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# Isolation of a New Subspecies, *Bartonella vinsonii* subsp. *arupensis*, from a Cattle Rancher: Identity with Isolates Found in Conjunction with *Borrelia burgdorferi* and *Babesia microti* among Naturally Infected Mice

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**Bacteremia with fever due to a novel subspecies of *Bartonella vinsonii* was found in a cattle rancher. The subspecies shared major characteristics of the genus *Bartonella* in terms of most biochemical features and cellular fatty acid profile, but it was distinguishable from other subspecies of *B. vinsonii* by good growth on heart infusion agar supplemented with X factor and by its pattern of enzymatic hydrolysis of peptide substrates. DNA relatedness studies verified that the isolate belonged to the genus *Bartonella* and that it was genotypically related to *B. vinsonii*. The highest level of relatedness was observed with recently characterized strains from naturally infected mice that were coinfected with *Borrelia burgdorferi* and *Babesia microti*. We propose the name *Bartonella vinsonii* subsp. *arupensis* subsp. nov. as the new subspecies to accommodate these human and murine isolates.**

*Bartonella vinsonii* was described as the Canadian vole agent in 1946 by Baker (1). It was further characterized by Weiss and Dasch (17), who proposed the name *Rochalimaea vinsonii* in 1982. The members of the genus *Rochalimaea* were then reclassified as *Bartonella* spp. on the basis of DNA relatedness and 16S rRNA sequence data by Brenner and coworkers in 1993 (5). *B. vinsonii* has not heretofore been implicated in human disease, although a subspecies (*Bartonella vinsonii* subsp. *berkhoffii*) causing canine endocarditis was recently described (3, 11).

The characterization of novel *Bartonella* species and subspecies continues (2, 7, 8, 10, 11, 13). A study originally intended to determine the prevalence of *Borrelia burgdorferi*, *Babesia microti*, and *Ehrlichia* species in Minnesota and Wisconsin mice identified, instead of any animals infected with *Ehrlichia* spp., four *Bartonella*-infected animals by using *Ehrlichia* PCR primers (9). Analysis of the citrate synthase gene sequence of one of the isolates showed that it was most closely related to *B. vinsonii*. Prior to that report, our laboratory received an isolate from the blood culture of a Wyoming man which we confirmed as *B. vinsonii*. In light of these two findings, we were led to compare the human isolate and the isolates from nature for DNA relatedness as well as to describe the Wyoming man's case and the isolate itself.

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## CASE REPORT

**Case history.** The patient is a 62-year-old Caucasian male who was admitted in May 1994 to a community hospital in Wyoming with acute onset of confusion, difficulty in walking, and facial numbness. The patient had been well 48 h prior to admission. He is a rancher by occupation, and he had been very active ranching and irrigating in the days prior to the onset of symptoms. Two days prior to admission he developed some low-grade "warm and cold" feelings and fatigue. He also experienced some dizziness, characterized as a feeling of instability when walking and doing chores. A mild headache and myalgias, but no stiffness or photophobia, were also reported. He took aspirin and ibuprofen for these symptoms. On the day of admission, his legs "got stiff," and he fell when trying to get up from a seated position while branding cattle. He was noted to have slurred speech and to be confused. There was no evidence of seizure activity. The pulse was rapid and irregular. No other symptoms were reported.

The medical history is significant for an ill-defined rheumatologic disease characterized by a high positive antinuclear antibody level, elevated erythrocyte sedimentation rate, positive rheumatoid factor, Sjögren's syndrome, and polyneuropathy, diagnosed in 1960 after a lengthy hospitalization in California. The rheumatologic syndrome is also characterized by variable neurological manifestations, including aseptic meningitis. Relapses are managed by high-dose intravenous steroids. In 1987 he was treated with prednisone for a presumed relapse of vasculitis when he presented with vertigo, headache, and numbness of the hands. Between exacerbations, he is relatively asymptomatic, and he does not require long-term steroid therapy.

