MOLECULAR EPIDEMIOLOGY OF BARTONELLA INFECTIONS IN PATIENTS WITH BACILLARY ANGIOMATOSIS–PELIOSIS

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ABSTRACT

Background Bacillary angiomatosis and bacillary peliosis are vascular proliferative manifestations of infection with species of the genus bartonella that occur predominantly in patients infected with the human immunodeficiency virus. Two species, *Bartonella henselae* and *B. quintana,* have been associated with bacillary angiomatosis, but culture and speciation are difficult, and there has been little systematic evaluation of the species-specific disease characteristics. We studied 49 patients seen over eight years who were infected with bartonella species identified by molecular techniques and who had clinical lesions consistent with bacillary angiomatosis–peliosis.

Methods In this case–control study, a standardized questionnaire about exposures was administered to patients with bacillary angiomatosis–peliosis and to 96 matched controls. The infecting bartonella species were determined by molecular techniques.

Results Of the 49 patients with bacillary angiomatosis–peliosis, 26 (53 percent) were infected with *B. henselae* and 23 (47 percent) with *B. quintana.* Subcutaneous and lytic bone lesions were strongly associated with *B. quintana,* whereas peliosis hepatis was associated exclusively with *B. henselae.* Patients with *B. henselae* infection were identified throughout the study period and were epidemiologically linked to cat and flea exposure ($P \leq 0.004$), whereas those with *B. quintana* were clustered and were characterized by low income ($P = 0.003$), homelessness ($P = 0.004$), and exposure to lice ($P = 0.03$). Prior treatment with macrolide antibiotics appeared to be protective against infection with either species.

Conclusions *B. henselae* and *B. quintana,* the organisms that cause bacillary angiomatosis–peliosis, are associated with different epidemiologic risk factors and with predilections for involvement of different organs. (N Engl J Med 1997;337:1876-83.)

BACILLARY angiomatosis was first described early in the epidemic of the acquired immunodeficiency syndrome (AIDS) in patients infected with the human immunodeficiency virus (HIV).[1-4] This unusual vascular lesion can involve many different organs, including skin, bone, and brain. A closely related angioproliferative lesion of the liver and spleen is known as bacillary peliosis.[5-7] These two vascular lesions are associated with infection with fastidious gram-negative bacilli of the genus bartonella and will be referred to as bacillary angiomatosis–peliosis.

The study of bacillary angiomatosis–peliosis in HIV-infected patients has led to the discovery of bartonella infections in the United States,[5,6,8] the naming of a new species, *Bartonella henselae,*[9,10] the cultivation of two bartonella species from cutaneous bacillary angiomatosis lesions,[11] documentation that *B. henselae* causes cat scratch disease,[12,13] identification of the domestic cat as the principal reservoir of *B. henselae* from which humans can acquire infection,[14] and identification of the cat flea as the vector of *B. henselae.*[15]

An initial case–control study to identify risk factors for bacillary angiomatosis–peliosis found a statistically significant association between cat bites or scratches and disease.[16] However, the pathogenic species were not identified, and 30 percent of the patients did not have exposure to cats, leading us to hypothesize that the disease might be caused by more than one species, each with different reservoirs and vectors. We subsequently found that, in addition to *B. henselae, B. quintana* can also cause bacillary angiomatosis in HIV-infected patients,[11] yet the risk factors, reservoirs, and vectors for *B. quintana* infection in immunocompromised patients with bacillary angiomatosis–peliosis remain unidentified.

In most studies of bacillary angiomatosis–peliosis, the infecting bartonella species has not been identified. To identify the microbiologic characteristics and risk factors associated with infection with particular bartonella species, we systematically evaluated 49 patients over eight years who had clinical lesions of bacillary angiomatosis–peliosis and who were infected with bartonella species identified by molecular techniques. The goal was to improve the recognition, diagnosis, and prevention of infection with this emerging human pathogen.

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METHODS

Study Design

Case Definition

A suspected case was defined as the presence of clinical symptoms and signs of bacillary angiomatosis, parenchymal bacillary peliosis, or both in a patient.[16] A case was confirmed when an infecting bartonella species was identified by molecular techniques.[5,11]

Eligible Study Participants

Persons with suspected bacillary angiomatosis–peliosis were identified by their health care providers between July 1987 and January 1995. Recovery of bartonella from cutaneous tissue was not accomplished until 1991[11]; therefore, for patients interviewed between July 1987 and October 1991, only those with archived tissue specimens containing well-visualized bacillary organisms[16] for molecular speciation were eligible for enrollment. Patients with suspected bacillary angiomatosis–peliosis who had biopsies or cultures undertaken between May 1991 and January 1995 were also eligible.

