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Coinfection with *Bartonella clarridgeiae* and *Bartonella henselae* and with Different *Bartonella henselae* Strains in Domestic Cats

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***Bartonella clarridgeiae* and several strains of *Bartonella henselae*, the agent of cat scratch disease, with variations in the 16S rRNA gene have been found to infect the blood of cats. An epidemiologic study of *Bartonella* infection in domestic French cats revealed that of 436 cats sampled, 5 cats (1.1%) were coinfected with *B. henselae* and *B. clarridgeiae* and 2 cats (0.5%) were coinfected with two strains of *B. henselae* with variations in the 16S rRNA gene, *B. henselae* type I and type II. In an indirect immunofluorescence assay, coinfected cats tested positive for both *Bartonella* species at titers of ≥128. Identification of the colonies was achieved by preformed enzyme analysis, PCR-restriction fragment length polymorphism analysis of the citrate synthase gene, and 16S rRNA gene sequencing. Colony size differences in mixed culture allowed differentiation of the *Bartonella* species. The coinfection of cats with two *Bartonella* species or variants of the same species raises concern about the possibility of dual infection in humans. The development of a polyvalent vaccine targeted against the most pathogenic or invasive strains may be a means of protecting cats and man from infection.**

The genus *Bartonella* is presently composed of 11 species. At least four species are known to be human pathogens: *B. bacilliformis*, the agent of Carrion's disease; *B. quintana*, the agent of trench fever and an agent of bacillary angiomatosis (BA); *B. henselae*, the agent of cat scratch disease (CSD) and an agent of BA (31); and *B. elizabethae*, which can cause endocarditis (11). *B. vinsonii* has been reported recently to be the cause of human endocarditis (29). *B. vinsonii* var. *berkoffii* has been found in a case of canine endocarditis as well (5). The other species, *B. clarridgeiae*, *B. doshiae*, *B. grahamii*, *B. peromysci*, *B. talpae*, and *B. taylorii*, have been isolated from the blood of various animals (4, 21) but are not known to induce disease in the infected mammal species.

Two *Bartonella* species, *B. henselae* and *B. clarridgeiae*, have been isolated from the blood of cats (21, 25). Epidemiological studies have implicated cats as a major reservoir of *B. henselae* (7, 18, 19, 25) and have shown that cats can remain asymptomatic and bacteremic for several months to several years (1, 18, 19). *B. henselae* DNA has been amplified from fleas found on bacteremic cats (16, 18), and transmission of *B. henselae* by the cat flea, *Ctenocephalides felis*, has been demonstrated (9). Recently, *B. clarridgeiae* has also been isolated from the blood of a cat (21). It was present in the bloodstream of a healthy cat involved in a human case of CSD caused by *B. henselae* (10). It is not known whether *B. clarridgeiae* can be transmitted to man nor whether it induces disease.

During the course of an epidemiologic study of 436 domestic French cats, we identified 5 cats that were coinfected with two *Bartonella* species, *B. henselae* and *B. clarridgeiae*, and 2 cats that were coinfected with two strains of *B. henselae*, type I and type II, with variations in the 16S rRNA gene, as described by Bergmans et al. (3). Implications for diagnostic procedures, vaccines, and risks to humans are discussed in light of these findings.

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MATERIALS AND METHODS

***Bartonella* isolation from cat blood.** The seven cats described in this study were presented at two veterinary clinics in the Paris area between February and May 1996. Three milliliters of blood were aseptically drawn from the external jugular vein. A 1.5-ml aliquot of the blood was placed into a pediatric lysis-centrifugation isolator tube (Isostat; Wampole Laboratories, Cranbury, N.J.), and the rest was collected into a serum-separating tube. The isolator tubes were spun at 1,800 × *g* for 75 min at ambient temperature, and the supernatant was decanted. The pellet was resuspended in 125 μl of inoculation medium (17), and the volume was recorded. Two hundred fifty microliters of the suspension was inoculated onto heart infusion agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% defibrinized rabbit blood, and the remaining suspension was inoculated onto a second plate. The plates were incubated for 1 month at 35.5°C with 5% CO_2 in a humid incubator and checked regularly for bacterial growth. After Gram staining and microscopic examination, colonies of different morphologies were subcultured, harvested, and frozen at −70°C in 100% fetal calf serum. The serum tubes were centrifuged at 500 × *g*, followed by removal and freezing of the serum at −20°C.

