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Infection with *Bartonella weissii* and Detection of *Nanobacterium* Antigens in a North Carolina Beef Herd

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Very recently, *Bartonella* organisms have been isolated from large ruminants (deer, elk, and dairy and beef cattle) located in the United States and in France. In this study, we report the serologic, microbiologic, and molecular findings related to the isolation of a *Bartonella* species in North Carolina beef cattle and the detection of nanobacterial antigen using a commercially available enzyme-linked immunosorbent assay. Between August 1998 and September 1999, blood was collected from 38 cattle ranging in age from 1 month to 6.5 years. After a 1-month incubation period, a *Bartonella* sp. was isolated on a 5% rabbit blood agar plate from three of six EDTA blood samples. PCR amplification of the 16S rRNA gene from all three isolates resulted in a DNA sequence that was 100% identical to that of *B. weissii* 16S rRNA (GenBank no. AF199502). By IFA testing, 36 of 38 cattle had antibodies (≥1:64) to *Bartonella weissii* (bovine origin) antigens. Nanobacterial antigen was detected in 22 of 22 serum samples. We conclude that infection with an organism similar or closely related to *B. weissii* can occur in North Carolina cattle and that although their actual existence is still controversial *Nanobacterium* antigens were detected with a commercially available test kit. The epidemiology, vector biology, and potential pathogenicity of these organisms in cattle deserve future consideration.

Bartonella spp. comprise an important group of vector-transmitted, intracellular bacterial pathogens (2). Diverse vectors, including sandflies, lice, mites, fleas, ticks, and potentially other insects can transmit these organisms among reservoir hosts (2, 6). Recently, other investigators have isolated *Bartonella* spp. from cattle (*Bos domesticus*) in Oklahoma and California (5; B. B. Chomel, R. W. Kasten, Y. Yamamoto, C. Chang, T. E. Honadel, and Y. Kikuchi, Abstr. First Int. Conf. *Bartonella* Emerg. Pathogens, p. 31, 1999) and from cattle in France (H.-J. Boulouis, R. Heller, F. Barrat, B. Van Laere, D. Thibault, F. Claro, S. Chastant, E. Plouzeau, A. Lecu, F. Ollivet, J. Rigoulet, X. Legendre, P. Moisson, M. Leclerc-Cassan, B. B. Chomel, and Y. Piemont, 2nd Int. Conf. Emerg. Zoonoses, 61, 1998). *Bartonella* spp. have also been isolated from wild ruminants, including mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*) from California and roe deer (*Capreolus capreolus*) from France (R. Heller, M. Kubina, G. Delacour, F. Lamarque, G. Van Laere, R. Kasten, et al., Int. Conf. Emerg. Infect. Dis., p 21.18, 1998).

Although Carrion's disease, caused by *B. bacilliformis*, and trench fever, caused by *B. quintana*, have long-standing medical histories, several new *Bartonella* species or subspecies (*Bartonella henselae, B. clarridgeiae, B. elizabethae, B. vinsonii* subsp. *berkhoffii* and *B. vinsonii* subsp. *arupensis*) have been implicated as human pathogens within the past decade (1, 14, 21). Based upon their very recent recognition, the pathogenic potential of *Bartonella* spp. in cattle, as well as in most other animal species, remains to be clarified. However, accumulating evidence indicates that *Bartonella* spp. can cause disease manifestations in some domestic animal species. In dogs, *B. vinsonii* subsp. *berkhoffii* causes granulomatous lymphadenitis, granulomatous rhinitis, myocarditis, and endocarditis (3, 4, 19). *B. henselae* has been associated with peliosis hepatitis in a dog (13). Of comparative medical interest, *B. henselae* is the only *Bartonella* species that has been associated with peliosis hepatitis in canine and human patients (13, 14). Despite the frequent isolation of *B. henselae* from seemingly healthy, flea-infested cats, evolving evidence from several laboratories indicates that *B. henselae* represents a previously unrecognized cause of chronic, insidious disease manifestations in cats (6, 9, 16).