Health care providers identified two controls per patient, matched according to HIV serologic status and hospital (for inpatients) or clinic (for outpatients) with inpatient-ward and outpatient-schedule registries, respectively.[16] Written consent was obtained from all participants, as approved by the Committee for Human Research of the University of California, San Francisco.

Questionnaire

A standardized questionnaire was administered; because of the increasing body of knowledge about bartonella over the eight-year study period, additional standardized questions were added pertaining to arthropod exposures and history of diarrheal illness (in 1991) and alcohol consumption and bathing habits (in 1993). Participants were asked about demographic and behavioral characteristics, medical history, and environmental exposures during specific periods preceding the onset of the illness (patients) or enrollment (controls). Additional clinical information was extracted from medical records with a structured data-collection instrument.

Statistical Analysis

Patients and matched controls were evaluated for differences. Matched analyses were repeated with stratification according to infecting bartonella species. Several subanalyses were conducted on unmatched data to maintain statistical power with diminished matched sets (for questions added in 1991 and 1993).

The Mann–Whitney U test was used to compare distributions of continuous variables between cases and controls. For dichotomous variables, univariate matched odds ratios with 95 percent confidence intervals were calculated by the Mantel–Haenszel method with Epi Info.[17] For univariate unmatched odds ratios, 95 percent confidence intervals were calculated by Yates' corrected method or a two-tailed Fisher's exact test (for analyses with small numbers of observations).

Molecular and Microbiologic Methods

Isolation of Bartonella Species from Humans and Pet Cats

Blood, tissue, or both were obtained for culture of bartonella species. Tissue was homogenized in inoculation medium[11] and plated onto both chocolate agar and heart infusion agar supplemented with 5 percent fresh defibrinated rabbit blood. Biopsy tissue from seven patients also was cocultivated with an endothelial-cell monolayer.[11] Human and cat blood was cultured in adult[8,11] and pediatric[14] lysis-centrifugation tubes (Wampole, Cranbury, N.J.), respectively. Control medium, agar plates, and endothelial-cell monolayers were processed in parallel.

Extraction of DNA from Archived Tissue and Speciation of Bartonella Isolates by Sequencing

The infecting bartonella species was determined by extraction of DNA from bartonella isolates (25 patients), frozen biopsy tissue (9 patients), or formalin-fixed, paraffin-embedded tissue (15 patients).[11,14] DNA was amplified under standard conditions by the polymerase chain reaction (PCR) with alpha-proteobacterium range-restricted 16S ribosomal RNA gene primers p24E and p12B.[5] Control tissues matched by type and simultaneously processed were included in each group. Negative and positive PCR controls (containing no DNA and *B. henselae* DNA, respectively) were included in each experiment.

The amplified fragment was cloned and sequenced.[11] Both strands of a minimum of two different clones were sequenced for each patient; if a sequence differed from the previously published bartonella sequence, additional clones were sequenced to resolve the difference. Speciation of each isolate was corroborated by PCR analysis of restriction-fragment–length polymorphisms.[9]

Molecular Subtyping of B. henselae *Isolates*

After a subspeciation method for *B. henselae* had been developed,[18] we studied the 13 cats belonging to the last three consecutively enrolled patients with confirmed *B. henselae* infection who had been exposed to cats (June to December 1994) to determine the relatedness between the human *B. henselae* isolate and the corresponding cat isolate or isolates. Repetitive-element PCR with oligonucleotide primers based on highly conserved repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) sequences was performed with whole-cell preparations.[18] DNA fingerprints of isolates were compared for similarity by visual inspection of band patterns, and the findings were confirmed by computer-assisted analysis (RFLPscan, Scanalytics, CSP, Billerica, Mass.).

RESULTS

Case–Control Study

Forty-nine patients infected with a bartonella species were enrolled: 33 at the onset of illness and 16 previously reported patients who had histologically confirmed bacillary angiomatosis–peliosis infection without identification of a bartonella species.[16] Forty-six patients (94 percent) had histologic evidence of bacillary angiomatosis–peliosis; two (4 percent) had histologic features (one in the spleen and one in a lymph node) of an acute, necrotizing, inflammatory process without angiomatosis; and one (2 percent) refused liver biopsy but had *B. henselae* bacteremia and clinical features of peliosis hepatis (a temperature of more than 39°C, hepatomegaly, and an elevated alkaline phosphatase level). Forty-five patients (92 percent) had HIV infection; four (8 percent) were HIV-negative (of whom three were immunocompetent[19] and one of whom had received immunosuppressive drugs[5]).