TABLE 1. DNA relatedness of strain OK 94-513 to *Bartonella* strains[a]

| Source of unlabeled DNA | OK 94-513 | | | *Bartonella* strain 95 0726 14a | | | *B. vinsonii* subsp. *vinsonii* ATCC VR-152 | | |
|---|---|---|---|---|---|---|---|---|---|
| | % R at 55°C | % D | % R at 70°C | % R at 55°C | % D | % R at 70°C | % R at 55°C | % D | % R at 70°C |
| OK 94-513 | 100 | 0.0 | 100 | 72 | 3.5 | 78 | 73 | 5.0 | 62 |
| *Bartonella* strain 95 0726 14a | 82 | 2.5 | 80 | 100 | 0.0 | 100 | 69 | 4.5 | |
| *Bartonella* strain 95 0726 14b | 81 | 2.0 | 78 | 99 | 0.0 | 98 | 68 | 5.5 | |
| *Bartonella* strain 95 0726 14c | 80 | 2.5 | 77 | 96 | 0.0 | 99 | 65 | 5.0 | |
| *B. vinsonii* subsp. *vinsonii* ATCC VR-152$^T$ | 81 | 6.5 | 71 | 85 | 6.0 | 64 | 100 | 0.0 | |
| *B. vinsonii* subsp. *berkhoffii* ATCC 51672$^T$ | 72 | 7.0 | 57 | 73 | 7.0 | | 73 | 5.5 | 63 |
| *B. vinsonii* subsp. *berkhoffii* 93-CO1 | 75 | 6.0 | 61 | | | | 77 | 5.0 | 68 |
| *B. quintana* ATCC VR-358$^T$ | 60 | 11.0 | 40 | 57 | 11.5 | | | | |
| *B. henselae* ATCC 49882$^T$ | 63 | 11.0 | 40 | 52 | 11.0 | | | | |
| *B. elizabethae* ATCC 49927$^T$ | 68 | 13.5 | 36 | 53 | 12.0 | | | | |
| *B. grahamii* NCTC 12860$^T$ | | | | 50 | 11.0 | | | | |
| *Bartonella doshiae* NCTC 12862$^T$ | | | | 50 | 11.0 | | | | |
| *Bartonella koehlerae* C29$^T$ | 65 | 10.0 | 46 | 52 | 13.5 | | | | |
| *Bartonella clarridgeae* ATCC 51734$^T$ | | | | 27 | 13.5 | | | | |
| *B. bacilliformis* ATCC 35685$^T$ | 42 | 13.0 | | 23 | 12.5 | | | | |

[a] *Bartonella* 95 0726 14a, -b, and -c represent second, third, and fourth passages of the same strain. % R, percent relatedness to labeled DNA.

On admission, the patient had a temperature of 100.9°F (38.3°C). Blood pressure was 150/90 mm Hg, the pulse was 120/min, and the respiratory rate was 16/min. He appeared encephalopathic but in no acute distress. A skin examination revealed no stigmata of vasculitis. Examination of the head, eyes, ears, nose, and throat was normal. The neck was supple, and there was no adenopathy. The heart rate was irregularly irregular without murmurs, rubs, or gallops. The lungs were clear to percussion and auscultation. There was no hepatosplenomegaly or mass found on abdominal examination. The neurological examination was significant for decreased sensation in the area innervated by the right-fifth-cranial nerve. There was a palsy of the right fourth cranial-nerve, and the gag reflex was absent. He had bilateral diplopia and slurred speech. There was no visual-field defect. He had hyperreflexia with bilateral clonus. He exhibited decreased concentration and emotional lability on mental-status examination but was otherwise fully oriented.

