Natural History of Infection with *Bartonella bacilliformis* in a Nonendemic Population

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An investigation was performed after an outbreak of bartonellosis in a region of Peru nonendemic for this disorder. Symptoms of acute and chronic bartonellosis were recorded. Serological analysis was performed on 55% of the affected population (554 individuals), 77.5% of whom demonstrated previous infection with *Bartonella bacilliformis*. The attack rate of Oroya fever was 13.8% (123 cases); the case-fatality rate was 0.7%. The attack rate of verruga peruana was 17.6%. A new specific immunostain was developed and used to confirm the presence of *B. bacilliformis* in the biopsied skin lesions. Most seropositive individuals (56%) were asymptomatic. The symptoms that were associated with prior infection, as determined by Western blot, included fever (37.2% of the seropositive vs. 17.2% of the seronegative population; \( P < .001 \)), bone and joint pain (27% vs. 9%; \( P < .001 \)), headache (27% vs. 12.3%; \( P < .001 \)), and skin lesions described as verruga peruana (26.8% vs. 4.9%; \( P < .001 \)). Our findings suggest that infection with *B. bacilliformis* causes a broad spectrum of disease that is significantly milder in severity than that frequently reported.

Bartonellosis is endemic in the river valleys of Peru, Ecuador, and Colombia at the elevations of 600–3200 m [1]. The causative agent *Bartonella bacilliformis* is a small, motile, pleomorphic, aerobic coccobacillus transmitted by sand flies (*Lutzomyia* species). Infection results in an illness divided into 2 distinct clinical phases. The first and acute phase of bartonellosis (Oroya fever) is characterized by fever, hemolytic anemia, headache, pallor, myalgia, and arthralgia. Mortality rates of up to 40%–88% in untreated patients have been reported [2, 3]. Most fatalities are associated with the presence of secondary infections, most notably with *Salmonella* or *Toxoplasma* [4, 5]. In a recent retrospective hospital-based study, the mortality rate was 8.8%, despite appropriate antibiotic therapy, in 96% of patients [6]. The second clinical phase of infection with *B. bacilliformis* occurs from 2 weeks to several years after clinical recovery from acute bartonellosis and is characterized by the eruption of crops of nodular skin lesions, verrugous skin lesions, or both, predominantly on the head and distal extremities. This eruptive and chronic phase of bartonellosis, called verruga peruana, has also been noted to occur in patients without a history of acute bartonellosis [7]. Epidemiological data on bartonellosis and an understanding of the clinical course of this biphasic illness are limited. Currently accepted mortality rates and descriptions of the disease are derived from hospital-based studies, which are likely to have underestimated the spectrum of clinical disease and overestimated disease-related morbidity and mortality. An outbreak of bartonellosis occurred in late 1996 in the Amazonas department in Northern Peru. Epidemiologic data was gathered after this outbreak, to determine risk factors for infection. Also, clinical history was correlated with histopathology and serological status, to determine the incidence and nature of clinical disease after infection.

Patients and Methods

**Study area.** The study area encompassed the communities of Churuja, Nuevo Horizonte, Cuchulla, Matiaza Rimachi, Cocahuaco, Palo Seco, and Donee in the Amazonas department of Peru at an altitude of 1372 m; this site is ~40 km from the department capital Chachapoyas (figure 1). This area represents an...
isolated contiguous string of small agricultural communities in the semitropical Utcubamba River Valley. During July 1996, patients began to present to the health post with a febrile illness characterized by severe bone and joint pain (in the most severely affected patients, pain limited ambulation), and headache. Pallor and anemia were also noted in a minority of individuals. Bartonellosis was suspected, and empiric therapy with chloramphenicol resulted in the resolution of symptoms in treated patients. Giemsa-stained peripheral blood smears examined at the regional laboratory in Chachapoyas were positive for *Bartonella*.

Two weeks after the first patient presented with fever and anemia, several patients presented to the health post with subcutaneous nodules and cutaneous exophytic angiogenic lesions. The communities did not recall a febrile illness characterized by the type of bone and joint pain that was occurring, nor had they ever seen similar cutaneous lesions. Records of reported cases for the entire department of Amazonas (population 311,800) revealed 0–15 cases per year of bartonellosis from 1968 through 1996. This outbreak was reported to the Ministry of Health and the Institute of Tropical Medicine at Universidad Peruana Cayetano Heredia, because it was suspected to represent the emergence of bartonellosis in a non-endemic zone.