Forty-five of the 49 patients (92 percent) and 89 of their 96 matched controls (93 percent) were residents of the San Francisco Bay area. Two controls were enrolled for each patient, except that one control each was enrolled for two of the three HIV-negative, immunocompetent patients. Relatives or friends answered questions about five patients who had died and one who was a child under 12 years old.

TABLE 1. SITE OF BACILLARY ANGIOMATOSIS–PELIOSIS ACCORDING TO BARTONELLA SPECIES.

SITE*	*B. QUINTANA* (N=23)	*B. HENSELAE* (N=26)	P VALUE†
Skin	21	19	0.15
Lymph node	1	12	<0.001
Bone	8	0	0.001
Liver or liver and spleen	0	6	0.02
Subcutaneous mass	8	1	0.008

*Twenty-four patients had bartonella infection at multiple sites.

†P values were calculated with Fisher's exact test.

Patient Characteristics

Matched Analyses

The patients were similar to the matched controls in median age, sex, race, ethnic group, and proportion with a preceding diagnosis of AIDS[20] (all $P>0.22$). The patients were less likely than the controls to be homosexual or bisexual (matched odds ratio, 0.2; 95 percent confidence interval, 0.1 to 0.5; $P=0.002$). Clinical comparisons between the patients with *B. quintana* infection and those with *B. henselae* infection suggest that *B. quintana* infections have a predilection for causing subcutaneous and deep soft-tissue disease and lytic bone lesions (Table 1). In contrast, *B. henselae* infections were strongly associated with lymph-node disease and parenchymal peliosis of the liver or liver and spleen.

The patients were significantly less likely than the controls to have received therapy with a macrolide antibiotic (such as erythromycin or clarithromycin) during the six-month reference period (matched odds ratio, 0.2; 95 percent confidence interval, 0.1 to 0.7; $P=0.009$). Trimethoprim–sulfamethoxazole, ciprofloxacin, dapsone, penicillins, and cephalosporins were not protective. Other antibiotics were not used with enough frequency for statistical evaluation. The patients were more likely than the controls to have poor appetite (matched odds ratio, 3.3; 95 percent confidence interval, 1.5 to 7.4; $P=0.004$), weight loss (matched odds ratio, 5.6; 95 percent confidence interval, 2.1 to 14.8; $P<0.001$), and recurrent fevers (matched odds ratio, 14.0; 95 percent confidence interval, 3.4 to 58.5; $P<0.001$), but the patients were similar to the controls in the frequency of malaise, nausea, vomiting, rash, headache, and joint pains during the six-month reference period (all $P>0.12$). The results were similar when the data were stratified according to bartonella species.

Unmatched Analyses

Patients with *B. henselae* infection (odds ratio, 6.5; 95 percent confidence interval, 1.1 to 51.1; $P=0.04$) but not *B. quintana* infection (odds ratio, 1.7; 95 percent confidence interval, 0.2 to 13.0; $P=0.68$) were more likely than controls to have had diarrhea.

Environmental Exposures

Matched Analyses

Table 2 shows environmental exposures and clinical characteristics associated with bacillary angiomatosis–peliosis, with stratification according to the infecting bartonella species. Patients with *B. henselae* infection but not those with *B. quintana* infection were more likely than matched controls to have owned a cat, been bitten or scratched by a cat, owned a cat with fleas, or been bitten by cat fleas (all $P\leq0.004$). Patients with *B. quintana* infection were 8.5 times as likely as matched controls to be homeless and 9.8 times as likely to have a low annual income. Neither *B. henselae* nor *B. quintana* infection was associated with alcohol consumption, intravenous drug use, or a prior AIDS-associated opportunistic infection or cancer.

Table 2 also shows differences in laboratory findings between patients and controls. CD4 lymphocyte counts were lower among the patients with *B. quintana* infection than among their matched controls, and patients with either *B. quintana* or *B. henselae* infection had lower hematocrit values than their matched controls. Significantly elevated alkaline phosphatase levels were associated only with *B. henselae* infection.

