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Rochalimaea henselae sp. nov., a Cause of Septicemia, Bacillary Angiomatosis, and Parenchymal Bacillary Peliosis

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Nine strains of *Rochalimaea* spp. that were isolated from patients over a period of 4.5 years were characterized for their enzyme activities, cellular fatty acid compositions, and DNA interrelatedness among *Rochalimaea* spp., *Bartonella bacilliformis*, and *Afipia felis* (cat scratch disease bacillus). All except one isolate, which was *Rochalimaea quintana*, were determined to belong to a newly proposed species, *Rochalimaea henselae* sp. nov. After recovery from clinical material, colonies required 5 to 15 days of incubation to become apparent. Cells were small, gram-negative, curved bacilli and displayed twitching motility. Enzyme specificities for amino acid and carbohydrate substrates showed that *R. henselae* could be distinguished from *Rochalimaea vinsonii* by L-arginyl-L-arginine and L-lysyl-L-alanine peptidases, but not all strains could be distinguished from *R. quintana* on the basis of peptidases or carbohydrate utilization. *R. henselae* also closely resembled *R. quintana* in cellular fatty acid composition, with both consisting mainly of $C_{18:1}$, $C_{18:0}$, and $C_{16:0}$ fatty acids. However, the strains of *R. henselae* all contained $C_{18:0}$ in amounts averaging ≥22%, in contrast to *R. quintana*, which contained this cellular fatty acid in amounts averaging 16 and 18%. DNA hybridization confirmed the identification of one clinical isolate as *R. quintana* and showed a close interrelatedness (92 to 100%) among the other strains. Under optimal conditions for DNA reassociation, *R. henselae* showed approximately 70% relatedness to *R. quintana* and approximately 60% relatedness to *R. vinsonii*. Relatedness with DNA from *B. bacilliformis* was 43%. *R. henselae* was unrelated to *A. felis*. *R. henselae* is the proposed species of a newly recognized member of the family *Rickettsiaceae*, which is a pathogen that may be encountered in immunocompromised or immunocompetent patients. Prolonged fever with bacteremia or vascular proliferative lesions are clinical manifestations of the agent.

Rochalimaea spp. belong to the order *Rickettsiales*. Among members of this order that are known to be human pathogens, only *Rochalimaea quintana* and *Bartonella bacilliformis* are cultivable on bacteriologic media. Both are regarded as rare causes of infection in most parts of the world, although recent epidemics of bartonellosis have occurred in regions of the Peruvian Andes, where the organism is endemic (16).

R. quintana is the agent of trench fever, which is also known as Volhynia fever, Meuse fever, His-Werner disease, shinbone fever, shank fever, and quintan or 5-day fever (27). Bartonellosis is also known as Carrion's disease, and in the septicemic phase it is known as Oroyo fever (15). A distinct manifestation of bartonellosis is the cutaneous phase of the disease, known as verruga peruana (3). This is characterized by a dermal eruption consisting predominantly of vascular endothelial cell proliferation. The clinical and histologic appearances of these lesions are similar to those of Kaposi's sarcoma (4), cat scratch disease (10, 13, 17), and bacillary angiomatosis (BA). While they can usually be distinguished histologically, several reports have addressed difficulties in diagnosis (2, 4, 10, 18). BA, referred to as epithelioid angiomatosis (12) before the association with bacilli was established histologically (5), is most commonly diagnosed in human immunodeficiency virus (HIV)-infected patients. It is distinguished from Kaposi's sarcoma by its absence of malignant cells and its response to antimicrobial therapy (5). Another condition, peliosis hepatis, is seen in some patients in conjunction with BA (23, 31), and it is also characterized by angiogenesis. The liver is the organ that is usually involved, but peliosis of the spleen has also been reported. The presence of bacteria in peliosis hepatis was recently documented by Perkocha et al. (23).

