American Journal of Pathology, Vol. 149, No. 6, December 1996

Cat Scratch Disease

Detection of Bartonella henselae *DNA in Archival Biopsies from Patients with Clinically, Serologically, and Histologically Defined Disease*

Margie A. Scott,* Thomas L. McCurley,* Cindy L. Vnencak-Jones,* Cynthia Hager,† Jennifer A. McCoy,* Burt Anderson,‡ Robert D. Collins,* and Kathryn M. Edwards†

From the Department of Pathology and the Department of Pediatrics,† Division of Pediatric Infectious Disease, Vanderbilt University Medical Center, Nashville, Tennessee, and the Department of Medical Microbiology and Immunology,‡ College of Medicine, University of South Florida, Tampa, Florida*

Serological and epidemiological studies suggest that* Bartonella henselae *is the etiological agent of cat scratch disease. We designed a study to detect* B. henselae *in archival biopsies by polymerase chain reaction amplification of the 16S rRNA gene followed by Southern blot hybridization. Forty-two histologically defined cat scratch disease biopsies and eighteen controls were selected for blinded analysis. After testing, charts were reviewed for clinical, immunological, and microbial evidence of infection. Results were correlated with duration of illness and antimicrobial therapy.* B. henselae *DNA was identified in 27 of 42 (64%) histologically defined patients and 23 of 34 (68%) patients defined both clinically and histologically. There were no false positives (0 of 18). A small subset (n = 14) had cat scratch disease serological tests performed.* B. henselae *was identified in 8 of 10 serologically positive patients. Polymerase chain reaction detected 50% of our DNA-positive cases (most of these early in the clinical course). Southern blotting of amplicons both doubled sensitivity (detecting patients >4 weeks into illness) and confirmed* B. henselae *as the causative species. Our study strongly associates* B. henselae *with cat scratch disease, suggesting that it may be the most likely etiological agent in the majority of patients with cat scratch disease. (Am J Pathol 1996, 149:2161–2167)

Cat scratch disease (CSD) is a common cause of subacute regional lymphadenitis in children and adults. Since the original description of CSD in 1950 by Debré and Mollaret, diagnosis has been based on a history of cat exposure with a recent scratch or papule, tender regional lymphadenitis, skin test positivity, and negative routine bacterial cultures.[1] Lymph node biopsy characteristically shows necrotizing lymphadenitis with stellate microabscesses.

There were no clues as to the etiological agent until Wear et al in 1983 identified small delicate pleomorphic gram-negative bacilli within capillary walls and microabscesses of infected lymph nodes.[2] Since that time, *Bartonella henselae* (formerly *Rochalimaea henselae*) and *Afipia felis* have emerged as probable etiological agents of CSD after being cultured from involved lymph nodes.[3–5] Serological data strongly suggests that *B. henselae* is the most common cause of CSD with 84 to 95% of patients demonstrating antibodies to *B. henselae*.[6–8] However, it remains unclear whether this immune response reflects current and/or past infection. Earlier attempts to determine the incidence of CSD caused by *B. henselae* using both polymerase chain reaction (PCR) and Southern blot (SB) methods in archival tissue have proven difficult to impossible.[9,10] These studies sug-

Accepted for publication July 30, 1996.

Address reprint requests to Dr. Margie A. Scott, Vanderbilt University Medical Center, Department of Pathology, Medical Center North C3321, 22nd Avenue and Garland, Nashville, Tennessee 37232.

gested that, despite the strong serological evidence of infection, failure to detect the bacterial DNA in affected tissues made *B. henselae* an unlikely or infrequent cause of CSD.

The role of *Afipia felis* in CSD remains unclear. One study using dot-blot hybridization techniques suggested that *A. felis* may play a role in some cases either as a single causative agent or as a co-infectious agent along with *B. henselae*.[11] This was not supported, however, in a much larger study of aspirated pus and fresh lymph node biopsies from researchers in The Netherlands.[12] In the later study, *A. felis* was not detected by PCR studies in any of the 89 aspirates or 137 fresh biopsies tested, whereas *B. henselae* was detected in 90% of aspirated pus and 60% of the fresh biopsy tissues.