Unmatched Analyses

Because only a subgroup of our 49 patients and 96 controls were queried about exposure to arthropods, use of alcohol, and bathing habits, these variables were also evaluated in an unmatched analysis (Table 2). The unmatched results were similar to the matched results for all variables. In the unmatched analysis, patients with *B. quintana* infection were significantly more likely than controls to have had recent infestations with head or body lice (21 percent of patients and 0 percent of controls, $P=0.03$), although the results of a matched analysis did not achieve significance ($P=0.11$), possibly because of the small number of patients and controls queried. *B. henselae* infection was not associated with exposure to lice in either matched or unmatched analyses.

Isolation and Speciation of Bartonella from Tissue

Twenty-six patients (53 percent) had *B. henselae* infection and 23 (47 percent) had *B. quintana* infection documented from either cultured tissues (25 patients) or archived tissues (24 patients). Figure 1 shows 44 patients residing in the San Francisco Bay area according to bartonella species and date of onset of illness (1 patient with onset of illness due to *B. quintana* in February 1987 is not shown). Disease due to *B. henselae* occurred at regular intervals

TABLE 2. ENVIRONMENTAL EXPOSURES AND CHARACTERISTICS OF PATIENTS WITH BACILLARY ANGIOMATOSIS–PELIOSIS AND THEIR CONTROLS.

VARIABLE	*B. QUINTANA*				*B. HENSELAE*			
	PATIENTS (N=23)	CONTROLS (N=46)	MATCHED ODDS RATIO (95% CI)*	P VALUE†	PATIENTS (N=26)	CONTROLS (N=50)	MATCHED ODDS RATIO (95% CI)*	P VALUE†
Cat exposure‡								
Cat ownership (% of subjects)	34.8	45.7	0.6 (0.2–1.8)	0.56	88.5	50.0	10.8 (2.0–56.7)	0.004
Mean no. of cats owned	0.5	0.8		0.21§	2.3	0.9		<0.001§
≥1 Cat bite or scratch (% of subjects)	13.0	32.6	0.3 (0.1–1.2)	0.10	69.2	22.0	6.2 (2.0–18.8)	0.001
Ownership of a cat with fleas (% of subjects)	8.7	6.5	1.3 (0.2–8.0)	0.75	46.2	6.0	Undefined (4.2–undefined)	<0.001
Arthropod exposure (% of subjects)¶								
Cat-flea bite	26.7	36.7	0.6 (0.1–3.0)	0.74‖	78.3	23.3	11.9 (3.0–49.8)	<0.001‖
Dog-flea bite	21.4	23.3	0.9 (0.2–5.1)	1.00**	13.6	19.0	0.7 (0.1–3.3)	0.74**
Head or body lice	21.4	0.0	Undefined	0.03**	4.3	0.0	Undefined	0.35**
Pubic lice	14.3	0.0	Undefined	0.10**	4.3	0.0	Undefined	0.35**
Socioeconomic characteristics (% of subjects)								
Homeless or no stable home‡††	43.5	10.9	8.5 (1.8–40.3)	0.004	11.5	6.1	2.5 (0.4–16.6)	0.64
Frequently drank alcohol‡‡‡§§	50.0	66.7	0.5 (0.1–3.7)	0.66**	53.8	58.3	0.8 (0.2–4.0)	0.93‖
Bathed <5 times/wk‡§§	66.7	26.7	5.5 (0.5–74.8)	0.15**	33.3	13.0	3.3 (0.5–25.9)	0.20**
Annual income <$10,000¶¶	63.6	22.2	9.8 (2.0–47.3)	0.003	34.6	31.9	1.3 (0.5–3.8)	0.79
HIV risk factors (% of subjects)								
Sexual behavior associated with acquiring HIV‖‖	86.4	97.8	0.0 (0.0–1.8)	0.15	80.8	78.0	4.0 (0.3–58.6)	0.64
Intravenous drug use	39.1	30.4	1.4 (0.5–3.7)	0.66	30.8	30.0	1.1 (0.4–3.3)	0.93
AIDS-associated diagnosis*** †††	56.5	50.0	1.3 (0.5–3.9)	0.80	61.5	64.0	1.0 (0.3–3.6)	0.76
Laboratory values†††								
CD4+ count <200 cells/mm³ (% of subjects)	95.7	61.4	16.0 (1.6–157.2)	0.008	79.2	70.8	2.1 (0.5–9.4)	0.46
Median CD4+ count (cells/mm³)	20	90		<0.001§	55	40		0.22§
Median hematocrit (%)	33	41		<0.001§	32	38		0.004§
Median alkaline phosphatase (U/liter)	112.0	87.0		0.23§	190.0	107.0		0.003§
Median aspartate aminotransferase (U/liter)	35.0	46.0		0.64§	62.0	43.5		0.17§

*CI denotes confidence interval. Conditional maximum-likelihood estimates were used to determine one-sided confidence intervals for undefined matched odds ratios or matched odds ratios equal to zero.