The peripheral leukocyte count was 8,700/μl with 81% neutrophils, 8% band forms, and 10% lymphocytes; platelet count was 166,000/μl and the hematocrit was 41.3%. A cerebrospinal fluid (CSF) analysis was performed, and there were three leukocytes, undifferentiated. CSF protein was 49 mg/dl, and glucose was 45 mg/dl (peripheral glucose, 120 mg/dl). Other CSF study results included a serum-to-CSF immunoglobulin G ratio of 0.31, positive oligoclonal bands, but a normal immunoglobulin G index. The erythrocyte sedimentation rate was 45 mm/h. A chemistry screen was normal except for a serum glutamic oxalacetic transaminase level of 53 μ/liter and a lactate dehydrogenase level of 190 μ/liter. An antinuclear antibody test was positive, showing a 1:2,560 titer with a speckled pattern. Magnetic resonance imaging of the head showed an area of increased signal in T2-weighted images in the middle and superior portions of the pons. A computerized axial tomography scan of the head was normal. An echocardiogram showed moderate mitral and tricuspid insufficiency and mild aortic insufficiency but no evidence of endocarditis. An electrocardiogram revealed atrial flutter. Blood cultures were obtained on admission, including one set in BACTEC bottles and one in an Isolator, which grew five colonies of a gram-negative bacillus at day 7 on chocolate agar. The organism was sent to the microbiology laboratory of Associated and Regional University Pathologists, Inc., for identification. The organism was identified as a *Bartonella* species, but not *B. henselae* or *B. quintana*. The organism was then sent to D. F. Welch for further characterization.

The patient initially had 24 to 36 h of temperatures as high as 39°C; he received a single 1-g dose of ceftriaxone, after which he defervesced. Prednisone (40 mg) was administered for presumptive cerebral vasculitis related to Sjögren's syndrome. Anticoagulation was begun because of the atrial flutter. He was discharged after 7 days of hospitalization with a significant improvement in neurologic symptoms. He has gradually been tapered off steroids; persistent neurologic deficits include mild dizziness, slight difficulty in swallowing, and mild ophthalmoplegia. He has not required hospital admission since 1994, and he continues an active lifestyle as a rancher in rural Montana.

## MATERIALS AND METHODS

**Strains.** The following type strains were obtained from the American Type Culture Collection: *Bartonella henselae* ATCC 49793, *Bartonella quintana* ATCC 51694, *Bartonella vinsonii* subsp. *vinsonii* ATCC VR-152, *B. vinsonii* subsp. *berkhoffii* ATCC 51672, and *Bartonella elizabethae* ATCC 49927. The isolate described in "Case history" was designated OK 94-513. Cultures were maintained on Columbia agar supplemented with 5% defibrinated sheep blood.

**DNA relatedness studies.** The strains used in DNA relatedness determinations are listed in Table 1. The methods used for isolation and purification of bacterial DNA and the hydroxyapatite method used for DNA relatedness determinations have been described previously (4). Divergence (D) was calculated to the nearest 0.5%. All reactions were done at least twice.

**Phenotypic studies.** Conventional biochemical tests and commercial panels of biochemicals were used as described elsewhere (15, 18). Briefly, inocula were obtained from cultures growing on Columbia blood agar that were incubated for 5 to 7 days at 35°C in 5 to 10% $CO_2$. For determining biochemical reactivity based on preformed enzymes, suspensions equivalent to a McFarland no. 2 turbidity standard were prepared in sterile water. A dehydrated panel containing 24 substrates (Rapid Anaerobe Panel; Dade International, Inc., West Sacramento, Calif.) was inoculated and incubated at 35°C for 4 h, after which the results were interpreted according to the manufacturer's directions. Suspensions equivalent to a 0.5 McFarland turbidity standard were prepared in saline for determining X-factor growth requirements, with strips (Difco, Detroit, Mich.) applied to heart infusion agar, or in Trypticase soy broth for susceptibility testing by the E-test technique (AB Biodisk, Culver City, Calif.) on Columbia blood agar. Cellular fatty acid compositions were analyzed by gas-liquid chromatography, and immunofluorescence tests were performed as previously described (15, 19).