Identification of isolates. Cat blood isolates were identified by using the MicroScan Rapid Anaerobe panel (30), PCR-restriction fragment length polymorphism (RFLP) analysis of a fragment of the citrate synthase gene (18, 24), and 16S rRNA gene sequence analysis (26).

Preformed enzymes were detected in the different isolates with the MicroScan panel (Baxter Diagnostics, Chicago, Ill.) according to the manufacturer's directions.

DNA was extracted from frozen bacterial suspensions via a Chelex extraction technique (12). An approximately 1,500 bp fragment of the 16S rRNA gene was amplified from the extracted DNA with two eubacterial universal primers specific for the 16S rRNA gene: P8 (5′-AGAGTTTGATCCTGGCTCAG-3′) and Pc1544 (5′-AAGGAGGTGATCCAGCCGCA-3′). Rigorous precautions were taken to not contaminate the samples prior to gene amplification. The presence of the amplified products was verified by electrophoresis on a 1% agarose gel followed by staining with ethidium bromide and visualization on a UV transilluminator.

The amplified fragment was purified via phenol extraction and isopropanol precipitation (6). The sequencing reaction was performed by using the 5′ fluo-

TABLE 1. Coinfected cat characteristics, level of bacteremia, and serologic titers

Cat no.	Sex	Age	Bacteremia (CFU/ml)	*B. henselae* titer	*B. clarridgeiae* titer	Coinfection[a]
1	M	7 mo	1,248	128	ND[b]	*B. clarridgeiae*, *B. henselae* type II
2	M	8 mo	624	1,024	512	*B. clarridgeiae*, *B. henselae* type II
3	M	9 mo	1,512	256	512	*B. clarridgeiae*, *B. henselae* type II
4	F	13 mo	18	128	ND	*B. henselae* type I, *B. henselae* type II
5	F	6 yr	252	128	512	*B. clarridgeiae*, *B. henselae* type I
6	ND	ND	2,667	256	256	*B. clarridgeiae*, *B. henselae* type I
7	F	9 yr	56	256	128	*B. henselae* type I, *B. henselae* type II

[a] The sequences of *B. henselae* type I and *B. henselae* type II are analogous to the sequences of *B. henselae* Houston-1 and *B. henselae* "BA-TF," respectively.
[b] ND, no data available.

rescein isothiocyanate-labeled internal primer Pc535 (5′-GTATTACCGCGGC TGCTGGCAC-3′), Thermo Sequenase DNA polymerase (Amersham Life Science, Buckinghamshire, England), and a cyclic sequencing protocol. Electrophoresis and reading of the sequence were performed with an A.L.F. DNA sequencer (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. The DNA sequences were compared to other *Bartonella* sequences contained in the EMBL GenBank database by using the Clustal method (15) with DNAStar Ltd. (London, England) software.

An approximately 400-bp fragment of the citrate synthase gene was amplified by using previously described primers and methods (24). The amplified products were verified by gel electrophoresis and then enzymatically digested with *Taq*I and *Hha*I restriction endonucleases (7). The banding patterns were compared with those obtained with the type strains of *B. henselae* Houston-1 (ATCC 49882) and *B. clarridgeiae* (ATCC 51734).

Serological analysis. Titers of antibodies against *B. henselae* type II and *B. clarridgeiae* were determined by an indirect fluorescent-antibody technique previously described (7, 8). Briefly, serum was serially diluted in phosphate-buffered saline and incubated on slides containing FCWF (*Felis catus* whole-fetus) cells infected with 16S rRNA sequence-confirmed strains of *B. henselae* type II (strain U4, University of California, Davis) and *B. clarridgeiae* (ATCC 51734). The slides were washed and probed with fluorescein isothiocyanate-labeled goat anti-cat immunoglobulin G (heavy plus light chains) conjugate (Cappel; Organon Teknika Corp., Durham, N.C.), and the fluorescence was graded, via a double-blinded protocol, by two readers. Any serum with a titer of ≥64 was considered positive (7).