†P values were calculated by Mantel–Haenszel chi-square test unless otherwise indicated.

‡Values represent exposure during the six months before diagnosis (patients) or enrollment (controls).

§P value was calculated by Mann–Whitney U test for two groups.

¶Exposure was during the 12 months before diagnosis (patients) or enrollment (controls). Unmatched odds ratios are given because of the low number of matched case and control sets: 14 patients and 30 controls for *B. quintana*, and 23 patients and 42 controls for *B. henselae*.

‖P value was calculated with Yates' correction.

**P value was calculated by Fisher's exact test.

††The subjects were living in streets, parks, shelters, hotels, or rehabilitation homes.

‡‡The subjects frequently drank beer, wine, cocktails, or shots of liquor.

§§Unmatched odds ratios are given because of the diminished number of matched case and control sets: 7 patients and 17 controls for *B. quintana*, and 13 patients and 24 controls for *B. henselae*.

¶¶Annual income before becoming ill was used.

‖‖Behavior was defined as sexual contact with homosexual or bisexual men, commercial sex workers, or intravenous drug users.

***AIDS-associated opportunistic infection or cancer was defined according to the 1993 expanded surveillance case definition for AIDS of the Centers for Disease Control and Prevention.

†††Values are those on the date of diagnosis (patients) or enrollment (controls).

over a period of 75 months; however, 15 of 22 cases of *B. quintana* infection were clustered in the 17-month period from February 1992 to June 1993.

Isolation of Bartonella Species from Humans

Thirty-six bartonella isolates were obtained from the blood, tissue, or both of 25 patients: *B. henselae* was obtained from 9 and *B. quintana* from 16 patients. Of 20 patients with focal bacillary angiomatosis–peliosis and simultaneous culture of blood and tissue, 10 (50 percent) had bacteremia, including 6 of 14 (43 percent) with *B. quintana* infection and 4 of 6 (67 percent) with *B. henselae* infection. No bartonella species other than *B. quintana* or *B. henselae* was isolated, and multiple isolates from the same patient were always the same species.

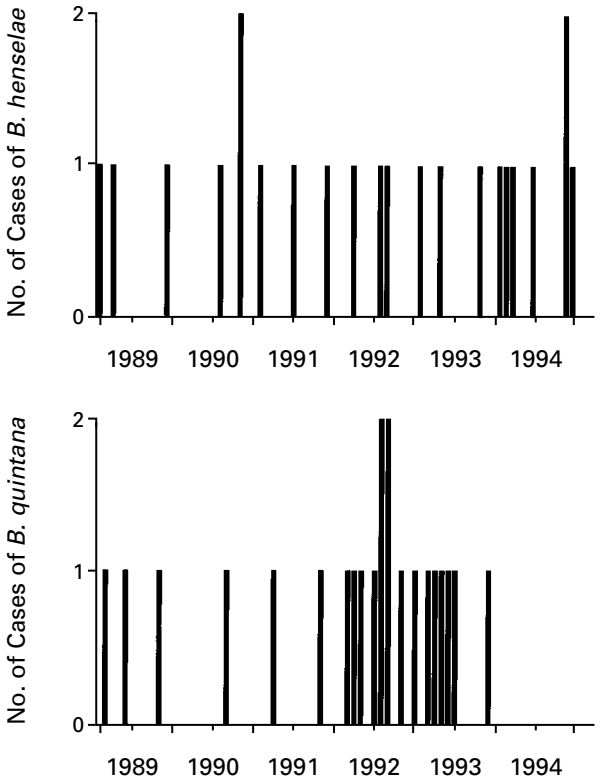


Figure 1. Cases of Bacillary Angiomatosis–Peliosis Caused by *B. quintana* and *B. henselae* in the San Francisco Bay Area, 1989 to 1995.

The graphs show the numbers of patients with bacillary angiomatosis–peliosis caused by *B. henselae* (upper panel) and bacillary angiomatosis caused by *B. quintana* (lower panel) over the 75-month period of the study. *B. henselae* infections were evenly distributed throughout the study period. In contrast, *B. quintana* infections were clustered in 1992 and the first six months of 1993. One patient with onset of symptoms due to *B. quintana* infection in February 1987 is not indicated.

