by PCR. The January 1997 point prevalence of asymptomatic
bacteremia was 0.5%. A reference laboratory in Lima confirmed
that no asymptomatic participant, including those with bacte-
remia, was positive for B. bacilliformis on Giemsa-stained thin
blood smear at study entry.

Prevalence of past B. bacilliformis infection by serologic test-
ing. On the basis of serological testing by IFA in February
1998, 45% of participants had IgG antibodies to B. bacillifo-
mis. The prevalence of IgG antibodies among volunteers at
study entry was also 45%. Seropositivity was most common
among volunteers <21 years old; after age 21 years, the rate of
seropositivity slowly began to decline, suggesting a long du-
ration of detectable IgG antibodies (figure 2).

Incidence of bartonellosis. Study participants were moni-
tored for an average of 1.6 years (range, 0.8–2.1 years); 1002.86
person-years of follow-up were accrued from 1997 to 1999 and
are the basis for the analysis. In February 1998, 476 (83%)
cohort members completed the first-year follow-up interviews,
and 241 (42%) participants contributed blood for serologic test-
ing. In February 1999, 512 (74%) second-year follow-up inter-
views were obtained, and 367 (53%) participants provided
blood specimens. Seventy-six subjects from the cohort (11%)
were lost to follow-up and thus contributed no person-time to
the analysis. Reasons for nonparticipation were as follows:
moved away (n = 30), not at home on repeated visits (n = 34),
refused further participation (n = 7), and death (n = 5).

We documented 127 incident cases of laboratory-confirmed

Figure 2. Prevalence in 1998 of IgG antibodies to Bartonella ba-
cilliformis, by age category, among participants in a bartonellosis co-
hort study, Caraz, Peru.
bartonellosis during the 25-month study (table 1). The incidence was 12.7/100 person-years. Thirty-two cases (25%) occurred during 1997, 24 during the first 6 months of the study. The remaining 95 cases (75%) occurred between January 1998 and February 1999. The temporal increase in incidence paralleled a rise in minimum monthly temperatures and rainfall recorded in the Caraz area from December 1997 through July 1998 (the El Niño event) [29].

At diagnosis, 14 case patients (11%) had acute hematic bartonellosis, 47 (37%) had verruga peruana preceded or accompanied by constitutional symptoms suggestive of hematic bartonellosis (i.e., fever, headache, bone and muscle pain, and malaise), 40 (31.5%) had verruga peruana with no history of a preceding acute hematic phase, and 26 (20.5%) were asymptomatic. Fifteen of the asymptomatic participants gave no history of bartonellosis at study entry, 10 gave a history of bartonellosis earlier in life, and 1 person’s medical history was uncertain. There was no statistically significant difference in the rate of asymptomatic infections among male and female participants (P = .56). B. bacilliformis was isolated by bacterial culture and PCR from 11 specimens collected from symptomatic participants.

Nine (7%) of the 127 incident case patients (2 male and 7 female patients) were hospitalized for their disease. There were no deaths among cohort participants that were attributable to B. bacilliformis infection during the study.

Risk factors for infection. Incidence decreased linearly with age, from 38/100 person-years in the <5-year-old group to 1/100 person-years in the >60-year-old group (χ² test for trend, 50.15; P < .001). Incidence did not differ by sex.

Cases were clustered in households. For instance, in 34 households we found up to 7 confirmed cases. In fact, 70% of the bartonellosis cases were clustered in only 18% of all study households.

On univariate analysis, several potential risk factors for B. bacilliformis infection were identified (table 2): age (P < .001), no history of prior infection (P = .03), recent immigration to the community (P < .001), and household case density (no. of cases in the household controlled for the number of household members sampled; P < .001). On multivariate analysis, age (RR, 0.96; 95% CI, 0.95–0.98; P < .001) and household case density (RR, 2.63; 95% CI, 2.1–3.3; P < .001) were the only variables to remain independently associated with the risk of infection.

Discussion

This is the first prospective, population-based study of bartonellosis in an area of endemicity. The incidence of bartonellosis in this cohort was 12.7/100 person-years. The age-related incidence pattern suggests an acquired immunity following exposure. With each year of age, the risk of infection diminished by 4%, conditional on not having acquired the infection at an earlier age. Other factors associated with acquired immunity, such as long-term residence in an area of endemic bartonellosis and a history of prior bartonellosis, were protective against infection with B. bacilliformis. However, of those predictors, age was the only factor to remain independently associated with infection after controlling for the other risk factors.

The risk of acquiring bartonellosis in this study population was substantially increased for those living in a household with a confirmed case patient. The risk of becoming infected for a family member of a confirmed case patient was 2.6 times that of a member of a disease-free household. As a result, case patients were clustered in households. Only 18% of the cohort households accounted for 70% of the case patients. This pattern of bartonellosis transmission seems to follow a statistical pattern known as the “20/80 rule” [30]. Seen also in malaria and leishmaniasis, the rule implies that 20% of households or individuals in a susceptible population account for 80% of the disease. Subgroups within a population vary in their exposures, resulting in a clustering of disease that affects a relatively small fraction of the total households or individuals.