Success in detecting *B. henselae* DNA has required fresh tissue or fresh aspirated pus for analysis.[12,13] We present a modification of these methods that is equally suited for detection of *B. henselae* DNA in fresh or fixed, paraffin-embedded tissue.

We designed a retrospective study to determine the incidence of CSD caused by *B. henselae* in a group of patients fulfilling both pre-established clinical and histological criteria for this diagnosis. As a starting point, 42 patients were identified by reviewing paraffin-embedded archival biopsies histologically characteristic of CSD. After DNA testing, we began chart reviews with comparative analysis of all clinical, immunological, and microbial evidence of *B. henselae* infection.

Materials and Methods

Case Selection and Study Design

Paraffin-embedded biopsies from 42 patients computer coded (SNOMED) as CSD during a period from July 1987 to April 1994 were retrieved from the surgical pathology files of Vanderbilt University Medical Center. All patients were from the Southeastern United States region, predominantly Tennessee. Biopsies were either 10% formalin fixed (n = 28) or B-5 fixed (n = 14). All biopsies fulfilled the following four pathological criteria for a diagnosis of probable CSD: necrotizing granulomatous inflammation, multiple microabscesses with formation of stellate microabscesses, negative special stains (Gomori methenamine silver, Modified Fite acid fast, and Brown & Brenn tissue gram), and negative cultures (routine bacterial, acid fast, and fungal). Only 4 of the formalin-fixed biopsies (14%) demonstrated rare pleomorphic bacilli compatible with the CSD bacillus by Warthin Starry stains. The 42 probable CSD biopsies were randomly intermixed with 18 control biopsies of other documented etiologies and assigned a number from 1 to 60 for testing in a blinded fashion. After completion of molecular testing, the study was unblinded and charts were reviewed in the 42 probable CSD patients.

For the purpose of the study, clinical CSD was defined as at least four of the five following criteria documented by chart review: exposure to cats, history of recent cat scratch or papule, febrile illness, persistent regional lymphadenopathy or persistent abscess elsewhere, and positive CSD immune response by skin test, enzyme-linked immunosorbent assay (ELISA), and/or indirect fluorescent antibody (IFA).

Skin Testing, ELISA, and IFA (Immunological Criteria)

At the time of chart review, patients in whom skin testing, ELISA, or IFA for *B. henselae* had been performed were identified and analyzed as a subgroup. These diagnostic techniques were compared with *B. henselae* DNA detection. Skin testing had been performed by intradermal injection of 0.1 ml of heat-inactivated CSD skin test antigen, as previously described.[14] A positive cell-mediated immune response was defined as ≥5 mm induration present at 48 to 72 hours. In addition, a serological response to *B. henselae* was determined by the ELISA method as described by Szelc-Kelly et al.[15] A positive ELISA was greater than or equal to the 95th percentile of the control group. Immunofluorescence for IgG-specific antibody levels (IFA) to *B. henselae* were determined using the method of Regnery et al.[6] A positive IFA was determined to be ≥64.

Controls for Molecular Testing

Negative controls included 18 paraffin-embedded lymph node and soft tissue biopsies, DNA that was purified from cultured *A.* (ATCC 53690, American Type Culture Collection, Rockville, MD), and 60 reagent blanks. The 18 paraffin-embedded controls included cases of *Mycobacterium tuberculosis* (n = 4), *Xanthomonas maltophilia* (n = 1), *Francisella tularensis* (n = 1), *Yersinia enterocolitica* (n = 1), *Toxoplasma gondii* (n = 1), *Histoplasma capsulatum* (n = 1), Whipple's disease (n = 1), Kaposi's sarcoma (n = 2), lymphoid follicular hyperplasia (n = 4), and sarcoidosis (n = 2). Positive controls included purified DNA from cultured bacterial strains of *B.*

henselae (Houston-1; ATCC 49882) and *B. quintana* (Oklahoma isolate 90–268).