Although their actual existence, is controversial, nanobacteria are purportedly intracellular pathogens that can persist within intracellular compartments of the host for years (8, 12). Nanobacteria appear to cause a variety of cytotoxic manifestations in cell culture, a feature that was responsible for their ultimate discovery (8, 12). Based upon the respective 16S rRNA gene sequences, *Bartonella* and *Nanobacterium* spp. are members of the alpha subdivision of the *Proteobacteria* and purportedly share cross-reacting epitopes (8, 12). In Europe, Kajander and colleagues have reported that more than 80% of commercial bovine serum lots contain *Nanobacterium* spp. (8, 12). To our knowledge, data related to *Nanobacterium* infection in cattle is not yet available from the United States.

In 1997, as a component of ongoing studies related to *Bartonella* spp., we detected seroreactivity to *B. vinsonii* subsp. *berkhoffii* antigens by immunofluorescent antibody testing of blood samples obtained from a beef herd in Wake County, N.C. Initial attempts in our laboratory to isolate *Bartonella* spp. from these seroreactive cattle, using lysis centrifugation or

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freeze-thaw techniques, were unsuccessful. Recent documentation of *Bartonella* bacteremia in cattle from the United States and France renewed efforts in our laboratory to isolate *Bartonella* spp. from seroreactive beef cattle on a farm in North Carolina. We now report serologic, microbiologic, and molecular findings, as related to a *Bartonella* sp. that was isolated from North Carolina beef cattle. We also report the detection of nanobacterial antigen using a commercially available enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Description of the herd. Between August 1998 and September 1999, blood was collected from 38 cattle ranging in age from 1 month to 6.5 years (mean age, 19.8 months). At the time of sampling, 25 calves were less than 1 year old, and the remaining 13 samples were obtained from adult cows ranging in age from 1 to 6.5 years, with a mean age of 4.4 years. The cow-calf herd, which consisted of full-blooded and crossbred Salers, was established in 1992, when adult cows and a Salers bull were purchased from another farm in Wake County, N.C. Although beef cattle were maintained on an adjacent farm, there had been no introduction of new cattle into the herd between 1992 and September 1999. During this time period, fescue toxicity—characterized by severe lameness, loss of the tail switch; and edema, swelling, and pain in the region of the coronary band—was the only serious medical disorder reported by the owner. Clinical manifestations of fescue toxicity affected approximately one-third of the herd, with full-blooded Salers more likely to be severely affected, perhaps reflecting a genetic susceptibility. During the 7-year period following introduction of the herd, reproductive performance was considered excellent. There were one aborted fetus, one calf born dead within the placenta, two instances of retained placenta, and an occasional dystocia, particularly in first-calf heifers that were born on the farm. Otherwise, each cow produced and raised a calf annually.

Microimmunofluorescence assay (IFA). Using a previously described technique (19), antigens derived from Vero cell culture-grown *B. vinsonii* subsp. *berkhoffii* and a *Bartonella* sp. isolate derived from cow 85 in this study were placed on Teflon-coated multiwell slides and stored at −20°C until used for analyses. Serially diluted sera were added to each well containing a fixed concentration of antigen. Antibody reactivity was determined by immunofluorescence using anti-bovine immunoglobulin G conjugated to fluorescein isothiocyanate. For diagnostic purposes, a titer of ≥1:64 was considered indicative of exposure to a *Bartonella* sp.

Bacteriological methods. For isolation of *Bartonella* spp., 1 ml of EDTA-anticoagulated blood was inoculated directly onto a blood agar plate (5% rabbit blood trypticase soy agar) and incubated for 2 months at 35°C, in 5% CO_2. Isolates were examined for morphological characteristics by light microscopy.

DNA extraction. DNA from three isolates obtained from three different cows was obtained using the QIAamp DNA Blood Minikit (Qiagen Inc., Valencia, Calif.), as recommended by the manufacturer.

16S rRNA gene amplification by PCR. Primers PC5A (5′CCTTGTTACGACTTCACCC) and PO-C (5′AGAGTTTGATCCTGG) were employed for 16S rRNA gene amplification. Primers were obtained from Integrated DNA Technologies, Inc., in Coralville, Iowa. PCR cycling parameters for the reactions were 95°C for 10 min, 35 cycles at 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min, followed by a 72°C extension for 5 min. DNA was analyzed on a 1% agarose gel, stained with ethidium bromide, and photographed. For PCR, 0.2 mM deoxynucleoside triphosphate, a 1 μM concentration of each primer, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 0.001% gelatin (wt/vol), 3 mM $MgCl_2$, and 1.25 U. of Ampli Taq Gold (PE Applied Biosystems, Foster City, Calif.) were combined in 50-μl reaction mixtures.