In 1986 we encountered the first of several isolates of *R. quintana*-like organisms (RLOs) in blood cultures of septicemic adults at the Oklahoma Medical Center (30). Subsequently, we and others have seen apparent cutaneous (BA) and visceral (splenic and hepatic peliosis [23, 31]) manifestations of disease produced by the organism as well as additional cases of septicemia (9, 19, 29). Evidence linking BA, peliosis, and bacteremia caused by this organism has been established by recovery of the organism from infected tissue, histopathology, and DNA sequence analysis of the partial 16S rRNA gene in situ and from the organisms in pure culture (25). The latter was accomplished after Relman et al. (26) and Slater et al. (29) independently concluded that the agent associated with BA and a newly recognized agent of bacteremia resembled *R. quintana* but were not identical to *R. quintana* in several respects.

This report extends the clinical and laboratory characterization of the isolates recovered to date. Relatedness to other species and genera determined by DNA hybridization

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is presented as the basis for the proposal of the name *Rochalimaea henselae* as an agent of septicemia, BA, and parenchymal peliosis.

MATERIALS AND METHODS

Sources of strains. *B. bacilliformis* KC583 (ATCC 35685) and KC584 (ATCC 35686) were acquired from Aristides Herrer in 1963. Strain KC583 was recently designated as the type strain (8). The type strains of *R. quintana*, ATCC VR 358, and *Rochalimaea vinsonii*, ATCC VR 152, were provided by Ted Tzianabos, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control. The cat scratch disease bacillus *Afipia felis* (6) BV (F6400, ATCC 49713), the proposed type strain, was provided by D. J. Wear, Armed Forces Institute of Pathology. Clinical isolates of RLOs obtained from patients were recovered in the clinical microbiology laboratories of the Oklahoma Medical Center (six strains), Presbyterian Hospital (one strain), and the Baptist Medical Center (one strain [Table 1]), all in Oklahoma City. Those isolated from blood were recovered by using lysis-centrifugation (Isolator Microbial Tube, Wampole Laboratories, Cranbury, N.J.). Agar media for primary isolation included Columbia base (Becton Dickinson, Cockeysville, Md.) with 5% sheep blood and chocolate agars prepared with GC base (Becton Dickinson). Plates were made no more than 2 weeks in advance of use.

Characterization. In view of their general lack of reactivity in standard biochemical tests (29), all isolates were subjected to testing for preformed enzyme activity toward amino acid–β-naphthylamide substrates (hydroxyproline, L-isoleucine, L-proline, L-tyrosine, glycine, glycylglycine, glycyl-L-arginine, glycyl-L-proline, L-arginyl-L-arginine, L-lysyl-L-alanine, L-alanine, L-seryl-L-tyrosine, L-histidine) and nitrophenyl substrates of α-D-glucopyranoside, β-D-glucopyranoside, β-D-galactopyranoside, β-D-fucopyranoside, α-D-galactopyranoside, *N*-acetyl-β-D-glucosamine, β-D-cellobiose, and *N*-acetyl-β-D-galactosaminide. Preparations of substrates were those contained in the Rapid Yeast Identification Panel (Baxter Healthcare Corp., West Sacramento, Calif.). A heavy inoculum (1 loopful) of RLOs from 5- to 7-day-old cultures on heart infusion agar with 5% rabbit blood or 11- to 14-day-old cultures on *Haemophilus* test medium (Becton Dickinson) was delivered in sterile water to the wells of the panel and was incubated for 4 h at 35°C. The addition of reagents and interpretation of reactions were then done as recommended by the manufacturer for identifying yeasts.

CFAs. CFA compositions were determined by gas-liquid chromatography as described previously (29). Numerical analysis of CFA data was also performed, resulting in a dendrogram (unweighted pair matching) showing the Euclidean distance (degree of dissimilarity) among strains and related species (28). The CFA profiles of all isolates were based on the average amounts of individual CFAs determined in at least two independent analyses. CFA profiles and the dendrogram calculations were produced by using Library Generation Software (MIDI, Newark, Del.).