A research team was dispatched to the area in early 1997. Case histories from the local health post that served as the primary-care site for all communities in the study area were reviewed. Clinical history, examination data, available laboratory findings, and the treatment and outcome data of patients who presented with a febrile illness or dermatologic lesions consistent with verruga peruana were collected and were reviewed with the treating physician (R.L.). Peripheral blood smears that had been obtained during the acute phase of illness were sent to the reference laboratory at the Institute of Tropical Medicine at Universidad Peruana Cayetano Heredia for quality assurance. A house-to-house census of all 997 residents from the 7 study communities was completed. The census data included symptoms selected from the review of case histories suspected as representing disease resulting from infection with *B. bacilliformis*. These symptoms included a history of fever, headache, pallor, osteoarticular pain, malaise, weakness, and verruga peruana in the year before the census. Patients with a history of any of the above symptoms reported the dates that these symptoms were present. All 997 members of the assessed communities were invited to participate in the study. A 5-mm punch biopsy of skin lesions consistent with verruga peruana was obtained, if such lesions were present.

**Specimen collection.** After participants provided informed consent, clinicians collected blood from 544 individuals. Peripheral blood smears were prepared, and serum samples were separated and frozen for transport to a central laboratory. The *B. bacilliformis* ATCC 510 strain was obtained from the Naval Medical Research Institute Detachment (NAMRID) and cultured in biphasic media consisting of a solid medium of 10% defibrinated sheep blood, glucose, tryptose, NaCl, and agar, and a liquid RPMI 1640 medium enriched with HEPES buffer, sodium bicarbonate, and 10% fetal bovine serum. The culture was incubated at 28°C for 14–20 days. Crude antigen preparation was by sonication, and immunoblot was performed as described elsewhere [8]. Blots were interpreted as positive if the 17-kDa or 18-kDa band was present. By use of this technique and interpretation, the immunoblot was 70% sensitive for acute disease, 94% sensitive in identifying chronic bartonellosis, and 100% specific, although significant cross-reactions occur with *Brucella* (40%), *Salmonella* (4%), *Chlamydia psittaci* (5.0%), and *Coxiella burnetii* (10%) [8]. There were no cross-reactions with *Bartonella henselae*. Serum agglutination tests for *Brucella* were done on 20 randomly selected serum samples found to be positive for *B. bacilliformis* by Western blot. Titers ≥1:160 were
considered positive. Two samples (10%) were positive (1:160 and 1:320).

Five-millimeter punch biopsy specimens from 58 patients were divided, and portions were fixed in buffered formalin and glutaraldehyde solutions. All the formalin-fixed tissues were embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin-eosin. Sections from 14 collected biopsy samples were stained by the Steiner and Steiner silver method. The same 14 biopsy samples were also examined immunohistochemically for \textit{B. bacilliformis}. Antiserum was obtained by the subcutaneous inoculation of 12-week-old rabbits with 0.5 mg of antigen prepared by sonification and combined with Freund complete adjuvant. Three booster inoculations of 0.5 mg of antigen with Freund partial adjuvant were done on days 15, 22, and 29, and sera were obtained on day 36, when ELISA confirmed nonspecific titers >1:4000. We used a 1:1500 dilution of antiserum with a Vectascan automatic stainer (Ventura Medical Systems, Inc., Tucson, AZ) after antigen retrieval. Potential cross-reaction with \textit{B. henselae} was investigated. Immunostaining of splenic tissue from a patient with bacillary angiomatosis, previously labeled with an immunostain using polyclonal anti-\textit{B. henselae} antiserum, revealed no labeling of organisms, despite a decrease in the dilution of anti-\textit{B. bacilliformis} antiserum to 1:500 (data not shown). Conversely, attempts to label organisms in a verruga peruana sample utilizing polyclonal anti-\textit{B. henselae} antiserum with and without prior \textit{Bartonella quintana} adsorption failed (L. N. Slater and K. W. Min, personal communication), although the same tissue was strongly positive with the anti-\textit{B. bacilliformis}-based immunostain.

Twelve of the glutaraldehyde-fixed samples were postfixed in 1.5% osmium tetroxide, dehydrated in alcohol, embedded in epoxy, and sectioned at 50 nm. Sections were then stained serially with lead hydroxide and uranyl acetate and were examined with a Philips electron microscope (Phillips Electronic Instruments, Eindhoven, The Netherlands) operating at 75 kV.