DNA sequencing. DNA fragments from three isolates were used directly for DNA sequencing, which was performed at the Central Sequencing Laboratory, University of North Carolina at Chapel Hill, on a model 373A DNA sequencer (Applied Biosystems) using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). All samples were submitted to the sequencing facility with 70 ng of DNA and 10 pmol of each primer (Integrated DNA Technologies, Inc [see below]) premixed at a total volume of 20 μl. Bidirectional sequencing was performed. Forward primers used previously[34] were POC (5′AGAGTTTGATCCTGG), P1 (5′ACTCCTACGGGAGGCAGCAGT), P3MOD (5′ATTAGATACCCTGGTAGTCC), and P4 (5′GAGGAAGGTGGGGATGACGTCAA). Primers used previously in the reverse direction were PC5A (5′CCTTGTTACGACTTCACCC), PC4 (5′TTGACGTCATCCCCACCTTCCTC), PC3 (5′GGACTACCAGGGTATCTAAT), and PC1 (5′ACTGCTGCCTCCCGTAGGAGT).

TABLE 1. Results of blood culture, bartonella serology, and nanobacterial antigen testing in a North Carolina beef herd[a]

Cow no.	Date of birth (mo/yr)	Sex	Blood culture result	Reciprocal titer of antibody to:		Reaction with nanobacterial antigen
				B. vinsonii subsp. *berkoffii*	*B. weissii*	
286	10/92	Heifer	POS	64	512	4+
432	10/92	Heifer	ND	32	256	4+
440	10/92	Heifer	ND	128	256	4+
495	10/92	Heifer	ND	16	128	4+
9	4/93	Heifer	ND	<16	16	4+
10	4/93	Heifer	POS	<16	32	4+
42	1/95	Heifer	ND	<16	128	4+
49	4/95	Heifer	ND	64	128	ND
85	6/96	Heifer	POS	16	512	4+
119	8/97	Heifer	ND	32	512	ND
127	3/98	Heifer	ND	32	1,024	ND
128	4/98	Bull	ND	64	512	ND
137	7/98	Bull	ND	16	256	ND
139	8/98	Bull	ND	<16	1,024	4+
140	8/98	Heifer	ND	<16	256	4+
144	12/98	Bull	ND	<16	1,024	ND
145	2/99	Heifer	ND	<16	256	4+
146	2/99	Heifer	ND	<16	128	ND
147	3/99	Bull	ND	<16	256	ND
148	3/99	Bull	ND	<16	256	ND
149	3/99	Bull	ND	<16	128	ND
150	3/99	Heifer	ND	<16	128	4+
141	3/99	Heifer	ND	<16	128	4+
151	4/99	Heifer	ND	<16	128	4+
152	4/99	Heifer	ND	<16	64	4+
153	4/99	Bull	ND	<16	256	4+
154	4/99	Bull	ND	<16	128	4+
155	5/99	Heifer	ND	<16	128	4+
156	5/99	Bull	ND	<16	256	ND
157	5/99	Bull	ND	128	128	4+
158	5/99	Bull	ND	128	256	4+
159	5/99	Bull	ND	128	512	4+
161	6/99	Bull	NEG	256	1,024	4+
162	6/99	Bull	NEG	256	512	4+
163	6/99	Heifer	ND	64	512	ND
164	6/99	Heifer	ND	128	512	4+
165	7/99	Bull	ND	128	512	4+
166	7/99	Heifer	ND	64	128	ND

[a] Abbreviations: POS, positive; ND, not determined; 4+, strongly positive reaction.

Each PCR product was purified with a QIAquick PCR purification kit (Qiagen). The GeneBee Service, from Moscow State University (www.genebee.msu.su), was used to compare the alignments of the sequences derived from the three cow isolates. BLAST analysis was used to compare the sequences to other 16S rRNA gene sequences in GenBank.