DNA relatedness. To obtain cells for DNA extraction, *B. bacilliformis*, at 25°C, and *Rochalimaea* species (including *R. henselae*), at 37°C, were grown on heart infusion agar with 5% rabbit blood. *A. felis* was grown on buffered charcoal-yeast extract agar at 30°C. Extraction and purification of DNA and the hydroxyapatite hybridization method for determining DNA relatedness have been described previously (7). DNAs were labeled enzymatically in vitro with [^{32}P]dCTP by using a nick-translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer.

RESULTS

Summary of cases. Details of the clinical illnesses of our first five patients were described previously (29). We have isolated *R. henselae* from a total of eight individuals and *R. quintana* from one individual over a 4.5-year period (Table 1). The organism was isolated from the blood of seven patients on multiple occasions and from the spleen of one patient (strain 91-148). Four patients were infected with HIV, with one patient having already developed AIDS. Two patients were recipients of allogeneic transplants (one bone marrow, one kidney) several years before the infection; both were receiving immunosuppressive agents. The remaining three patients were immunocompetent. No epidemiologic association existed among any of these patients. They developed their illnesses in widely separate locales in Oklahoma. Only one patient had a documented history of previous tick exposure, which occurred 1 week prior to the onset of his illness.

In the HIV-infected patients, *R. henselae* infection was characterized by an insidiously developing, prolonged symptom complex of malaise, fatigue, weight loss, and recurring fevers of gradually higher elevations. These symptoms spanned weeks to months before a diagnosis was made. In the first two patients, brief courses of antibiotic treatment with agents to which the isolates ultimately proved susceptible in vitro resulted in transient improvement in symptoms, but this was followed by relapse. In these patients, 1 month or more of antibiotic treatment was necessary before the fever was eradicated, but no further blood cultures were positive. The first of these patients was also ultimately shown to have bacillary peliosis hepatis (25, 31). In the third patient, prompt lysis of fever occurred within days of the institution of doxycycline, which was administered for 1 month. However, 1 week after its discontinuation, both clinical and bacteriologic relapses occurred, requiring a second month-long course of high-dose erythromycin.

In contrast, the HIV-uninfected patients, whether pharmacologically immunosuppressed or immunocompetent, all had abrupt onsets of febrile illnesses. The three patients which we described in detail previously (29) all had rapid clinical improvements with short courses of antimicrobial treatment (no longer than 10 days) and no subsequent clinical recurrences. We have since followed another immunocompetent patient with fever and bacteremia who had clinical and bacteriologic relapses after his first course of 10 days of tetracycline and after his second course of 10 days of doxycycline. He was cured after 2 weeks of chloramphenicol

TABLE 1. Clinical isolates of RLOs

Strain no.	Source/underlying disease[a]
87-66	Blood, 31-yr-old man/HIV infection
88-64	Blood, 33-yr-old man/HIV infection
88-712	Blood, 28-yr-old woman/CML, bone marrow transplant recipient
89-674	Blood, 43-yr-old man/none
89-675	Blood, 40-yr-old woman/none
90-268	Blood, 55-yr-old man/HIV infection
90-615	Blood, 30-yr-old man/none
90-782	Blood, 30-yr-old man/HIV infection
91-148	Spleen, 44-yr-old woman/renal transplant recipient

[a] CML, chronic myelogenous leukemia.

TABLE 2. Peptidase activities of *Rochalimaea* spp.

Strain	Activity on the following substrate[a]:												
	HIS	HPR	ILE	PRO	TYR	GLY	GGLY	GLAR	GLPR	AARG	LYAL	ALA	STY
R. quintana	−	−	−	−	−	+	+	+	−	+	+	+	+ (ω)
R. vinsonii	−	−	−	−	−	+	+	+	−	−	−	+	+
RLO													
87-66	−	−	+	−	+	+	+	+	−	+	+	+	+
88-64	−	−	+	−	+	+	+	+	−	+	+	+	+
88-712	−	−	−	−	−	+ (ω)	+	+	−	+	+	+	+ (ω)
89-675	−	−	+	−	+ (ω)	+	+	+	−	+	+	+	+
90-268	−	−	−	−	−	+	+	+	−	+	+	+	−
90-615	−	−	−	−	−	+	+	+	−	+	+	+	−
90-782	−	−	−	−	−	+	+	+	−	+	+	+	−
91-148	−	−	−	−	−	+	+	+	−	+	+	+	−