DNA Isolation and Quality Assurance

One 10-μm-thick section was obtained from each paraffin block for DNA isolation using sterile techniques, and 4-μm-thick sections flanking the target section were stained with hematoxylin and eosin (H&E) and held for later review to assure that lesional tissue had been tested. Crude DNA extracts were prepared as previously described.[16]

Adequate DNA isolation was insured by successful PCR amplification of a 98-bp sequence specific for exon 10 of the human cystic fibrosis transmembrane conductance regulator gene on chromosome 7q as analyzed by gel electrophoresis of amplified products on a 10% polyacrylamide gel. To maximize the efficiency of the amplification reaction and minimize the effects of co-purified inhibitors, varying amounts of crude extract were added to the human cystic fibrosis transmembrane conductance regulator amplification reaction and the volumes of extract required to yield maximal band intensity were utilized for subsequent CSD testing.

Polymerase Chain Reaction and Southern Blotting

Primer pair CAT1 (5′ GATTCAATTGGTTTGAA (G/A)GAGGCT 3′) and CAT3 (5′ TTGCAATACGCTTTGCTAGATCACGG 3′) designed by Anderson et al, which defines a 153-bp segment of the 16S rRNA genes shared by *B. henselae* and *B. quintana*, were used for PCR amplification as previously described.[17] The specificity of this primer pair was found to be identical to the previously designed CAT 1, CAT 2 primer pair (unpublished data), and amplicons differ only in the length of DNA amplified with the 3′ end of CAT 3 being located further upstream.[11] The 100-μl reactions contained 1 μmol/L of each primer, 10 μl of buffer (500 mmol/L KCL, 100 mmol/L Tris, pH 8.3, 15 mmol/L $MgCl_2$, dH_2O), 200 μmol/L dNTPs, 2.5 U of *Taq* polymerase, template DNA, and sterile water. Each sample was run in duplicate at previously determined concentrations, separated by a reagent blank used to eliminate the possibility of cross-over contamination. Amplicons were electrophoresed on 1.5% agarose gels, and products were visualized under ultraviolet light after ethidium bromide staining. The presence of a 153-bp band was considered a positive reaction. Photographs were obtained before overnight capillary transfer of the products to nylon-charged membranes for SB.

To increase the sensitivity of the assay and confirm the specificity of amplified products, membranes were hybridized with ^{32}P-end-labeled internal probes specific for *B. henselae* (Rh1: 5′ GGTGCGTTAATTACCGATCC 3′) and *B. quintana* (Rq1: 5′GGCGCTTTGATTACTGATCC 3′) as described by Anderson et al.[11] After standard end labeling with 20 U of T_4 kinase and [γ-^{32}P]ATP, each membrane was hybridized at 40°C overnight with 3×10^6 counts/ml of ^{32}P-end-labeled probe. Unbound probe was removed by stringent multiple washes, and autoradiography was performed using intensifying screens with exposure for 4 and 24 hours at −70°C. Positive bands were compared with PCR gels. After hybridizing for *B. henselae*, the membranes were stripped and then hybridized for the presence of the closely related species *B. quintana*.

Results

Of the 42 probable CSD patients initially selected by histological criteria alone, chart review identified 34 fulfilling the pre-established clinical criteria for this diagnosis as outlined in case selection and study design (Table 1); 8 patients failed to meet the minimal pre-established clinical criteria and were excluded from the study at time of chart review. In this group of 8 patients, 7 had no record of cat exposure, 8 had no history of a cat scratch or papule, 5 had no documentation of a febrile illness, 1 had no record of regional adenopathy, and 6 had no CSD immunological testing performed. The documented clinical impressions in these 8 excluded patients were rule out malignant neoplasm (n = 6), probable tuberculosis or CSD (n = 1), and probable osteomyelitis (n = 1). Although molecular testing in these 8 patients confirmed that 50% (n = 4) were positive for *B. henselae* DNA, these 8 patients were dropped from the study to maintain a rigidly defined group (Table 1). All calculations and conclusions were based on the final population of 34 patients.