RESULTS

Of a group of 436 domestic French cats, 5 (1.1%) were coinfected with two species of *Bartonella*, *B. henselae* and *B. clarridgeiae*, and 2 others (0.5%) were coinfected with two strains of *B. henselae*, type I and type II, with variations in the 16S rRNA gene. The ages of the cats ranged from 8 months to 9 years (Table 1). Three cats (cats 2, 5, and 7) had common histories of living outdoors more than 50% of the time during the preceding month. Cats 1, 3, and 4 had lived indoors for the preceding 2 to 7 months without other pets. Fleas were either observed at the time of presentation to the clinics or detected by the owners during the preceding 6 months on four of the seven cats (57%) (cats 1, 3, 5, and 7). Except for cat 5, which had a retrobulbar mass, all cats were clinically normal. Life style and flea data were unavailable for cat 6. The amount of serum obtained from cats 1 and 4 was insufficient to determine their *B. clarridgeiae* antibody titers. Cat 1 was traced and retested 9 months later but was not bacteremic and was seronegative for *B. henselae* and *B. clarridgeiae* at that time. The other coinfected cats had titers of antibodies to *B. henselae* and *B. clarridgeiae* at levels greater than or equal to 1:128 (Table 1).

The number of *Bartonella* isolates varied from 18 to greater than 2,600 CFU per ml of blood. Colonies of two sizes were observed in the mixed-species coinfected cultures: 2- to 4-mm-diameter colonies and ≤1-mm-diameter colonies. By using the methods described previously, the larger colonies were subsequently identified as *B. henselae* and the smaller colonies were identified as *B. clarridgeiae*. Colony sizes appeared smaller and more uniform when the colony density on the culture plate was high (>10^3 CFU/ml). Gram staining of *B. henselae* colonies and Gram staining of *B. clarridgeiae* colonies both revealed small gram-negative rods.

Hydrolysis of preformed enzymes by *B. henselae* and *B. clarridgeiae* isolates was observed for the following substrates: bis-*p*-nitrophenyl-phosphate (BPO_4), L-leucine-β-naphthylamide (LEU), L-tryptophane-β-naphthylamide (TRY), DL-methionine-β-naphthylamide (MET), glycylglycine-β-naphthylamide (GGLY), L-arginine-β-naphthylamide (ARG), L-lysine-β-naphthylamide (alkaline) (LYB), and glycine-β-naphthylamide (GLY). Sixty percent of the *B. henselae* and 75% of the *B. clarridgeiae* isolates were also positive for *p*-nitrophenyl-phosphate (PO_4). All *B. henselae* isolates caused positive reactions for L-proline-β-naphthylamide (PRO) and L-lysine-β-naphthylamide (acid) (LYA), whereas none of the *B. clarridgeiae* isolates were positive for PRO and only one (25%) was slightly positive for LYA.

PCR-RFLP analysis by digestion of the citrate synthase gene amplicon with *Taq*I (Fig. 1) and *Hha*I (Fig. 2) restriction endonucleases was used to correlate the profiles of our isolates (lanes 5 to 8, Fig. 1 and 2) with those of reference *Bartonella* species or subspecies (lanes 2 to 4). The isolate in lane 5 (Fig. 1 and 2) had a profile identical to the profile of *B. clarridgeiae* (ATCC 51734), and the isolates in lanes 6, 7, and 8 had profiles similar to that of *B. henselae*. After *Taq*I and *Hha*I endonuclease digestion of *B. henselae* strains, occasional faint bands (110 to 120 bp) occurred in our isolates and in the reference strains.