[a] HIS, L-histidine; HPR, hydroxyproline; ILE, L-isoleucine; PRO, L-proline; TYR, L-tyrosine; GLY, glycine; GGLY, glycylglycine; GLAR, glycyl-L-arginine; GLPR, glycyl-L-proline; AARG, L-arginyl-L-arginine; LYAL, L-lysyl-L-alanine; ALA, L-alanine; STY, L-seryl-L-tyrosine. ω, weak reaction.

treatment followed by 2 weeks of erythromycin treatment. The renal transplant recipient from whom the organism was cultured from the spleen may have been bacteremic, but her blood cultures were still negative when they were discarded after only 7 days of incubation. She was found to have large nodular lesions of the liver and spleen as well as retroperitoneal lymphadenopathy. The histologic abnormalities found included pyogranulomata, BA, and bacillary peliosis of both the liver and spleen. She was cured with a month-long course of high-dose erythromycin.

The *R. quintana* isolate was from a 55-year-old HIV-infected man. His record contained no description of louse infestation at the time of his presentation with a history of several days of high fever, confusion, and ataxia. He was found to have cryptococcal meningitis and was treated successfully with amphotericin B. Because an ulcer on the penis was interpreted as being consistent with a lesion of primary syphilis, he also received a 10-day course of high-dose penicillin before *R. quintana* (3 CFU/ml) was isolated on day 13 from a lysis-centrifugation blood culture obtained on admission. Repeat blood cultures, obtained after the completion of penicillin therapy, were negative.

Phenotypic characteristics. The blood culture isolates (both *R. henselae* and *R. quintana*) were recovered after 5 to 15 days of incubation of lysis-centrifugation (Isolator) culture plates. The average interval to the detection of growth was 9 days. RLO 91-148 (patient 8) was recovered from spleen tissue in 11 days. The whitish colonies were dry, autoadherent, and heterogeneous with respect to size and consistency. When they were first observed, colonies were generally pinpoint in size. On subculture, isolates grew at 35 to 37°C, required 5 to 10% CO_2, and did not grow anaerobically or at temperatures of 25 or 42°C. The property of adherence was also observed after centrifugation of a suspension in a glass tube or attempting propagation in broth cultures, which resulted in a film and pellicle of organisms on the glass and broth surfaces. Microscopically, organisms were small, curved, gram-negative bacilli. The size and morphology strongly suggested *Campylobacter* spp. on the initial examination of the primary cultures. The organisms were motile by wet mount, which was thought to distinguish them from *R. quintana*. However, the RLOs displayed twitching motility, and attempts, including electron microscopy, to further characterize the motility did not reveal the presence of flagella. On examination of *R. quintana* VR358, this same twitching motility was also observed. Motility was confirmed for RLO 87-66 in the Enteric Bacteriology Section at the Centers for Disease Control.

Utilization of carbohydrates was undetectable by conventional tests (29) or the use of the nitrophenyl substrates. Enzyme activities on the amino acid substrates are given in Table 2. *R. quintana* and all of the RLO strains could be distinguished from *R. vinsonii* by the absence of L-arginyl-L-arginine and L-lysyl-L-alanine peptidases in the latter. Three RLO strains (87-66, 88-64, 89-675) hydrolyzed L-isoleucine and L-tyrosine, distinguishing them from *R. quintana*, but none of the other strains could be distinguished clearly from *R. quintana* on the basis of specificity for the other substrates. A weakly positive L-seryl-L-tyrosine reaction was the only test that separated *R. quintana* from RLOs 90-268, 90-615, 90-782, and 91-148.