Nanobacterial antigen detection by ELISA. A commercially available monoclonal antibody kit (ELISA) from Nanobac Oy, Kuopio, Finland, was used to detect *Nanobacterium* antigens in EDTA-anticoagulated plasma or serum samples. Due to limited kit availability, only a subset of the herd that included 22 cows ranging in age from 1 month to 6.5 years (mean age, 18.9 months) were tested, as recommended by the manufacturer. In addition to the kit's negative control, stored frozen sera from two 13-month-old female specific-pathogen-free beagles that were raised and maintained in controlled vector-free conditions were tested as nonbovine controls. In addition, sera from 30 sick dogs with a wide range of disease manifestations were also tested. The standards, prepared by the company, and the test sera were added in the wells, washed with the buffer supplied by the manufacturer, and allowed to react with the tracer (horseradish peroxidase-conjugated mouse monoclonal antibody). Reactors were detected by a substrate solution supplied by the manufacturer and graded as negative (no reaction), 1+, 2+, 3+, or 4+.

RESULTS

When initially tested by IFA, only 15 of 38 cattle were seroreactive (≥1:64) to *B. vinsonii* subsp. *berkhoffii* antigens (Table 1). Repeat testing using a *Bartonella* sp. isolate obtained from the herd (isolate from cow 85) identified 36 of 38 serum samples as seroreactive (≥1:64) to *B. weissii* antigens. Reciprocal titers to *B. weissii* antigens ranged from 16 to 1,024, whereas cross-reactive reciprocal antibody titers to *B. vinsonii* subsp. *berkhoffii* ranged from 16 to only 256. Twenty-one samples that were originally nonseroreactive to *B. vinsonii* subsp. *berkhoffii* were subsequently found to be seroreactive to *B. weissii* at reciprocal titers of ≥1:64. Of the 25 calves ranging in age from 1 to 8 months, reciprocal titers were 64 ($n = 1$), 128 ($n = 8$), 256 ($n = 7$), 512 ($n = 6$) or 1,024 ($n = 3$). In the two youngest calves (1 month of age at the time of sampling), reciprocal titers were 256 and 512, respectively. The distribution of antibody titers in adult cows (ranging in age from 1 to 6.5 years) was similar to the distribution in calves that were less than 1 year old. Two of the older cows sampled (cows 9 and 10, both 6.5 years old) had the lowest reciprocal titers (16 and 32, respectively) detected in the herd, despite the concurrent isolation of bartonella from cow 10.

After at least 1 month of incubation, *B. weissii* was isolated on a 5% rabbit blood agar plate from three of six randomly selected EDTA blood samples. Colonies ($n = 1$ for cow 85, $n = 3$ for cow 10, and $n = 30$ for cow 286) became visible on blood agar plates between 3 and 6 weeks following inoculation. The reciprocal *B. weissii* IFA titers for cows 85, 10, and 286, at the time of culture, were 512, 32, and 512, respectively. The colonies were small, irregular, approximately 1 to 1.5 mm in diameter, opaque, rough, raised, and white. On subsequent passages, the colonies became smooth and round in appearance. By Gram staining, the microorganisms were found to be small gram-negative rods. Due to the relatively inert nature of bartonella organisms, biochemical characterization was not performed in our laboratory.

PCR amplification and DNA sequencing resulted in at least 1,400 base segments, from each of the three isolates (cows 85, 10, and 286), that were identical to each other and were 100% identical to the 16S rRNA gene of *B. weissii* (GenBank accession no. AF199502). The GenBank accession number for the 16S rRNA gene sequence derived from the *B. weissii* isolate from cow 10 is AF291746. As a component of a larger multiinstitutional study, all three isolates have been sent to the Centers for Disease Control and Prevention, Atlanta, Ga., for total genomic DNA hybridization and for pan-genomic fragment pattern analysis.

Nanobacterial antigen was detected using a commercially available ELISA kit in 22 of 22 cow plasma or serum samples (Table 1). All 22 samples resulted in very strong (4+) reactions, regardless of the age of the animal or the reciprocal antibody titer to *B. wessii* antigens. In contrast, nanobacterial antigen was not detected in the serum of two healthy specific-pathogen-free dogs or in sera from 27 of 30 sick dogs with a wide range of disease manifestations. Weak positive reactions (1+ or 2+) were obtained for three dogs evaluated for endocarditis, neutrophilic polyarthritis, or a history of extensive tick exposure, and antibodies to *B. vinsonii* antigens were detected only in the dog with a history of heavy tick exposure (reciprocal titer, 256).