Results

\textit{Weather data.} Weather data from 1994 to 1996 were obtained from Jazan, located 8 km from the study communities. Mean monthly temperature and humidity in 1995 and 1996 were not significantly different. Mean temperatures during 1996 were 4% higher (20.7°C vs. 19.9°C; 95% confidence interval [CI], 2.8–5.1; \(P < .001\)) than during 1994. Mean humidity was 7.2% lower in 1996 than in 1994 (77.5% vs. 83.7%; 95% CI, 4.8–9.6; \(P < .001\)). The peak occurrence of acute bartonellosis occurred late in the dry season.

\textit{Peripheral blood smears.} The reference laboratory confirmed only 2 positive blood smears of the 208 obtained from June through October 1996 that were interpreted as positive by the laboratory of Chachapoyas. All 544 smears obtained in March 1997 during the chronic or asymptomatic phase of illness were negative.

\textit{Pathology.} Biopsies of 58 lesions resembling that seen in figure 2.4 demonstrated histiocytic infiltrates with angioblastic proliferation characteristic of verruga peruana (figure 2B). Immunostain using polyclonal rabbit antiserum successfully labeled the bacteria in all 14 lesions previously identified as being verruga peruana by histopathologic appearance (figure 2C). Transmission electron microscopy of 3 specimens identified organisms consistent with \textit{B. bacilliformis} (figure 2D) [9].

\textit{Patient histories.} The 4 patients presented were selected to demonstrate the spectrum of clinical disease after infection, which includes acute bartonellosis, disease symptomatic for the cutaneous phase only, biphasic disease, and the one fatality associated with the outbreak.

\textit{Patient 1.} A 13-year-old male presented to the health post with a 4-day history of fever, headache, and progressively worsening diffuse bone and joint pain. On examination, he was alert but pale and appeared moderately ill. At the time of the examination, he was afebrile. The chest was clear to auscultation. His cardiac examination was notable for a hyperdynamic precordium, tachycardia, and an early systolic murmur. An abdominal examination revealed mildly tender hepatomegaly with the liver edge palpable 1–2 cm below the costal margin. Musculoskeletal examination revealed no localizing tenderness, joint effusions, or impaired range of motion. Laboratory studies revealed a hemoglobin of 4 g/L and a Giemsa-stained peripheral smear positive for \textit{B. bacilliformis}. Chloramphenicol therapy was started, and the patient was transferred to the regional hospital, where he received 1 unit of packed red blood cells, with rapid subsequent improvement. Three months after his acute illness, the patient was well, with a hematocrit of 37%. A Western blot performed on a blood specimen obtained 3 months after his acute illness was positive. When seen 15 months after his acute illness, he denied ever having a skin lesion consistent with verruga peruana.

\textit{Patient 2.} A 33-year-old male presented with a 2-month history of a painless red lesion on the lateral aspect of his neck. He also reported a history of 3–4 similar lesions on his upper extremities that had resolved spontaneously. He denied having had fever, headache, and bone or joint pain. He was afebrile and well when he presented to the health post for the removal of this lesion. Physical examination revealed a well-appearing man with a well-defined 2–3-cm subcutaneous nodule that was not tender to palpation. Superimposed on the nodular lesion was a highly vascular pedunculated lesion 3 mm in diameter. There was no surrounding erythema or local lymphadenopathy. Histopathologic examination of the lesion was consistent with verruga peruana. Serological examination at the time of biopsy was positive for \textit{B. bacilliformis}.