Most members of cohort households were long-term residents of the community, and the lack of significant differences seen in other risk factors may be due, in part, to the homogeneous environment and behaviors of this community. For example, 90% of families kept their chickens, guinea pigs, and/or dogs inside their homes at night, making it difficult to assess the risk of nocturnal animal exposures. In addition, other fac-

Table 1. Incidence of Bartonella bacilliformis infection among 690 volunteers participating in a bartonellosis cohort, Caraz, Peru.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of subjects at risk</th>
<th>No. of cases</th>
<th>Incidence/100 person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire study population</td>
<td>690</td>
<td>127</td>
<td>12.7</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>405</td>
<td>72</td>
<td>12.1</td>
</tr>
<tr>
<td>Male</td>
<td>285</td>
<td>55</td>
<td>13.5</td>
</tr>
<tr>
<td>Age, years</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>146</td>
<td>48</td>
<td>33.8</td>
</tr>
<tr>
<td>5–10</td>
<td>139</td>
<td>35</td>
<td>25.3</td>
</tr>
<tr>
<td>11–20</td>
<td>128</td>
<td>17</td>
<td>13.4</td>
</tr>
<tr>
<td>21–30</td>
<td>89</td>
<td>13</td>
<td>14.9</td>
</tr>
<tr>
<td>31–40</td>
<td>66</td>
<td>8</td>
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</tr>
<tr>
<td>41–50</td>
<td>39</td>
<td>4</td>
<td>10.3</td>
</tr>
<tr>
<td>51–60</td>
<td>29</td>
<td>1</td>
<td>3.4</td>
</tr>
<tr>
<td>&gt;60</td>
<td>50</td>
<td>1</td>
<td>2.0</td>
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<tr>
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<td>23</td>
<td>7.8</td>
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<tr>
<td>Agricultural worker</td>
<td>98</td>
<td>6</td>
<td>6.0</td>
</tr>
<tr>
<td>Other</td>
<td>38</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>History of bartonellosis</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>374</td>
<td>71</td>
<td>12.9</td>
</tr>
<tr>
<td>Yes</td>
<td>248</td>
<td>39</td>
<td>10.4</td>
</tr>
<tr>
<td>Uncertain</td>
<td>68</td>
<td>17</td>
<td>22.7</td>
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<td>Occupation providing family support</td>
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<tr>
<td>Other than farming</td>
<td>178</td>
<td>27</td>
<td>9.6</td>
</tr>
<tr>
<td>Farming</td>
<td>385</td>
<td>83</td>
<td>13.9</td>
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<tr>
<td>Unknown</td>
<td>127</td>
<td>17</td>
<td>13.8</td>
</tr>
</tbody>
</table>

NOTE. Characteristics are at study entry. All participants were of Quechua-Spanish descent.
Table 2. Risk factors for *Bartonella bacilliformis* infection: crude and final adjusted model.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Crude model</th>
<th>Adjusted model</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
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<tr>
<td>Female</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>1.1 (0.8–1.6)</td>
<td>1.1 (0.8–1.7)</td>
</tr>
<tr>
<td>Age, per year increase</td>
<td>0.96 (0.95–0.98)</td>
<td>0.96 (0.95–0.98)</td>
</tr>
<tr>
<td>Duration of residence, per year increase</td>
<td>0.98 (0.97–0.99)</td>
<td>0.9 (0.9–1.0)</td>
</tr>
<tr>
<td>Household case density</td>
<td>2.8 (2.3–3.4)</td>
<td>2.63 (2.1–3.3)</td>
</tr>
<tr>
<td>History of bartonellosis at study entry</td>
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<td></td>
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<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>0.6 (0.3–0.9)</td>
<td>0.9 (0.5–1.7)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0.5 (0.3–0.8)</td>
<td>0.9 (0.4–1.8)</td>
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<tr>
<td>Occupation</td>
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<tr>
<td>Other</td>
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</tr>
<tr>
<td>Homemaker</td>
<td>1.3 (0.4–4.2)</td>
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<tr>
<td>Agricultural worker</td>
<td>0.6 (0.2–2.6)</td>
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<td>Self-reported health status</td>
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<td>Fair or poor</td>
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<tr>
<td>Good to excellent</td>
<td>0.8 (0.5–1.2)</td>
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<tr>
<td>History of neighbor with bartonellosis during study period</td>
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</tr>
<tr>
<td>Yes</td>
<td>0.9 (0.6–1.6)</td>
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<td>1.3 (0.8–2.1)</td>
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<td>Other than farming</td>
<td>1</td>
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<tr>
<td>Farming</td>
<td>0.7 (0.4–1.1)</td>
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<tr>
<td>Formal education of head of household, per year increase</td>
<td>0.9 (0.9–1.1)</td>
<td></td>
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<tr>
<td>Family size, per member increase</td>
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<td>Daily sightings of rodents in home</td>
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<td>No</td>
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</tr>
<tr>
<td>Yes</td>
<td>1.2 (0.7–1.9)</td>
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<tr>
<td>Daily sightings of reptiles near home</td>
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<tr>
<td>Yes</td>
<td>0.9 (0.7–1.5)</td>
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<tr>
<td>Indoor use of insect spray or powder during study period</td>
<td>0.7 (0.4–1.1)</td>
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<td>1</td>
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<tr>
<td>Yes</td>
<td>0.7 (0.4–1.1)</td>
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</tr>
<tr>
<td>Reported more sand fly bites during study period</td>
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</tr>
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</tr>
<tr>
<td>Yes</td>
<td>1.6 (0.9–2.5)</td>
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<tr>
<td>Head of household able to recognize sand flies</td>
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<tr>
<td>Yes</td>
<td>1.3 (0.8–2.1)</td>
<td></td>
</tr>
<tr>
<td>Sleeps outdoors at night</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.8 (0.4–1.6)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Data are relative risk (95% confidence interval).