TABLE 2. Differential phenotypic characteristics of the case isolate compared to those of representative strains of other *Bartonella* species or subspecies[a]

| Test | Case isolate OK 94-513 | *B. vinsonii* subsp. *vinsonii* ATCC VR-152 | *B. vinsonii* subsp. *berkhoffii* ATCC 51672 | *B. quintana* ATCC 51694 | *B. henselae* ATCC 49793 | *B. elizabethae* ATCC 49927 |
|---|---|---|---|---|---|---|
| Growth on heart infusion agar + X factor | + | w | − | w | − | + |
| Twitching motility | − | − | − | + | + | − |
| L-Leucine-β-naphthylamidase | + | + | + | + | + | + |
| L-Lysine-β-naphthylamidase (alkaline) | + | + | + | + | + | + |
| L-Lysine-β-naphthylamidase (acidic) | − | − | + | − | + | w |
| L-Proline-β-naphthylamidase | − | − | + | + | + | − |
| L-Tryptophan-β-naphthylamidase | + | + | + | + | + | + |

[a] +, positive; −, negative; w, weakly positive.

## RESULTS

**DNA relatedness studies.** Labeled DNAs from the patient strain, OK 94-513, and from three separate passages of mouse strain 95 0726 14a were reacted with unlabeled DNAs from each other and from type strains of *Bartonella* species (Table 1). Relatedness of the patient strain and the passages of the mouse strain was 72 to 82% at 55°C (optimal criterion for DNA reassociation), with a D of 2.0 to 3.5%. Relatedness at the stringent (70°C) reaction temperature was 77 to 80%. Reciprocal DNA relatedness of the human and mouse strains to *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *berkhoffii* was 65 to 85% at 55°C, with a D of 4.5 to 7%; relatedness in 70°C reactions was 57 to 71%. Relatedness of the human and mouse strains to all other *Bartonella* species for which strains are available (there are no strains for *Bartonella talpae*, *Bartonella peromysci*, and *Bartonella taylorii*) was 23 to 68% at 55°C, with a D of 10 to 13.5%. Similar results were obtained when labeled DNAs from the type strains of *B. vinsonii* subsp. *vinsonii* (Table 1) and *B. vinsonii* subsp. *berkhoffii* were used.

**Phenotypic characterization of isolate.** The organism was initially recognized as a fastidious, small, gram-negative rod, based on its Gram staining properties and its recovery on chocolate agar after 7 days of incubation in an Isolator-processed blood culture. It was catalase and oxidase negative, and it was unreactive with immunodiagnostic reagents specific for *Brucella*, *Francisella*, *B. henselae*, and *B. quintana*. The cellular fatty acid profile was consistent with *Bartonella* spp., however, characterized by a large amount (~50%) of $C_{18:1\omega7C}$ and only four other cellular fatty acids in significant amounts: $C_{16:0}$, 13%; $C_{17:0}$, 9%; $C_{17:1\omega6C}$, 3%; and $C_{18:0}$, 16%.

Biochemical analysis with preformed enzyme-dependent substrate utilization assays revealed reactivity with substrates bis *p*-nitrophenyl-phosphate, L-leucine-β-naphthylamide, L-lysine-β-naphthylamide (alkaline), glycylglycine-β-naphthylamide, glycine-β-naphthylamide, L-arginine-β-naphthylamide, L-tryptophan-β-naphthylamide, and DL-methionine-β-naphthylamide (weakly positive); there was no reactivity with substrates *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, *p*-nitrophenyl-*N*-actelyl-β-D-glucosaminide, *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-phosphate, *p*-nitrophenyl-α-L-fucopyranoside, *p*-nitrophenyl-α-D-mannopyranoside, L-lysine-β-naphthylamide (acid), L-prolyl-β-naphthylamide, L-pyrrolindonyl-β-naphthylamide, 3-indoxyl phosphate, trehalose, urea, indole, and nitrate. A comparison of these results with those which could help distinguish other *Bartonella* species or subspecies is shown in Table 2. Also shown in Table 2 are the results of motility testing by wet mount and growth tests on heart infusion agar plus X factor, which also distinguish the isolate from *B. henselae* and from *B. quintana*, although the latter grew weakly in the presence of the X factor.