B. henselae was isolated from the tissue of two patients taking prophylactic oral trimethoprim–sulfamethoxazole. *B. quintana* was isolated from one patient who had received nafcillin and gentamicin intravenously for several days before biopsy and from another patient pretreated with a first-generation cephalosporin. No bartonella isolate was obtained from any patient who was treated with a macrolide or tetracycline.

Microbiologic Characteristics Distinguishing Bartonella Species

Phenotypic differences were readily apparent between primary isolates of *B. henselae* and *B. quintana* from humans. Colonies of *B. henselae* were usually rough and deeply embedded in agar (Fig. 2A); the primary isolate was rarely smooth. In contrast, *B. quintana* primary isolates were always smooth and flat and never pitted the agar (Fig. 2B). All isolates could be passaged on agar indefinitely, although the rough *B. henselae* isolates became smooth with continued passage. There was also preferential growth of *B. henselae* on heart infusion agar with 5 percent rabbit blood and of *B. quintana* on chocolate agar. *B. henselae* isolates from cat blood were often detected on agar at three days, but neither *B. henselae* nor *B. quintana* isolates from human blood or tissue could be detected before eight days.

Molecular Subtyping of Bartonella Isolates

Isolation of Bartonella Species from Pet Cats

Ten patients still lived with the same 25 cats to which they had been exposed before bacillary angiomatosis–peliosis developed. Nine of these 10 patients were infected with *B. henselae*, and 1 with *B. quintana*. *B. henselae* was isolated from 18 of the 25 cats (72 percent) and from at least 1 cat in each household of a patient infected with *B. henselae*, with one exception. This patient's banked serum samples demonstrated the presence of bartonella antibodies several years before he acquired his two culture-negative cats (unpublished data). The cat linked by household to the sole *B. quintana*–infected patient was culture-negative for bartonella.

Molecular Subtyping of B. henselae *Isolates and Comparison of Isolates from Patients and Their Cats*

Subspeciation of the *B. henselae* isolates from the last three consecutively enrolled patients and their 13 cats was performed by PCR with REP and ERIC primers.[18] A bartonella species was isolated from the only cat of one patient, 1 of the 3 cats of the second patient, and 8 of the 9 cats of the third patient (overall, 10 of 13 cats [77 percent]). By REP PCR (Fig. 3, upper panel), similar patterns were obtained for all isolates but one (lane 15); by ERIC PCR (Fig. 3, lower panel), three different patterns were observed (pattern B in lanes 2 to 4, pattern C in lanes 5 to 14, and pattern I in lane 15).[18] Subspeciation based on the composite pattern of REP PCR and ERIC PCR[18] revealed that the three human isolates belonged to two different subtypes of *B. henselae:* Patient 1 had subtype II, and Patients 2 and 3 had subtype III. The patient's subtype matched the subtype obtained from the cats to which the patient was exposed, except for one of the eight cats of Patient 3, which was infected with *B. clarridgeiae* (Fig. 3, lane 15). The subtype III isolate pattern for Patient 2 and Cat 2 was consistently distinguishable from the other subtype III isolates by a distinct band at 910 base pairs on REP PCR (Fig. 3, upper panel). Although this finding does not meet the criteria for a different genomic fingerprint,[18] it enabled us to match the Cat 2–Patient 2 pair when all isolates were initially run as blinded samples.