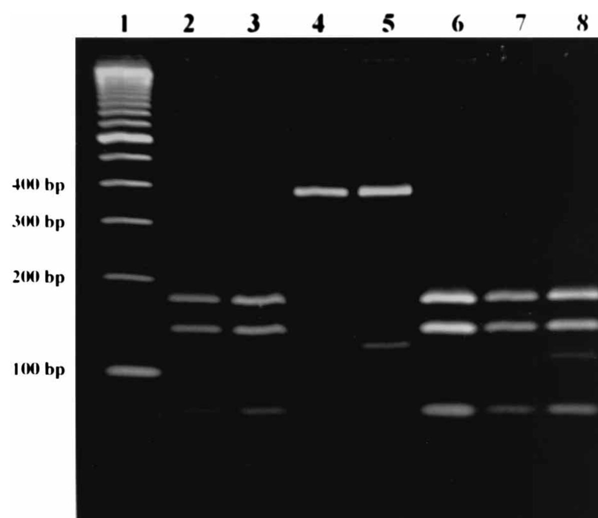

FIG. 1. PCR-RFLP *Taq*I digestion of *Bartonella* isolates. Lane 1, 100-bp molecular-size ladder; lane 2, *B. henselae* type I (Houston-1, ATCC 49882); lane 3, *B. henselae* type II (isolate U4, University of California, Davis); lane 4, *B. clarridgeiae* (ATCC 51734); lane 5, *B. clarridgeiae* (cat 5); lane 6, *B. henselae* type I (cat 5); lane 7, *B. henselae* type I (cat 7); lane 8, *B. henselae* type II (cat 7).

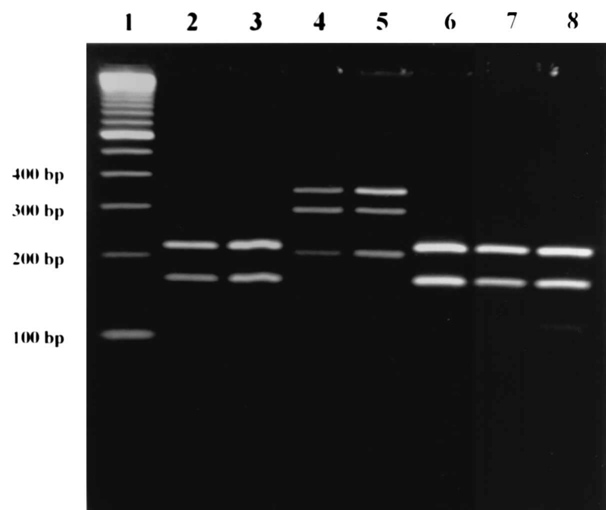

FIG. 2. PCR-RFLP *Hha*I digestion of *Bartonella* isolates. Lane 1, 100-bp molecular-size ladder; lane 2, *B. henselae* type I (Houston-1, ATCC 49882); lane 3, *B. henselae* type II (isolate U4, University of California, Davis); lane 4, *B. clarridgeiae* (ATCC 51734); lane 5, *B. clarridgeiae* (cat 5); lane 6, *B. henselae* type I (cat 5); lane 7, *B. henselae* type I (cat 7); lane 8, *B. henselae* type II (cat 7).

B. henselae type I, *B. henselae* type II, and *B. clarridgeiae* demonstrated unique sequences of the 16S rRNA gene. *B. henselae* type I contained the base sequence TAG, *B. henselae* type II contained ATTT, and *B. clarridgeiae* contained AC-T from bases 194 to 197 (*Escherichia coli* numbering system).

DISCUSSION

Bartonella coinfection in French cats has not been reported previously. *Bartonella* species and types can be identified by analysis of the citrate synthase and 16S rRNA genes (23, 24, 28). PCR-RFLP patterns of the citrate synthase gene distinguish *Bartonella* species, and sequencing of a 16S rRNA amplicon can identify variants within the same species. Using a PCR-based restriction endonuclease analysis of the 16S-23S rRNA intergenic spacer region, Matar et al. (22) demonstrated seven composite *Alu*I and *Hae*III RFLP types in *B. henselae* strains cultured from patients with BA, septicemia, and parenchymal bacillary peliosis. Bergmans et al. (3) found that immunocompetent Dutch patients with CSD showed only two RFLP patterns, A and B. The two types of *B. henselae* found to coinfect some of our cats are similar to the type I and type II *B. henselae* variants described by Bergmans et al. (3); the sequences of type I and type II are analogous to the sequences of *B. henselae* Houston-1, described by Regnery et al. (24), and *B. henselae* "BA-TF," described by Relman et al. (27), respectively.

PCR-RFLP analysis by digestion of the citrate synthase gene amplicon with the *Taq*I and *Hha*I restriction endonucleases correlated the profiles of our isolates with those of *B. henselae*, as previously described by Regnery et al. (24). However, the occasional appearance of a faint extra band at 110 to 120 bp requires further investigation to determine the nature of its importance. No previous PCR-RFLP analysis of the citrate synthase gene amplicon with the *Taq*I and *Hha*I restriction endonucleases for *B. clarridgeiae* has been published, but the profile of our isolate from cat 5 (lane 5 in Fig. 1 and 2) was similar to the profile of the *B. clarridgeiae* ATCC strain (ATCC 51734). The low-molecular-weight bands from the *B. clarridgeiae* strains, although often faint, were seen in all strains tested.