DISCUSSION

Recently, *Bartonella* spp. have been isolated from 5 of 12 (42%) Oklahoma cattle, from 58 of 116 (50%) California cattle, and from 39 of 42 (93%) California mule deer (5). In one California beef herd, *Bartonella* was isolated from 25 of 26 bulls (96%) and 22 of 27 (82%) cows, when sampled at a single point in time. In the present study, *B. weissii* or a closely related organism was isolated from 3 of 6 (50%) blood samples. As previous efforts to isolate a *Bartonella* sp. from cows in this herd using lysis centrifugation or freeze-thaw techniques were not successful, samples from only a subset of the herd were cultured as a component of this study. As such, colony numbers were extremely low (1, 3, or 30 colonies) in the three cows from which *B. weissii* was isolated. As seroprevalence, based upon *B. weissii* reciprocal titers of ≥1:64, was 95%, exposure to the organism appears to be substantial. Presumably culture of the organism from blood is not a very sensitive diagnostic technique for the detection of bacteremia. As illustrated in Table 1, seroprevalence increased from 39 to 95% when samples were tested against a *B. weissii* isolate obtained from a cow in the herd, indicating that the use of homologous antigens for serologic testing can be of diagnostic importance.

On an evolutionary basis, cattle have presumably developed a highly sophisticated immunologic response to *B. weissii*, which may be protective against serious pathology. However, the extent to which this previously unrecognized organism might represent a cofactor in the development of the recurrent disease manifestations, such as fescue toxicity, in this herd or the severe decompensation experienced by some cattle following the stress of prolonged transport should be examined in future seroepidemiologic studies. Although transplacental transmission of *B. weissii* antibodies or transplacental transmission of the organism was not assessed in this study, calves ranging in age from 1 to 8 months had similar, relatively high titers of antibody to *B. weissii*. Although there was probably maternal transfer of antibodies through the colostrum, these results appear to support the argument for exposure to a putative vector early in life, followed by chronic infection. Chronic *Bartonella* bacteremia, months in duration, has been reported in cats (28 months), dogs (14 months), and immunocompetent humans (4 months to perhaps years) (2, 15, 16). Collectively, these observations indicate that *Bartonella* spp. are highly adapted to causing persistent infection in the animal host. Based upon the high rate of isolation in cattle from Oklahoma (42%), California (50%), and North Carolina (50%), persistent infection seems likely in cattle, as is the case in other animal species (15).

B. weissii, originally isolated from domestic cats, was provisionally described by Regnery and colleagues at the Centers for Disease Control in Atlanta, Ga. (R. Regnery, N. Marano, P. Jameson, E. Marston, D. Jones, S. Handley, C. Goldsmith, and C. Greene, 15th Meet. Am. Soc. Rickettsiol., abstr. 4, 2000). In conjunction with *B. henselae, B. clarridgeiae*, and *B. koehlerae, B. weissii* represents the fourth *Bartonella* species found to infect cats in North America. To date, *B. weissii* isolates from cats have been obtained from Utah and from Illinois. Unlike *B. henselae, B. bacilliformis, B. clarridgeiae*, and *B. weis-*

sii are flagellated bacteria. As most *Bartonella* species appear to be more highly adapted to a specific host, detection of *B. weissii* in both cats and cattle may reflect an unusual evolutionary adaptation for this particular *Bartonella* species. Alternatively, infection in cats may represent an infrequent occurrence rather than adaptation in both cats and cows.

Recently, *B. vinsonii* subsp. *arupensis* was isolated from the blood of a febrile encephalopathic cattle rancher who became ill shortly after processing cattle (22). Although presumably infrequent, zoonotic infection obtained while processing or butchering cattle infected with *B. weissii* or *B. vinsonii* subsp. *arupensis* may occur. Epidemiologic studies have shown that seroprevalence to bartonella antigens is higher in veterinarians than in the general public, which may represent an increased risk of exposure associated with more frequent animal contact (17, 18). Based upon the recent discovery of bartonella infection in cattle, a seroprevalence study involving individuals working with cattle or working in meat processing plants might be indicated.