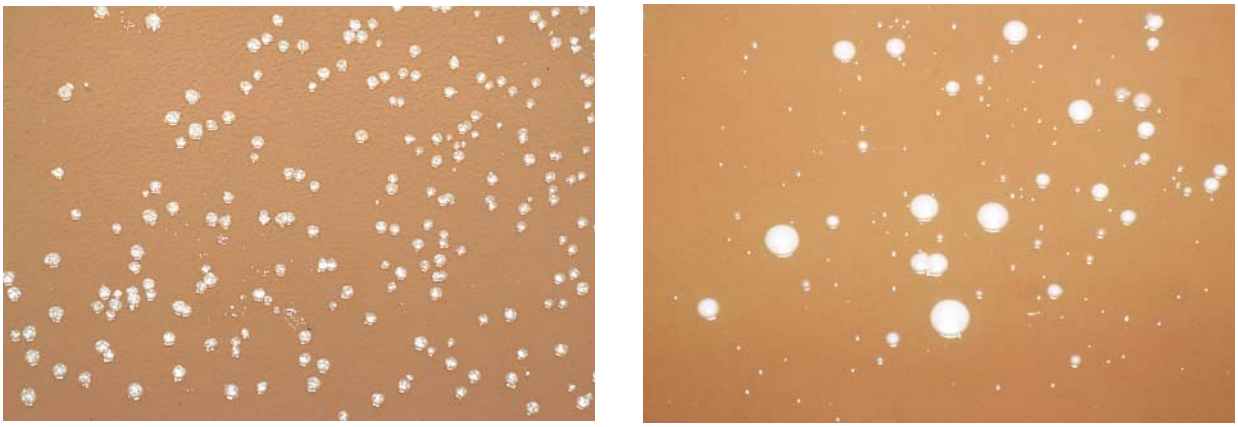
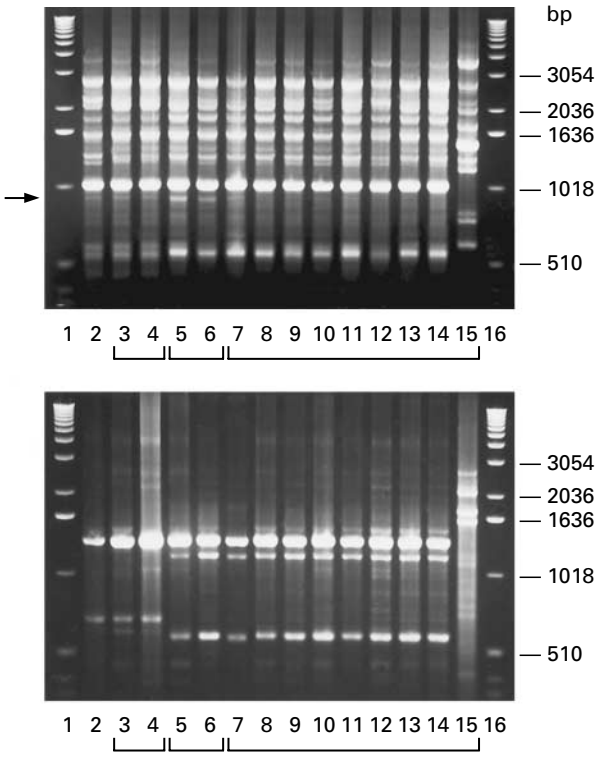


Figure 2. Primary Isolation of *B. henselae* and *B. quintana* from Biopsy Tissue of Cutaneous Bacillary Angiomatosis Lesions.

Primary isolation of *B. henselae* from a cutaneous bacillary angiomatosis lesion reveals colonies that are uniform in size, elevated, rough, gray, and deeply embedded in the chocolate agar (Panel A). Primary isolation of *B. quintana* from a cutaneous bacillary angiomatosis lesion on chocolate agar reveals colonies that are flat, round, smooth, shiny, opaque, and of heterogeneous size and that never appear rough or cause pitting of the agar (Panel B).

Figure 3. Fingerprint Patterns of Repetitive Extragenic Palindromic (REP) and Enterobacterial Repetitive Intergenic Consensus (ERIC) Polymerase-Chain-Reaction (PCR) Analysis of *B. henselae* Isolates from Patients with Bacillary Angiomatosis–Peliosis and *B. henselae* or *B. clarridgeiae* Isolates from Their Pet Cats.

The DNA fragments were separated by electrophoresis on an agarose gel and stained with ethidium bromide. Lanes 1 and 16 show DNA size standards. The upper panel shows the results of the REP PCR amplification and the lower panel the results of the ERIC PCR amplification of whole-cell DNA template for the following isolates: lane 2, *B. henselae* American Type Culture Collection strain 49882; lane 3, Patient 1; lane 4, cat of Patient 1; lane 5, Patient 2; lane 6, cat of Patient 2; lane 7, Patient 3; and lanes 8 to 15, cats of Patient 3. The patients and the respective cats to which they were exposed are grouped by brackets at the bottom of the figure. All isolates were identified as *B. henselae* by DNA sequencing except the isolate shown in lane 15, which is *B. clarridgeiae.* Two composite patterns were identified for the *B. henselae* isolates, and each patient's pattern matched that of the cat or cats to which he was exposed. The only exception was one of the cats belonging to Patient 3, which was infected with *B. clarridgeiae.* A distinct band of approximately 910 bp (arrow) that was unique to Patient 2 and Cat 2 was consistently identified by computer-assisted scanning.