Our results confirm the findings of Bergmans (2) that cats can carry a variety of different *B. henselae* types and indicate that such a variety of *Bartonella* types can be present at the same time within the same host. All permutations of coinfection by the two cat-infecting *Bartonella* species, i.e., *B. clarridgeiae* and *B. henselae*, and the two *B. henselae* species types, i.e., I and II, were evidenced. It is unknown whether the cats were simultaneously or successively infected by the different *Bartonella* species and types. The presence of a dual infection suggests that cross-protection between *Bartonella* species and/or types may not occur.

It is important to determine if one species or subspecies is predominant within the same infected cat and, if possible, whether human coinfection by accidental inoculation of two *Bartonella* species can occur. The isolation of *B. clarridgeiae* was reported from a cat that was implicated in a human *B. henselae* CSD case (10). Could that cat have been coinfected by both *B. henselae* and *B. clarridgeiae*? No human CSD case has yet been reported to be caused by *B. clarridgeiae*. It is possible that cats carry a variety of *Bartonella* species or variants and that only a limited number of these types can cause disease in immunocompetent human hosts (3). The lack of isolation of *B. clarridgeiae* from immunocompromised individuals up to the present would support such a hypothesis. Different prevalences of strain types at different geographic locations may also explain why different studies have found different strain prevalences in CSD patients.

The colony sizes of the two *Bartonella* species differed on the primary culture plate. *B. clarridgeiae* was isolated from the smaller of the two colony types when a multiple species infection was observed. Colonies became smaller and more uniform at higher plating densities and upon subculture. Other investigators have observed different *Bartonella* colony morphologies in cat blood cultures (19), but it is unknown if they represented different species.

Previous epidemiological studies have identified young cats as being the highest risk group for sustained *Bartonella* infection (7, 18). The ages of the coinfected cats in this study, however, ranged from 8 months to 9 years, thus demonstrating that a cat of any age may be coinfected. Also, prior studies have demonstrated that the cat flea, *C. felis*, can transmit *B. henselae* to cats (9). Most of our cats had a history of flea infestation or of a partially outdoor lifestyle which would facilitate flea exposure. It is interesting to note, though, that cats 1, 3, and 4 had lived indoors for the preceding 2 to 7 months, without contact with other animals. Although it is possible that fleas living within the owner's domicile may have infected the cat with *Bartonella*, one can speculate that the cats were infected with both *Bartonella* species before residing at the owner's apartment and thus maintained a nonclinical, dual bacteremia for up to six months or more. It is striking that the cats presented with such high bacteremias and yet demonstrated no clear evidence of overt disease. The mechanisms of tolerance of this bacteremia warrant further study.

The diversity of *Bartonella* isolates raises important concerns regarding the sensitivity of diagnostic procedures. Already, multiple *B. henselae* strains with differing seroreactivity patterns have been discovered. Drancourt et al. (13) have documented CSD patients seronegative on standard indirect fluorescent-antibody diagnostic tests for *B. henselae* Houston-1 antigen but seropositive for a Marseille strain of *B. henselae*. Kordick et al. (20) reported a CSD patient seronegative for *B. henselae* but seropositive for a novel *Bartonella* strain isolated from the cat implicated in the exposure. Studies have shown that up to 60% of CSD patients may test negative in conventional *B. henselae* seroassays (14, 32). Accurate diagnosis of

CSD, therefore, requires development of serologic assays that detect the various serogroups of *Bartonella.*

The number of cats found to be coinfected in this study is more likely to represent a minimum percentage of cats that are coinfected. Recognition of mixed infections requires techniques sensitive and specific for each of the variants possible. The identification methods currently available, i.e., observing colony size, MicroScan methods, and the use of genus-specific *Bartonella* PCR probes, are not sufficiently reliable to pick up all mixed infections. These methods favor identification of the predominant variant but detect the minority variant with less reliability. Furthermore, differences in the geographical distribution of strains will dictate which probes are required in different regions. Continued epidemiological studies are necessary to identify other variants and to elucidate the frequencies at which and the areas in which they occur. The development of a polyvalent vaccine targeted against the most pathogenic or invasive strains may be a means of protecting cats and humans from infection. Further work to define which *Bartonella* strains and/or species need to be included remains to be done.