The isolate displayed in vitro susceptibility (MIC < 0.5 μg/ml) to ampicillin, penicillin, cefotaxime, ciprofloxacin, chloramphenicol, erythromycin, clarithromycin, and tetracycline. The MIC of gentamicin was 3.0 μg/ml.

## DISCUSSION

The exact contribution of the *Bartonella* isolate to this patient's illness is unclear. He clearly was bacteremic, and he clearly had very high fever. The infection may have prompted a relapse of the vasculitis and the associated neurologic manifestations of his ill-defined rheumatologic syndrome. Occupational exposure seems to have been the likely means of acquiring the infection. There is anecdotal evidence of an abundance of voles in the locales of Montana in which this man worked. Ample opportunity probably existed for ectoparasitic exposure (ticks, fleas, and biting flies), but none was documented.

In a few parts of the United States where rodent populations have been studied, *Bartonella* spp. have been found to be prevalent among mice and rats. Kosoy et al. (12) detected *Bartonella* in roughly 50% of the rodent populations in the southeastern United States, and Hofmeister et al. (9) detected a novel *Bartonella* species in the blood of mice in Minnesota and Wisconsin in the course of studies on the reservoirs of tick-borne pathogens. The new *Bartonella* species was detected incidentally, along with *Borrelia burgdorferi* and *Babesia microti*, when PCR primers targeting *Ehrlichia* spp. resulted in the amplification of *Bartonella*-like 16S ribosomal DNA segments. These findings, as well as those of this case, suggest that *Bartonella* spp. may occasionally be transmitted by ticks. Prior to the current understanding of the epidemiology of cat scratch disease, Lucey et al. (14) reported two cases of bacteremia due to *B. henselae* in immunocompetent men who had sustained tick bites prior to their illnesses. In addition to the well-documented role of fleas in the transmission of *B. henselae* (6) and the classic examples of louse-borne trench fever due to *B. quintana* and oroya fever due to *Bartonella bacilliformis* acquired from sand flies, it is possible that more than one arthropod vector of *Bartonella*-associated diseases exists.

The *Bartonella* strains isolated from mice by Hofmeister et al. (9) were most closely related to *Bartonella grahamii* (2) on the basis of 16S rRNA sequence analysis and were most closely related to *B. vinsonii* subsp. *vinsonii* on the basis of citrate synthase gene sequence analysis. Our DNA relatedness studies indicated that mouse isolate 95 0726 14a from the study of Hofmeister et al. and human strain OK 94-513 fulfilled the molecular definition of a species as strains with 70% or more DNA relatedness (at an optimal reassociation criterion) and a D of 5% or less within related sequences (16). On the basis of this definition, the human and mouse strains were easily differentiated from all *Bartonella* species other than *B. vinsonii*.

The relatedness to *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *berkhoffii* was at the species level, except that D was almost always 5 to 7%. These data left us with the choice of creating a new species for these strains or creating a third subspecies for them within *B. vinsonii*. We chose the second alternative because of the similarity of relatedness of the new strains to that previously seen within existing *B. vinsonii* subspecies (11). We therefore now describe *Bartonella vinsonii* subsp. *arupensis* subsp. nov.

**Description of *B. vinsonii* subsp. *arupensis* subsp. nov.** *Bartonella vinsonii* subsp. *arupensis* (a.rup.en.′sis. N.L. fem.adj. *arupensis*, from Associated and Regional University Pathologists, Inc., the laboratory where the type strain was initially characterized). Exhibits characteristics of the species *B. vinsonii*. Grows on heart infusion agar in the presence of X factor. Catalase, oxidase, and motility negative. Does not react with monospecific antisera to *B. henselae* or *B. quintana*. Biochemical characteristics that may be used to distinguish it from other *Bartonella* spp. are shown in Table 2. Isolated from a human and mice (*Peromyscus leucopus*). Presumptively pathogenic for humans.

The type strain is OK 94-513 (= ATCC 700727), isolated from a 62-year-old bacteremic man.

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