In the 34 patients fulfilling both the histological and clinical criteria for CSD, ages ranged from 3 to 48 years (mean, 12.6) and duration of illness before biopsy ranged from 1 to 28 weeks (mean, 5.7). Diagnostic biopsies included lymph nodes (28), liver (4), bone (1), and skin (1). The 28 lymph nodes were distributed as follows: 14 cervical, 5 axillary, 3 epitrochlear, 4 inguinal, and 2 abdominal. Of the 34 patients, 12 (35%) had co-existing skin papules noted on physical exam at time of biopsy and 26

Table 1. *CSD Retrospective Study: Overview of All Patients and Subgroups*

Groups	Patients (code number for blinding)	CSD clinical criteria*	CSD skin test	*B. henselae* ELISA	*B. henselae* IFA	*B. henselae* PCR +	*B. henselae* SB
Patients fulfilling pathological and clinical criteria in whom immunological testing was available for correlation							
	1 (8)	5/5	+	+	ND	+	+
	2 (14)	5/5	+	+	+	+	+
	3 (32)	5/5	−	+	ND	+	+
	4 (33)	5/5	+	+	+	+	+
	5 (60)	4/5	−	ND	ND	+	+
	6 (18)	5/5	+	+	+	−	+
	7 (19)	5/5	+	+	+	−	+
	8 (23)	5/5	−	+	+	−	+
	9 (42)	4/5	ND	+	ND	−	+
	10 (2)	4/5	−	−	−	−	−
	11 (3)	4/5	−	−	−	−	−
	12 (11)	4/5	−	−	−	−	−
	13 (4)	5/5	+	+	+	−	−
	14 (22)	4/5	+	+	+	−	−
Patients fulfilling pathological and clinical criteria in whom no serological testing was available for correlation							
	1 (34)	4/5	ND	ND	ND	+	+
	2 (36)	4/5	ND	ND	ND	+	+
	3 (37)	4/5	ND	ND	ND	+	+
	4 (40)	4/5	ND	ND	ND	+	+
	5 (51)	4/5	ND	ND	ND	+	+
	6 (55)	4/5	ND	ND	ND	+	+
	7 (5)	4/5	ND	ND	ND	−	+
	8 (10)	4/5	ND	ND	ND	−	+
	9 (16)	4/5	ND	ND	ND	−	+
	10 (28)	4/5	ND	ND	ND	−	+
	11 (43)	4/5	ND	ND	ND	−	+
	12 (52)	4/5	ND	ND	ND	−	+
	13 (56)	4/5	ND	ND	ND	−	+
	14 (57)	4/5	ND	ND	ND	−	+
	15 (59)	4/5	ND	ND	ND	−	−
	16 (7)	4/5	ND	ND	ND	−	−
	17 (24)	4/5	ND	ND	ND	−	−
	18 (39)	4/5	ND	ND	ND	−	−
	19 (44)	4/5	ND	ND	ND	−	−
	20 (50)	4/5	ND	ND	ND	−	−
Patients fulfilling pathological criteria alone (these eight were dropped from study after chart review failed to document clinical criteria of CSD)							
	1 (25)	2/5	+	+	+	+	+
	2 (38)	2/5	ND	+	ND	+	+
	3 (27)	3/5	ND	ND	ND	−	+
	4 (12)	1/5	ND	ND	ND	−	+
	5 (15)	2/5	ND	ND	ND	−	−
	6 (21)	2/5	ND	ND	ND	−	−
	7 (48)	1/5	ND	ND	ND	−	−
	8 (49)	1/5	ND	ND	ND	−	−

*Number of clinical criteria fulfilled out of a possible total of 5.
**Number of CSD immunologic tests performed out of a possible total of 3.
− = negative; + = positive; ND = not done.