To our knowledge, data related to *Nanobacterium* infection in cattle are not available from United States. As *Nanobacteria* purportedly share similar surface antigens with *Bartonella* spp., there may be serologic cross-reactivity; however, the extent to which cross-reactivity occurs has not been well characterized (11, 12). Because of the potential for serologic cross-reactivity among these two organisms, we tested a subgroup of the herd with a commercially available antigen ELISA test kit. Surprisingly, 100% (22 of 22) of the cattle tested in this herd had very high levels of circulating nanobacterial antigens. In contrast, 29 of 32 dog samples did not contain detectable levels of nanobacterial antigens. Assuming the specificity of the monoclonal antibody for the detection of nanobacterial antigens, these results would support a high rate of nanobacterial infection in this herd. Whether a correlation exists between the detection of nanobacteria antigens and infection with *B. weissii* in this herd was not determined, due to the presumed relative insensitivity of bartonella blood culture as used in this study.

In humans and experimentally infected laboratory animals, *Nanobacteria* can cause chronic infection (1, 8, 12). For example, *Nanobacteria* were reportedly isolated from the blood of one person for five years, despite the concurrent presence of antibodies. Following experimental infection of rabbits with *Nanobacteria* derived from fetal bovine sera, organisms can be isolated from urine or cerebrospinal fluid for up to 1 year (1, 12). Most recently, a role for *Nanobacterium* in the development of human kidney stones and polycystic kidney disease has been proposed (8, 10). Future studies should examine the potential relationship between exposure to and infection with *Bartonella* and *Nanobacterium* species, the extent to which these organisms might contribute to disease manifestations in cattle and the extent to which cattle might serve as a reservoir for human infection.