* Multivariate final model.

tors influence infection rates, such as host factors and variability in virulence within bacterial populations. There also may be other (unrecognized) *Bartonella* species causing disease.

During El Niño weather cycles, the rainy season is extended, and the humidity and minimum monthly temperatures rise, as occurred in Peru during late 1997 through mid-1998. Nearly 75% of incident cases in this study appear to have been associated with this climatic anomaly that allowed potential vector populations to flourish, and unusually high numbers of sand flies were collected during El Niño (authors’ unpublished data). Weather extremes have played a significant role in the emergence and resurgence of diseases in the past. The predictive value of climatic risk factors is important in designing control programs, and studies of vector ecology and its impact on bartonellosis are under way.

Sand flies are weak fliers and feed at dusk and during the evening, when temperatures drop and relative humidity rises [31]. Participants reporting sand fly bites indicated that the bites occurred indoors during the evening, directly before and during the hours of sleep. *L. verrucarum* in the Caraz area feed readily indoors and preferentially on humans [32]. Thus, both vector and human data suggest that transmission occurs inside the home during the evening and the night.

Bartonellosis is classically described to be a biphasic illness...
characterized by acute bacteremia with profound anemia and by a benign, chronic phase with verrucous skin lesions. However, nearly one-third of patients in this study presented with verruga peruana as their initial symptom of disease, and nearly one-fourth of infections were asymptomatic. The ratio of subclinical-to-clinical infections was ~1:4. The asymptomatic subjects with a prior history of bartonellosis may have been partially protected from clinical disease, whereas those without a prior history may have had subclinical infections or may have been diagnosed at a preclinical stage.

Although the initial point prevalence of asymptomatic infection was low (0.5%), >18% of participants became infected during follow-up. Patients with verruga peruana generally have milder symptoms and frequently do not seek treatment, yet 47% are bacteremic at the time of testing (authors’ unpublished data) and may serve as reservoirs of infection for prolonged periods. Community surveillance, focusing on households with diagnosed cases, and early treatment of symptomatic patients might significantly diminish the reservoir of infection.

No asymptomatic participant was found to be slide-positive by Giemsa-stained thin smear, despite rigorous scrutiny. This finding is surprising, because published reviews have reported the slide-positive rate of asymptomatic bacteremia in populations in areas of endemicity to be 12%–16% [4]. The sensitivity of the peripheral blood thin smear procedure, compared with bacterial isolation as the reference standard, is 36% [3], and low-level invasion of red blood cells during the asymptomatic and verruga peruana phases makes diagnosis by peripheral blood thin smear difficult.

Despite our efforts to identify all incident bartonellosis cases, laboratory confirmation was not obtained for 51 participants reporting symptoms suggestive of bartonellosis during follow-up. Twenty (39%) of these 51 nonconfirmed case patients lived in the house of a laboratory-confirmed case patient, so they were probably at increased risk of exposure to *B. bacilliformis*. Matched serum samples were unavailable from these volunteers because of volume inadequacy for children <5 years old and the frequent unwillingness of asymptomatic cohort members to contribute specimens. The site’s remote location and participant reluctance to seek medical care because of time, transportation, and financial constraints resulted in the lack of confirmatory blood specimens that would have enabled comparative sensitivity and specificity testing by thin smear, bacterial culture, or dried-blood spot card PCR.

This study has several implications for Bartonellosis control. First, vector-control efforts, such as residual insecticide spraying, fine-mesh screens, and bed nets, need to target the homes of incident case patients, especially those homes where the majority of cases are clustered. Second, control efforts need to be particularly stringent during El Niño weather events. Third, community-based case detection and treatment programs should focus on infants and children, with an added objective to increase surveillance in the homes of incident case patients. In addition, attention must focus on methods to more effectively eradicate bacteremia in infected persons. The most cost-effective control program may involve a combination of these strategies, focusing surveillance, case detection and treatment, and vector control efforts in the 18% of homes where bartonellosis is clustered. The fact that most cases occurred in only a small percentage of the homes is important, and, clearly, further studies are warranted to better understand the dynamics of transmission and the biologic and environmental factors that contribute to the risk of acquiring *B. bacilliformis* infection.

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References