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REFERENCES

1. **Abbott, R. C., B. B. Chomel, R. W. Kasten, K. A. Floyd-Hawkins, Y. Kikuchi, J. E. Koehler, and N. C. Pedersen.** 1997. Experimental and natural infection with *Bartonella henselae* in cats. Comp. Immunol. Microbiol. Infect. Dis. **20:**41–51.
2. **Bergmans, A. M. C.** 1996. Cat scratch disease: studies on diagnosis and identification of reservoirs and vectors. Ph.D. thesis, University of Utrecht, The Netherlands.
3. **Bergmans, A. M. C., J. F. P. Schellekens, J. D. A. van Embden, and L. M. Schouls.** 1996. Predominance of two *Bartonella henselae* variants among cat-scratch disease patients in The Netherlands. J. Clin. Microbiol. **34:**254–260.
4. **Birtles, R. J., T. G. Harrison, N. A. Saunders, and D. H. Molyneux.** 1995. Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci* comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. Int. J. Syst. Bacteriol. **45:**1–8.
5. **Breitschwerdt, E. B., D. L. Kordick, D. E. Malarkey, B. Keene, T. L. Hadfield, and K. Wilson.** 1995. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J. Clin. Microbiol. **33:**154–160.
6. **Brow, M. A. D.** 1990. Sequencing with *Taq* DNA polymerase, p. 189–196. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, Calif.
7. **Chomel, B. B., R. C. Abbott, R. W. Kasten, K. A. Floyd-Hawkins, P. H. Kass, C. A. Glaser, N. C. Pedersen, and J. E. Koehler.** 1995. *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. J. Clin. Microbiol. **33:**2445–2450.
8. **Chomel, B. B., A. N. Gurfield, H. J. Boulouis, R. W. Kasten, and Y. Piemont.** 1995. Réservoir félin de l'agent de la maladie des griffes du chat, *Bartonella henselae*, en région parisienne: résultats préliminaires. Recl. Med. Vet. **171:**841–845.
9. **Chomel, B. B., R. W. Kasten, K. Floyd-Hawkins, B. Chi, K. Yamamoto, J. Roberts-Wilson, A. N. Gurfield, R. C. Abbott, N. C. Pedersen, and J. E. Koehler.** 1996. Experimental transmission of *Bartonella henselae* by the cat flea. J. Clin. Microbiol. **34:**1952–1956.
10. **Clarridge, J. E., III, T. J. Raich, D. Pirwani, B. Simon, L. Tsai, M. C. Rodriguez-Barradas, R. Regnery, A. Zollo, D. C. Jones, and C. Rambo.** 1995. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus-positive patient and unique *Bartonella* strain from his cat. J. Clin. Microbiol. **33:**2107–2113.
11. **Daly, J. S., M. G. Worthington, D. J. Brenner, C. W. Moss, D. G. Hollis, R. S. Weyant, A. G. Steigerwalt, R. E. Weaver, M. I. Daneshvar, and S. P. O'Connor.** 1993. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J. Clin. Microbiol. **31:**872–881.
12. **De Lamballerie, X., C. Zandotti, C. Vignoli, C. Bollet, and P. de Micco.** 1992. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res. Microbiol. **143:**785–790.
13. **Drancourt, M., R. Birtles, G. Chaumentin, F. Vandenesch, J. Etienne, and D. Raoult.** 1996. New serotype of *Bartonella henselae* in endocarditis and cat-scratch disease. Lancet **347:**441–443.
14. **Dupon, M., A. M. Savin de Larclause, P. Bouqui, M. Drancourt, D. Raoult, A. de Mascarel, and J. Y. Lacut.** 1996. Evaluation of serological response to *Bartonella henselae*, *Bartonella quintana* and *Afipia felis* antigens in 64 patients with suspected cat-scratch disease. Scand. J. Infect. Dis. **28:**361–366.
15. **Higgins, D. G., and P. M. Sharp.** 1988. Clustal: a package for performing multiple sequence alignment on a microcomputer. Gene **73:**237–244.
16. **Higgins, J. A., S. Radulovic, D. C. Jaworski, and A. F. Azad.** 1996. Aquisition of the cat scratch disease agent *Bartonella henselae* by cat fleas (Siphonaptera: Pulicidae). J. Med. Entomol. **33:**490–495.