\textit{Patient 3.} A 10-year-old female initially presented with an 8-day history of fever, anorexia, and mild sore throat. At the health post, she was afebrile and appeared only mildly ill. Her physical examination was unremarkable. The clinical diagnosis of viral upper respiratory tract infection was made, and the patient was not given specific therapy. She returned 16 days later with a history of fever persistent since the initial evaluation, headache, nausea, vomiting, and abdominal pain. Physical examination revealed an alert, pale, mildly ill–appearing child. The chest was bilaterally clear to auscultation. An abdominal
examination revealed moderately tender hepatosplenomegaly, with the liver and spleen edges palpated 2 cm below the costal margins. Laboratory findings included a white blood cell count of 9900 mm$^3$ with 43% lymphocytes and a hematocrit of 24%. Typhoid O antigen was negative; typhoid H antigen was 1:160; and a Giemsa-stained peripheral smear analyzed at the local laboratory was found to be positive for B. bacilliformis, although the same blood smear was later interpreted as negative by the reference laboratory. The patient was given chloramphenicol for the treatment of acute bartonellosis complicated by typhoid fever; symptoms subsequently resolved. Three months after her acute illness, she returned to the health post with multiple subcutaneous nodules on her knees and elbows and small exophytic angiogenic lesions on her face. She was afebrile and otherwise well. She was given rifampin. The lesions initially responded to treatment, but 2–3 lesions reappeared and persisted. Ciprofloxacin was given; the lesions resolved for a time, but a small number of verruga peruana recurred on her lower extremities. A biopsy taken on March 1997 confirmed the diagnosis of verruga peruana, and Western blot analysis done on a specimen obtained at that time was positive for B. bacilliformis. One year after her acute illness, all verrugas had resolved.

Patient 4. A 6-year-old female was evaluated at school as part of the health post’s case-finding attempts. There she appeared well, but the patient’s mother reported that the patient had a 2-day history of fever, headache, and abdominal pain. At the time of examination, the patient was afebrile. Physical examination revealed no pallor, but 40–50 exophytic angiogenic lesions were present on her lower extremities. The next night, the physician was contacted to re-evaluate the patient. On the doctor’s arrival, the patient was disoriented, irritable, and appeared acutely ill. Her oral temperature was 39.5°C. Her physical examination was notable for tachycardia and diffuse abdominal pain. Hepatosplenomegaly and peritoneal signs were absent. Her neurologic examination was nonfocal. Intravenous chloramphenicol was started, and the patient was evacuated to the regional hospital, where she died the following day. A peripheral blood smear was tested and found to be positive for B. bacilliformis. No autopsy was performed.
The treating physician had previously diagnosed 65 patients with acute bartonellosis. All febrile patients were given chloramphenicol, as were patients with severe osteoarticular pain, malaise, or pallor. Fifteen of 65 patients (those felt to be experiencing milder disease) were not given antibiotic treatment.

Population survey. The population survey indicated the presence of a febrile illness characterized by severe headache and osteoarticular pain that had a peak prevalence in September 1996 and which had remitted by the time of data and specimen collection in March 1997 (figure 3). Single or multiple cutaneous lesions consistent with the miliary, malar, or nodular form of verruga peruana were reported by 17.6% of the population. The peak prevalence for the appearance of new skin lesions occurred 1 month after the peak prevalence of the febrile illness (figure 4). Fever was reported by 28.2% of the population. The incidence of fever was 323.1 per 1000 person-years and did not vary significantly by sex, age group, educational level, or labor category (data not shown).

Twenty-seven percent of seropositive individuals developed verruga peruana. The incidence of skin lesions was greater in females than in males (349.7 vs. 237.5 cases per 1000 person-years; 95% CI, 1.09–1.99; \( P = .011 \)). The risk for developing verruga peruana increased with age and was significantly higher in the 7–12-years age group than in the 0–1-years age group (relative risk, 4.6; \( P = .02 \); 95% CI, 1.11–19.09). Incidence was highest in children aged 7–12 years (419 of 1000 patients per year) and fell decrementally to a rate of 187.3 in individuals aged >55 years, although, after the age of 2 years, there was no significant difference in the incidence rate of verruga peruana among age groups. People who had reported a history of fever were 9.43 times (95% CI, 6.14–14.49) as likely to develop verruga peruana as sex- and age (± 5 years)–matched control subjects.

Serological data. Serological analysis was available for 544 individuals in the surveyed population (55%). Characteristics of sampled versus nonsampled populations are compared in table 1. The sample population was younger (mean age, 25.3 vs. 28.5 years; \( P = .16 \), Student’s \( t \) test) and more predominantly comprised females (53.5% vs. 46.5%; \( P < .001 \), Pearson’s correlation) than the unsampled population. Significant differences also existed in educational status (\( P = .026 \)) and labor category (\( P < .001 \)). Those who were less represented in the serological sampling can be described as older male agricultural workers or those with more education.

Of the population for which serological data were available, 77.5% were found to have evidence of prior infection with \( B. \) bacilliformis, as determined by Western blot. Seroprevalence ranged from 70.4% in children aged <6 years to 85.1% in those aged >55 years. Adults aged >55 years were more likely to be seropositive than were younger individuals.