(76%) had completed at least one course of antibiotic therapy. Systemic complications included liver lesions in 6 (18%), lytic bone lesions in 4 (12%), and seizures in 2 (6%).

Of the 34 patients, 11 (32%) had PCR-amplified 153-bp fragments visible on ethidium-bromide-stained agarose gel after amplification with primer pair CAT 1 and CAT 3 for *B. henselae/B. quintana.* No amplified DNA from *B. henselae* or *B. quintana* was identified in the 18 patient controls. After transferring amplicons to nylon membranes, hybridization with ^{32}P-end-labeled Rh1 identified 23 of 34 (68%) patients positive for *B. henselae* DNA, including all 11 patients with visible amplified 153-bp bands on agarose gel. All specimens were negative for the *B. quintana* probe Rq1. None of the negative control samples were positive in either the PCR or SB procedures, for *B. henselae* or *B. quintana* DNA, for a specificity of

Table 2. *CSD Study Group (n = 34), Comparing Demographic and Clinical Features in the B. henselae DNA confirmed Cases (n = 23) and DNA-Negative Cases (n = 11)*

	Number of patients	Mean age in years (range)	Mean duration to biopsy (range)	Number (%) of patients						
				Antibiotic therapy	Lympho-denitis	Skin papule	Conjunc-tivitis	Liver lesion	Lytic bone lesions	Seizures
DNA$^+$ cases (SB for *B. henselae*)	23	14.1 (3–48)	5.7 weeks (2–28)	17 (74%)	22 (95%)	6 (26%)	2 (9%)	5 (22%)	4 (17%)	1 (4%)
DNA$^-$ cases (SB for *B. henselae*)	11	9.5 (3–26)	5.9 weeks (1–20)	9 (82%)	10 (91%)	6 (55%)	1 (9%)	1 (9%)	0	1 (9%)

100%. Table 2 compares the demographic characteristics of the 23 confirmed *B. henselae*-related CSD patients to the 11 cases in which no *B. henselae* DNA was identified.

Chart review identified 14 of 34 patients (41%) who had additional diagnostic studies for CSD (skin testing, ELISA, and/or IFA) performed (Table 1). Of these 14, 9 (64%) were positive for *B. henselae* DNA. In these 9 DNA-positive patients, 5 of 8 (62.5%) were skin test positive; 8 of 8 (100%) were ELISA positive, and 5 of 5 (100%) were IFA positive. In the group of 5 DNA-negative patients, 3 were negative by skin test, ELISA, and IFA, and 2 DNA-negative patients were positive by skin test, ELISA, and IFA. These 2 patients (4 and 22 in Table 1) had prolonged illnesses of 8 and 10 weeks and had completed two courses of amoxicillin and rifampin. Histologically, these 2 patients showed abundantly scarred, resolving stellate granulomas with scant residual neutrophilic infiltrates.

Discussion

The clinical and pathological features of CSD have been recognized for the past 40 years, but the etiological agent or agents have been unclear, primarily due to an inability to routinely culture the causative agent.[1] Modified silver stains have aided in our recognition of these bacilli in tissue but remain technically difficult and nonspecific. Since 1988, two fastidious gram-negative rods, *B. henselae* and *A. felis*, have been cultured from a small number of patients with CSD and have thus emerged as potential causative agents.[3–5]

By ELISA and IFA studies, 84 to 95% of CSD patients are seropositive for *B. henselae*, suggesting that *B. henselae* is the most frequent etiological agent for CSD.[6–8] Furthermore, retrospective DNA sequencing of two CSD skin test antigens have shown 99.7 and 100% homology for the 16S rRNA gene of *B. henselae*.[18] Epidemiological studies performed in the San Francisco Bay area have confirmed that the domestic cat serves as a major persistent reservoir for *B. henselae*, and this same study suggests that the ubiquitous cat flea may serve as a vector.[19] *B. henselae* has been recognized as a causative agent in not only CSD but also bacillary angiomatosis, peliosis hepatis, *Rochalimaea* bacteremic syndrome and culture-negative endocarditis.[20–22]