DISCUSSION

We found that the risk factors and organ tropism associated with bacillary angiomatosis–peliosis differed according to the infecting species, *B. henselae* or *B. quintana*. In addition, the temporal pattern of occurrence of bacillary angiomatosis–peliosis in the San Francisco Bay area differed for these two species (Fig. 1). Interestingly, clustered *B. quintana* infection occurred simultaneously in Seattle (bacteremia in predominantly Native American, non–HIV-infected men)[21] and San Francisco (bacillary angiomatosis in predominantly white, HIV-infected persons) from January to June 1993. *B. quintana* was associated with homelessness, low socioeconomic status, and exposure to head or body lice in our study. Cases of *B. henselae* infection occurred sporadically and were epidemiologically linked with cat bites and scratches and owning a cat with fleas. Finally, treatment with macrolide antibiotics (e.g., erythromycin or clarithromycin) appeared to be protective.

Of nine bartonella species, only two, *B. quintana* and *B. henselae,* caused bacillary angiomatosis–peliosis in our 49 patients. Although *B. henselae* and *B. quintana* were equally likely to cause cutaneous bacillary angiomatosis (Table 1), only *B. henselae* was associated with hepatosplenic disease. Every patient in our study with peliosis hepatis was infected with *B. henselae,* and one fourth of our patients with *B. henselae* infection had peliosis. In addition, all six speciated cases of peliosis hepatis reported in the literature were associated with *B. henselae* infection, and none with *B. quintana*.[5,10,22,23] There was also a strong predilection for *B. henselae,* but not *B. quintana,* to cause lymph-node disease, whereas *B. quintana* was associated with osseous and subcutaneous infections.

A mammalian reservoir has been identified for most bartonella species: the domestic cat for *B. henselae*[14] and *B. clarridgeiae,*[24] the vole for *B. vinsonii,*[25] *B. grahamii,* and *B. doshiae,*[26] and the field mouse for *B. taylorii*.[26] Arthropod vectors have been identified for several bartonella species: the sand fly for *B. bacilliformis,* the body louse for *B. quintana,* and, most recently, the cat flea for *B. henselae*.[15] Transmission of *B. henselae* by the cat flea occurs readily among cats,[15] but epidemiologic data implicate the domestic cat as a major vector (by a scratch or bite) of *B. henselae* from cat to human.[16,27-29] The role of the flea in infection of humans by *B. henselae* may either be indirect, because of the higher prevalence of *B. henselae* infection in cats exposed to infected fleas, or direct, by transmission to humans from fleas, although this has not been documented. Strong evidence for direct transmission of *B. henselae* from infected cats to owners is provided by the match between the DNA-fingerprint patterns of the isolates from cats and their respective owners.

B. quintana infection occurred in thousands of troops in Europe during World War I, when it caused a relapsing febrile illness known as trench fever.[30] The principal vector during this epidemic of *B. quintana* was the body louse, *Pediculus humanus,* from a human reservoir. In our study, unmatched subanalysis for lice infestation revealed that only *B. quintana* infection was associated with lice infestation ($P = 0.03$), providing an epidemiologic association between lice and contemporary *B. quintana* infection. In addition, patients with *B. quintana* infection but not those with *B. henselae* infection were more often homeless and were of lower socioeconomic status than their matched controls and were thus at risk for exposure to lice.

We observed distinct differences between *B. henselae* and *B. quintana* with regard to optimal isolation medium and colony morphology. Inoculation of both agar types was necessary to optimize the recovery of bartonella from patient tissues and blood. Half the patients with bacillary angiomatosis–peliosis had bacteremia, which substantiates the systemic nature of this disease. Because culture of bacillary angiomatosis–peliosis lesions is extremely difficult, blood culture may be the most accessible technique for isolating bartonella species from patients with bacillary angiomatosis–peliosis.

We found that many of the in vitro susceptibilities reported in the literature[31,32] do not correlate with the in vivo response to treatment. In our case–control study, trimethoprim–sulfamethoxazole, ciprofloxacin, penicillins, and cephalosporins were not protective, and we were able to isolate bartonella from patients taking most of these antibiotics. In contrast, macrolide therapy was protective, and we were never able to isolate bartonella from a patient taking doxycycline, tetracycline, rifampin, or a macrolide (even a single dose[33]). Prophylaxis or treatment regimens for *Mycobacterium avium* complex that include a macrolide may provide simultaneous prophylaxis or treatment of bartonella infection.

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