Serostatus was compared with symptoms reported in the community survey. The majority (56.4%) of the patients who were found to be seropositive reported no illnesses or new skin lesions during the past year. Of the symptoms that were found to be correlated with serostatus (table 2), fever was the most
commonly reported symptom and was noted in 37.2% of the seropositive population versus 17.2% of the seronegative population \((P < .001)\). An episode of bone and joint pain was reported by 27% of the seropositive and 9% of the seronegative population \((P < .001)\). Headache was noted in 27% of seropositive and 12.3% of seronegative individuals \((P = .007)\). Pallor was infrequently reported in this population; only 8.3% of the seropositive individuals recalled an episode of pallor, compared with 3.3% of the seronegative population \((P = .05)\). Skin lesions that were described as verruga peruana had the strongest association with serostatus; 26.8% of those who had a positive Western blot described these lesions, as compared with 4.9% of the population that was Western blot negative \((P < .001)\).

More than ninety percent (90.7%) of individuals in whom serological examination was available and who had reported a history of fever accompanied by headache and bone and joint pain had a positive Western blot. This symptomatic triad of fever, headache, and bone and joint pain was used as a case definition of symptomatic Oroya fever and applied to the entire population of these communities. With this case definition, the attack rate for Oroya fever in this nonendemic population was 13.8% (123 individuals). There was 1 fatality. The classic biphasic course of bartonellosis with verruga peruana following symptomatic Oroya fever was observed in only 4.8% of this population \((n = 48)\) as a whole and in 8.1% of those with evidence of previous infection, as determined by Western blot \((n = 34)\). Individuals who had Oroya fever were 5.1 times (95% CI, 3.14–8.28) as likely to go on to develop verruga peruana as were sex- and age (±5 years)-matched control subjects.

**Discussion**

This is the first population-based study on bartonellosis. It remains unclear whether the majority of disease seen in the Utcubamba outbreak was mild, compared with that described elsewhere, because it was a population-based study, because the strain of *B. bacilliformis* was relatively avirulent, or because bartonellosis existed previously in this region and partial immunity had altered the severity of illness.

Previous reports from which disease-related morbidity and mortality have been calculated are either hospital-based [4, 5] or reflect outbreak investigations of severe Oroya fever focused on life-threatening disease [3]. Therefore, these reports likely represent only the most severe disease resulting from infection and may fail to describe the milder, and perhaps more common, forms of disease. Indeed, there have been multiple recent reports of mild febrile disease or verruginous disease in the absence of preceding Oroya fever [7, 10], which would seem to support this view. Further support for *Bartonella* causing a mild or subclinical disease elsewhere was the identification of *Bartonella*-specific antibodies in 63.6% of a small sample (1.2%) of the population in an endemic area in Peru [11]. Although we failed to see the frequently reported mortality rate of 10%–40%, the strain that caused this outbreak was sufficiently virulent to cause a hemolytic disease severe enough to result in a hemoglobin of 4 g/L in one child and the death of another.

However, diminished virulence of the strain implicated in the Utcubamba outbreak is supported by 2 observations. The first is the remarkably low number of blood smears found to be
positive in the acute phase of illness when examined by an experienced laboratory. The second is the failure of the patients with a history of fever, headache, and osteoarticular pain, as well as serological evidence of prior infection with \textit{B. bacilliformis}, to develop fulminant hemolytic disease. Only 50 members of the community were diagnosed with acute bartonellosis and were treated with chloramphenicol, although 123 met the strict case definition for Oroya fever. The failure of these patients to clinically progress in the absence of antibiotic treatment is significantly different from that described in the preantibiotic era during the Oroya fever outbreak of 1871. The Oroya fever outbreak resulted in 7000 deaths among railway workers and a fatality rate of 30\%–80\%, depending on the historical report used to estimate the number of railway workers.

The Utcubamba outbreak also differed from that of the Shumpillan outbreak, in which the patient fatality rate in untreated cases was 88\% [3]. We hypothesize that both the population-based methodology and a relatively avirulent strain of \textit{B. bacilliformis} contributed jointly to our findings of a relatively mild clinical course in the majority of patients with symptomatic bartonellosis. Further population-based studies in other populations will be required to determine whether the spectrum of disease following infection in the Utcubamba River Valley is characteristic for bartonellosis.