Despite the seemingly overwhelming epidemiological and serological evidence pointing toward *B. henselae* as the most likely cause of CSD, researchers have been unable to support these data with successful detection of *B. henselae* bacterial DNA in archival tissues.[9,10] One of these studies concluded that "these bacilli may be responsible for only a small proportion of these characteristic lesions of unknown etiology, or the typical CSD histology, including the pleomorphic bacillary structures, may be nonspecific."[10] After recent success in detecting *B. henselae* DNA in fresh lymph node tissues, modifications of these same methods have allowed detection in archival paraffin-embedded tissues.[11] This enabled evaluation of a larger group of clinically well defined patients in which to determine the incidence of *B. henselae* infection in CSD.

The current study is based on detection of a 153-bp DNA fragment of the 16S rRNA gene shared by both *B. henselae* and *B. quintana* using CAT 1 and CAT 3 PCR primers.[17] The specificity of this primer pair was confirmed in purified cultures and was then applied to an archival source of paraffin-embedded tissues. Only 32% of our study group was positive by PCR analysis alone. However, 68% of our study group was positive for *B. henselae* DNA when the additional steps of DNA transfer and SB were applied using species-specific ^{32}P-labeled probes. No false positives were found within our 18 patient controls. Interestingly, we found no cases of CSD attributable to *B. quintana* in light of the single case report

of *B. quintana* isolation from an infected lymph node in a woman with cats.[23] This suggests that, if *B. quintana* causes CSD, it is uncommon.

In the 11 patients positive by PCR, the average duration of illness was 3.1 weeks; 36% had co-existing skin papules and 55% had completed one course of antibiotic therapy. Histologically, the PCR-positive patients showed smaller stellate granulomas with increased neutrophils and minimal scarring. In the 12 patients positive only after amplicon transfer and SB analysis, the average duration of illness was 7.3 weeks; no residual skin papules were found and 75% had completed two courses of antibiotic therapy. Histologically the cases requiring amplicon transfer and SB for DNA detection appeared to be older resolved or resolving lesions with abundant scar formation and scant residual neutrophilic aggregates. These findings are felt to reflect the progressive paucity of target DNA contained within the tissues as the duration of illness extends. This supports the long-held belief that bacterial forms are most numerous early in the course of CSD, with numbers dropping after 4 to 8 weeks. In addition, by 4 to 8 weeks of clinical illness, nearly all patients have completed at least one course of antibiotic therapy. Whether this represents clearance of organisms remains unclear.

Skin tests and ELISA and IFA studies were identified at the time of chart review and correlated with the detection of *B. henselae* DNA in fourteen (41%) of our patients. Inherent problems encountered with skin testing include insufficient standardization and lack of a safe commercially available antigen for application. Thirteen patients had received a single lot of CSD skin test antigen previously prepared at Vanderbilt University. Subsequent DNA sequencing of this skin test antigen confirmed 100% homology with *B. henselae.*[18] We found skin testing to be the least sensitive indicator of CSD, with positivity found in only five of eight (62.5%) DNA-positive patients. It is not surprising that the duration of illness in the three DNA-positive, skin-test-negative patients were all 3 weeks or less. This most likely reflects the time required for mounting a detectable delayed-type hypersensitivity reaction (Table 1). Another factor may include inherent variability when using any non-standardized skin test reagent. On the other hand, ELISA and IFA were found to be the most sensitive immunological indicators of CSD. ELISA and IFA were positive in 8 of 8 and 5 of 5 patients, respectively, in whom *B. henselae* DNA was detected. Although numbers are small, it is encouraging that no false negatives were encountered in the ELISA and IFA subgroup. We failed to confirm the presence of *B. henselae* DNA in two patients who were skin test, ELISA, and IFA positive. Both of these patients had prolonged illnesses before biopsy, multiple courses of antibiotics, and histologically scarred resolving granulomas. This raises the possibility that perhaps *B. henselae* had been cleared from the sampled tissues or that the DNA remaining was degraded or below detectable limits. Three of fourteen patients (21%) were negative for *B. henselae* by skin test, ELISA, IFA, PCR, and SB. This small group is significant in pointing toward a subset of patients with CSD the etiology of which remains unidentified. It is in this group that other agents such as *A. felis* may be responsible.