17. **Koehler, J. E., D. Q. Frederick, T. G. Berger, P. E. LeBoit, and J. W. Tappero.** 1992. Isolation of Rochalimaea species from cutaneous and osseous lesions of bacillary angiomatosis. N. Engl. J. Med. **327:**1625–1631.
18. **Koehler, J. E., C. A. Glasser, and J. W. Tappero.** 1994. *Rochalimaea henselae* infection. A new zoonosis with the domestic cat as reservoir. JAMA **271:**531–535.
19. **Kordick, D. L., K. H. Wilson, D. J. Sexton, T. L. Hadfield, H. A. Berkhoff, and E. B. Breitschwerdt.** 1995. Prolonged *Bartonella* bacteremia in cats associated with cat-scratch disease patients. J. Clin. Microbiol. **33:**3245–3251.
20. **Kordick, D. L., T. L. Hadfield, K. H. Wilson, and E. B. Breitschwerdt.** 1996. Inoculation papule, fever and lymphadenopathy (cat scratch disease) associated with a novel *Bartonella* isolate from a domestic cat, abstr. B-151, p. 180. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
21. **Lawson, P. A., and M. D. Collins.** 1996. Description of *Bartonella clarridgeiae* sp. nov. isolated from the cat of a patient with *Bartonella henselae* septicemia. Med. Microbiol. Lett. **5:**64–73.
22. **Matar, G. M., B. Swaminathan, S. B. Hunter, L. N. Slater, and D. F. Welch.** 1993. Polymerase chain reaction-based restriction fragment length polymorphism analysis of a fragment of the ribosomal operon from *Rochalimaea* species for subtyping. J. Clin. Microbiol. **31:**1730–1734.
23. **Maurin, M., V. Roux, A. Stein, F. Ferrier, R. Viraben, and D. Raoult.** 1994. Isolation and characterization by immunofluorescence, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western blot, restriction fragment length polymorphism-PCR, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis of *Rochalimaea quintana* from a patient with bacillary angiomatosis. J. Clin. Microbiol. **32:**1166–1171.
24. **Regnery, R. L., B. E. Anderson, J. E. Clarridge III, M. C. Rodriguez-Barradas, D. C. Jones, and J. H. Carr.** 1992. Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. J. Clin. Microbiol. **30:**265–274.
25. **Regnery, R. L., M. Martin, and J. G. Olson.** 1992. Naturally occurring *Rochalimaea henselae* infection in domestic cat. Lancet **340:**557–558.
26. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
27. **Relman, D. A., P. W. Lepp, K. N. Sadler, and T. M. Schmidt.** 1992. Phylogenetic relationships among the agent of bacillary angiomatosis, *Bartonella bacilliformis*, and other alpha-proteobacteria. Mol. Microbiol. **6:**1801–1807.
28. **Roux, V., and D. Raoult.** 1995. Inter- and intraspecies identification of *Bartonella* (*Rochalimaea*) species. J. Clin. Microbiol. **33:**1573–1579.
29. **Schwartzman, W.** 1996. *Bartonella* (*Rochalimaea*) infections: beyond cat scratch. Annu. Rev. Med. **47:**355–364.
30. **Welch, D. F., D. M. Hensel, D. A. Pickett, V. H. San Joaquin, A. Robinson, and L. N. Slater.** 1993. Bacteremia due to *Rochalimaea henselae* in a child: practical identification of isolates in the clinical laboratory. J. Clin. Microbiol. **31:**2381–2386.
31. **Welch, D. F., D. A. Pickett, L. N. Slater, A. G. Steigerwalt, and D. J. Brenner.** 1992. *Rochalimaea henselae* sp. nov., a cause of septicemia, bacillary angiomatosis, and parenchymal bacillary peliosis. J. Clin. Microbiol. **30:**275–280.
32. **Yoshida, H., N. Kusaba, K. Omachi, N. Miyazaki, M. Yamawaki, Y. Tsuji, K. Nakahara, M. Sumino, M. Noudomi, Y. Shimokawa, and K. Tanikawa.** 1996. Serological study of *Bartonella henselae* in cat scratch disease in Japan. Microbiol. Immunol. **40:**671–673.