Strain heterogeneity demonstrated in recent restriction-fragment length polymorphism–based studies on the citrate synthase gene [7] presents the possibility that strain differences in \textit{B. bacilliformis} may, in part, account for the disparity in disease-related morbidity and mortality reported to date. It would seem possible that \textit{B. bacilliformis} strains exhibit a spectrum of virulence ranging from the mild monophasic verruginous disease seen in Ecuador, the type of virulence seen in this epidemic, to the virulence seen in the Oroya and Shumpillan outbreaks. Further studies that address strain heterogeneity and the isolation of virulence factors that vary among strains may improve our understanding of the course of disease that follows infection.

Prior endemicity of \textit{B. bacilliformis} in the Utcubamba River Valley is unlikely, although there is no serobank that would allow for the demonstration of recent seroconversion in this population. If bartonellosis was either holoendemic or previously endemic, it would be expected that seroprevalence would increase with age, reflecting a greater cumulative risk of exposure. At the time of sampling in the Utcubamba River Valley, seroprevalence rates were high (70\%–85\%) across the age groups; although patients aged >35 years were slightly more likely to be positive, the difference was small. Community members are insistent in their denial of a prior illness that resembled either the febrile or the cutaneous phase of bartonellosis. Indeed, memories of the disease were lucid. Bone pain was significant enough to limit ambulation in a significant amount of the population. Furthermore, the lesions of cutaneous bartonellosis, verruga peruana, are highly characteristic and generally numerous, and are unlikely to be either overlooked or confused with other cutaneous eruptions by patients or local physicians.

Though bartonellosis is typically described as a biphasic illness in which patients first experience Oroya fever and then go on to exhibit one of the cutaneous forms of bartonellosis, a minority of this study population with symptomatic disease

\begin{table}
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\caption{Prevalence of symptoms characteristic of bartonellosis in patients with and without serological evidence of previous infection.}
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Symptom & Symptomatic seropositive population, n (%) & Symptomatic seronegative population, n (%) & Pearson's \textit{P} \\
\hline
Fever & 157 (37.2) & 21 (17.2) & <.001 \\
Osteoarticular pain & 114 (27.0) & 11 (9.0) & <.001 \\
Headache & 114 (27) & 15 (12.3) & <.001 \\
Pallor & 35 (8.3) & 4 (3.3) & 0.50 \\
Verruga peruana & 113 (8.3) & 6 (4.9) & <.001 \\
\hline
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demonstrated this pattern. Although populations, such as railroad workers during the Oroya outbreak or residents of the Utcubamba River Valley in this outbreak, tend to go through febrile and cutaneous phases of the disease sequentially, individuals in the communities often fail to manifest both Oroya fever and verruga peruana.

The immunoblot used does cross-react with Brucella and, to a lesser extent, with Chlamydia psittaci, Coxiella burnetii, and Salmonella, raising the possibility that a portion of the seropositive individuals had prior infection with one of these organisms rather than with B. bacilliformis. The high rate of cross-reactivity with Brucella is of particular concern because of the high rate of cross-reactivity (40%) and the similarity between the symptoms of brucellosis and the acute phase of bartonellosis. However, when sera found to be positive by immunoblot were randomly selected for serum agglutination for Brucella, only 10% (2 of 20) were positive. Furthermore, since brucellosis has no cutaneous manifestations similar to verruga peruana, the high prevalence of these skin lesions in the study communities and the high relative prevalence of skin lesions in the seropositive, as compared with the seronegative, population strongly suggests that, in this population, a positive immunoblot reflects prior infection with B. bacilliformis, rather than with Brucella. However, in populations where brucellosis is common and there are no skin lesions consistent with verruga peruana, caution should be used in the interpretation of immunoblot results.

We also describe the development of a B. bacilliformis-specific immunostain that is useful for distinguishing the miliary form of verruga peruana from bacillary angiomatosis and may also prove useful in ultrastructural studies.

The large discrepancy between the results of peripheral blood smears examined in the regional laboratory in a nonendemic area and a reference laboratory was almost certainly the result of inexperience of the regional laboratory and reflects a need for the evaluation and confirmation of smears reported from nonendemic areas.

The cause of the outbreak in this semitropical valley remains unclear. The available evidence suggests that this outbreak represents the emergence of bartonellosis in a nonendemic area. Epidemics have been reported during the last year in 2 other nonendemic areas, Cusco and La Libertad. Bartonellosis appears to be an emerging disease with an expanding geographical range.

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