In summary, we were able to confirm the presence of *B. henselae* DNA in paraffin-embedded tissues from 23 of 34 patients (68%) fulfilling pre-established clinical criteria of CSD using a combination of PCR and SB methods. In addition, *B. henselae* DNA was detected in 80% of patients with both clinical and serological evidence of infection. Only 11 of 34 (32%) cases were detectable by PCR alone, and the mean duration of illness in this group was 3.1 weeks. These findings suggest that *B. henselae* organisms are more numerous early in the course of CSD and the likelihood of detection by PCR is accordingly high; after 4 to 8 weeks, there are fewer organisms, so detection of target DNA requires the added sensitivity of SB techniques. These findings also seem to support a previous hypothesis by Wear et al about the natural history of CSD and the ability to visualize causative bacillary forms best early in the course of disease when organisms are more numerous.[24] *B. henselae* DNA was not detected in any of the patient control group, including a variety of cases of infectious lymphadenitis, for a specificity of 100%. Finally, our study shows that a small but significant subgroup of patients with clinical and histological features of CSD are negative serologically and by molecular methods for *B. henselae*. These cases emphasize the need for additional epidemiological and clinical investigation of CSD.

References

1. Carithers HA: Cat scratch disease. Am J Dis Child 1970, 119:200–203
2. Wear DJ, Margileth AM, Hadfield TL, Fischer GW, Schlagel CJ, King FM: Cat scratch disease: a bacterial infection. Science. 1983, 221:1403–1405
3. English CK, Wear DJ, Margileth AM, Lissner CR, Walsh GP: Cat-scratch disease: isolation and culture of the bacterial agent. JAMA 1988, 259:1347–1352
4. Brenner DJ, Hollis DG, Moss CW, English CK, Hall GS,

Vincent J, Radosevic J, Birkness KA, Bibb WF, Quinn PD, Swaminathan B, Weaver RE, Reeves MW, O'Connor SP, Hayes PS, Tenover FC, Steigerwalt AG, Perkins BA, Daneshvar MI, Hill BC, Washington, JA, Woods TC, Hunter SB, Hadfield TL, Ajello GW, Kaufmann AF, Wear DJ, Wenger JD: Proposal of *Afipia* gen. nov., with *Afipia felis* sp. nov. (formerly the cat scratch disease bacillus), *Afipia clevelandensis* sp. nov. (formerly the Cleveland Clinic Foundation strain), *Afipia broomeae* sp. nov., and three unnamed genospecies. J Clin Microbiol 1991, 29:2450–2460