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REFERENCES

1. **Akerman, K. K., J. T. Kuikka, N. Ciftcioglu, J. Parkkinen, K. A. Bergstrom, I. Kuronen, and E. O. Kajander.** 1997. Radiolabeling and *in vivo* distribution of *Nanobacteria* in rabbits. Proc. SPIE Int. Opt. Eng. **3111:**436–442.
2. **Anderson, B. E., and M. A. Neuman.** 1997. *Bartonella* spp. as emerging human pathogens. Clin. Microbiol. Rev. **10:**203–219.
3. **Breitschwerdt, E. B., D. L. Kordick, D. E. Malarkey, B. Keene, T. H. Hadfield, and K. Wilson.** 1995. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J. Clin. Microbiol. **33:**154–160.
4. **Breitschwerdt, E. B., C. E. Atkins, T. T. Brown, D. L. Kordick, and P. S. Snyder.** 1999. *Bartonella vinsonii* subspecies *berkhoffii* and other alpha *Proteobacteria* associated with cardiac arrhythmias, endocarditis, or myocarditis in dogs. J. Clin. Microbiol. **37:**3618–3626.
5. **Chang, C.-C., B. B. Chomel, R. W. Kasten, R. Heller, K. M. Kocan, H. Ueno, K. Yamamoto, V. C. Bleich, B. J. Gonzales, P. K. Swift, W. M. Boyce, S. S. Jang, H.-J. Boulouis, and Y. Piemont.** 2000. *Bartonella* spp. isolated from wild and domestic ruminants in North America. Emerg. Infect. Dis. **6:**306–311.
6. **Chomel, B. B., R. W. Kasten, K. Floyd-Hawkins, B. Chi, K. Yamamoto, J. Roberts-Wilson, A. N. Gurfield, R. C. Abbott, N. C. Pederson, and J. E. Koehler.** 1996. Experimental transmission of *Bartonella henselae* by the cat flea. J. Clin. Microbiol. **34:**1952–1956.
7. **Ciftcioglu, N., A. Pelttari, and E. O. Kajander.** 1997. Extraordinary growth phases of *Nanobacteria* isolated from mammalian blood. Proc. SPIE Int. Opt. Eng. **3441:**429–435.
8. **Ciftcioglu, N., M. Bjorklund, K. Kuorikoski, K. Bergstrom, and E. O. Kajander.** 1999. *Nanobacteria*: an infectious cause of kidney stone formation. Kidney Int. **56:** 1893–1898.
9. **Guptill, L., L. Slater, C. Wu, T. Lin, L. Glickman, D. Welch, and H. Hogen-Esch.** 1997. Experimental infection of young specific pathogen-free cats with *Bartonella henselae*. J. Infect. Dis. **176:**206–216.
10. **Hjelle, J. T., M. A. Miller-Hjelle, I. R. Poxton, E. O. Kajander, N. Ciftcioglu, M. L. Jones, R. C. Caughey, R. Brown, P. D. Millikin, and F. S. Darras.** 2000. Endotoxin and *Nanobacteria* in polycystic kidney disease. Kidney Int. **57:** 2360–2374.
11. **Kajander, E. O., and N. Ciftcioglu.** 1998. *Nanobacteria*: an alternative mechanism for pathogenic intra- and extracellular calcification and stone formation. Proc. Natl. Acad. Sci. USA **95:**8274–8278.
12. **Kajander, E. O., I. Kuronen, K. Akerman, A. Pelttari, and N. Ciftcioglu.** 1997. *Nanobacteria* from blood, the smallest culturable autonomously replicating agent on Earth. Proc. SPIE Int. Opt. Eng. **3441:**420–428.
13. **Kitchell, B. E., T. M. Fan, G. Walenberg, D. L. Kordick, and E. B. Breitschwerdt.** 2000. Peliosis hepatitis in a dog associated with *Bartonella henselae*. J. Am. Vet. Med. Assoc. **216:**519–523.
14. **Koehler, J. E., M. A. Sanchez, C. S. Garrido, M. J. Whitfeld, F. M. Chen, T. G. Berger, M. C. Rodriguez-Barradas, P. E. LeBoit, and J. W. Tappero.** 1997. Molecular epidemiology of *Bartonella* infections in patients with bacillary angiomatosis-peliosis. N. Engl. J. Med. **337:**1876–1883.
15. **Kordick, D. L., and E. B. Breitschwerdt.** 1998. Persistent infection of pets within a household with three *Bartonella* species: *Bartonella clarridgeiae, Bartonella henselae*, or *Bartonella vinsonii* subsp. *berkhoffii*. Emerg. Infect. Dis. **4:**325–328.
16. **Kordick, D. L., T. T. Brown, K. Shin, and E. B. Breitschwerdt.** 1999. Clinical and pathologic evaluation of chronic *Bartonella henselae* or *Bartonella clarridgeiae* infection in cats. J. Clin. Microbiol. **37:**1536–1547.
17. **Noah, D. L., C. M. Kramer, M. P. Verbsky, J. A. Rooney, K. A. Smith, and J. E. Childs.** 1997. Survey of veterinary professionals and other veterinary conference attendees for antibodies to *Bartonella henselae* and *B. quintana*. J. Am. Vet. Med. Assoc. **210:** 342–344.
18. **Nowotny, N., A. Deutz, K. Fuchs, W. Schuller, F. Hinterdorfer, H. Auer, and H. Aspock.** 1997. Prevalence of swine influenza and other viral, bacterial and parasitic zoonoses in veterinarians. J. Infect. Dis. **176:**1414–1415.
19. **Pappalardo, B. L., M. T. Correa, C. C. York, C. Y. Peat, and E. B. Breitschwerdt.** 1997. Epidemiologic evaluation of the risk factors associated with exposure and seroreactivity to *Bartonella vinsonii* in dogs. Am. J. Vet. Res. **58:**467–471.
20. **Pappalardo, B. L., T. Brown, J. L. Gookin, C. L. Morrill, and E. B. Breitschwerdt.** 2000. Granulomatous disease associated with *Bartonella* infection in two dogs. J. Vet. Intern. Med. **14:**37–42.
21. **Welch, D. F, K. C. Carroll, E. K. Hofmeister, D. H. Persing, D. A. Robinson, A. G. Steigerwalt, and D. J. Brenner.** 1999. 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. J. Clin. Microbiol. **37:**2598–2601.
22. **Wilson, K. H., R. B. Blitchington, and R. C. Greene.** 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J. Clin. Microbiol. **28:**1942–1946.