5. Dolan MJ, Wong MT, Regnery RL, Jorgensen JH, Garcia M, Peters J, Drehner D: Syndrome of *Rochalimaea henselae* adenitis suggesting cat scratch disease. Ann Intern Med 1993, 118:331–336
6. Regnery RL, Olson JG, Perkins BA, Bibb W: Serological response to *R. henselae*: antigen in suspected cat-scratch disease. Lancet. 1992, 339:1443–1445
7. Barka NE, Hadfield T, Patnaik M, Schwartzman WA, Peter JB: EIA for detection of *Rochalimaea henselae*-reactive IgG, IgM, and IgA Antibodies in patients with suspected cat-scratch disease. J Infect Dis 1993, 167: 1503–1504
8. Zangwill KM, Hamilton DH, Perkins BA, Regnery RL, Plikaytis BD, Hadler JL, Cartter ML, Wenger JD: Cat scratch disease in Connecticut: epidemiology, risk factors, and evaluation of a new diagnostic test. N Engl J Med 1993, 329:8–13
9. Erler BS, Jiminez AM, Gedebou ML, Said JW, Nichols WS: Absence of *Rochalimaea henselae* sequences in cat scratch disease lymph nodes using a polymerase chain reaction. Mod Pathol 1993, 6:605 (abstract)
10. Jevon GP, Dunne WM, Finegold MJ: An analysis of lymph node DNA for possible bacterial agents of cat-scratch disease. Pediatr Pathol Lab Med 1995, 15:3–9
11. Alkan S, Morgan M, Sandin R, Moscinski L, Ross C: Dual role for *Afipia felis* and *Rochalimaea henselae* in cat-scratch disease. Lancet 345:385
12. Bergmans A, Groothedde J, Schellekens J, van Embden J, Ossewaarde J, Schouls L: Etiology of cat scratch disease: comparison of polymerase chain reaction detection of *Bartonella* (formerly *Rochalimaea*) and *Afipia felis* DNA with serology and skin tests. J Infect Dis 1995, 171:916–823
13. Anderson B, Sims K, Regnery R, Robinson L, Schmidt MJ, Gorel S, Hager C, Edwards K: Detection of *Rochalimaea henselae* DNA in specimens from cat-scratch disease patients by using polymerase chain reaction. J Clin Microbiol 1994, 32:942–948
14. Moriarity RA, Margileth AM: Cat scratch disease. Infect Dis Clin North Am 1987, 1:575–592
15. Szelc-Kelly CM, Goral S, Perez-Perez GI, Perkins BA, Edwards KM: Serologic responses to *Bartonella* and *Afipia* antigens in patients with cat scratch disease. Pediatrics 1995, 96:1137–1142
16. Sukpanichnant S, Vnencak-Jones CL, McCurley TL: Determination of B-cell clonality in paraffin-embedded endoscopic biopsy specimens of abnormal lymphocytic infiltrates and gastrointestinal lymphoma by polymerase chain reaction. Am J Clin Pathol 1994, 102(3): 299–305
17. Waldvogel K, Regnery RL, Anderson BE, Caduff R, Caduff J, Nadal D: Disseminated cat-scratch disease: detection of *Rochalimaea henselae* in affected tissue. Eur J Pediatr 1994, 153:23–27
18. Anderson B, Kelly C, Threlkel R, Edwards K: Detection of *Rochalimaea henselae* in cat-scratch disease skin test antigens. J Infect Dis 1993, 168:1034–1036
19. Koehler JE, Glaser CA, Tappero JW: *Rochalimaea henselae* infection: a new zoonosis with the domestic cat as reservoir. JAMA 1994, 271(7):531–535
20. Koehler JE, Quinn FD, Berger TG, LeBoit PE, Tappero JW: Isolation of *Rochalimaea* species from cutaneous and osseous lesions of bacillary angiomatosis. N Engl J Med 1992, 327:1625–1631
21. Adal KA, Cockerell CJ, Petri WA: Cat scratch disease, bacillary angiomatosis, and other infections due to *Rochalimaea*. N Engl J Med 1994, 330:1509–1515
22. Hadfield TL, Warren R, Kass M, Brun E, Levy C: Endocarditis caused by *Rochalimaea henselae*. Hum Pathol 1993, 24:1140–1141
23. Raoult D, Drancourt M, Carta A, Gastaut JA: *Bartonella* (*Rochalimaea*) *quintana* isolation in patient with chronic adenopathy, lymphopenia, and a cat. Lancet 1994, 343:977
24. Wear DJ, Malaty RH, Zimmerman LE, Hadfield TL, Margileth AM: Cat scratch disease bacilli in the conjunctiva of patients with Parinaud's oculoglandular syndrome. Ophthalmology 1985, 92:1282–1287