CFA compositions. In general, the RLO strains resembled *R. quintana* in their CFA compositions (Table 3). All were characterized by a relatively simple profile made up largely of $C_{18:1}$ (>50%), $C_{18:0}$ (16 to 25%), and $C_{16:0}$ (16 to 22%). Minor (up to 4%) amounts of $C_{13:1}$ and $C_{17:0}$ were found in most strains, including *R. quintana*. *R. quintana* was distinguished from all strains of RLO except 90-268 by the presence of $C_{18:0}$ in amounts of greater than 20% in the latter. Qualitative differences in CFA compositions of the RLOs and *R. quintana* from *R. vinsonii* and *B. bacilliformis* were the presence of $C_{15:0}$ and $C_{12:0}$, respectively, in the last two organisms. Cluster analysis of the CFA results, as seen in a dendrogram showing the relationships among *Rochalimaea* spp. and *B. bacilliformis*, also suggested that strain 90-268 was more closely related to *R. quintana* than to the other RLOs (Fig. 1). In accord with the qualitative and quantitative differences in CFA composition, a relatively distant relationship of all *Rochalimaea* spp. to *B. bacilliformis* was observed.

DNA hybridization. DNA hybridization results are given in Table 4. RLO 90-268 was definitely *R. quintana*, exhibiting 100% relatedness, with 1.5% divergence in related sequences, to the *R. quintana* type strain. The eight other strains formed a single, highly related hybridization group indicative of a single species. This species was 55 to 71% related to *R. quintana* and *R. vinsonii* and was 43% related to *B. bacilliformis*. *R. quintana* and *R. vinsonii* were 55 to 68% related in reciprocal reactions. *R. quintana* was 41 to 45% related to two strains of *B. bacilliformis*.

DISCUSSION

The initial reports (23, 26, 29) which raised the possibility (14) of a single newly recognized *Rochalimaea*-like agent that produces at least three different manifestations of infection have been corroborated by subsequent observations

TABLE 3. CFA compositions of *Rochalimaea* spp. and *B. bacilliformis*[a]

Strain	Amount of CFA (% of total)[b]										
	$C_{12:0}$	$C_{13:1}$	$C_{14:0}$	$C_{15:0}$	$C_{16:1\omega7}$[c]	$C_{16:0}$	$C_{17:1}$	$C_{17:0}$	$C_{18:1\omega9}$	$C_{18:1\omega7}$	$C_{18:0}$
RLO											
87-66		<1	<1		1	19		<1	<1	54	22
88-64			<1		<1	20			1	52	23
88-712		1			<1	22		<1	<1	52	22
89-674		<1			<1	16		2	<1	52	25
89-675		1			<1	17	<1	5	<1	51	22
90-268		3			<1	17		2	<1	58	18
90-615		2			<1	16		3	<1	52	24
90-782		1			<1	17		3	<1	51	24
91-148		1			<1	19		2	<1	53	23
R. quintana		1	<1		<1	20		1	<1	58	16
R. vinsonii		6		2	2	18	3	13		45	9
B. bacilliformis	4	2	4		18	25			2	35	7

[a] Fatty acid methyl esters were derived from the cells of each strain grown under the same conditions (5 to 7 days at 35°C and in CO_2 on rabbit heart infusion agar; however, *B. bacilliformis* was cultivated on Trpyticase soy blood agar at 30°C for 5 days.
[b] Amounts of ≤1% for some CFAs are not included.
[c] ω, weak reaction.

(25, 31) and the findings of the present study. The recovery of *R. henselae* depends on extended (at least 2 weeks) incubation of cultures using blood-enriched and, probably, freshly prepared media. A further requirement, from a practical standpoint, is use of the lysis-centrifugation method when culturing blood. These practices have pertained to all cultures of blood specimens from adults in our hospital since 1988, when a dramatic increase in the frequency of isolation of pathogens such as the *Mycobacterium avium* complex and *Histoplasma capsulatum* was noticed. The standard blood culture set consists of a biphasic (Septi-Chek) system in addition to lysis-centrifugation, but none of the *Rochalimaea* spp. was initially detected in the biphasic medium.

The technique that achieves the isolation of this organism is not very different from that described by Vinson (34) in

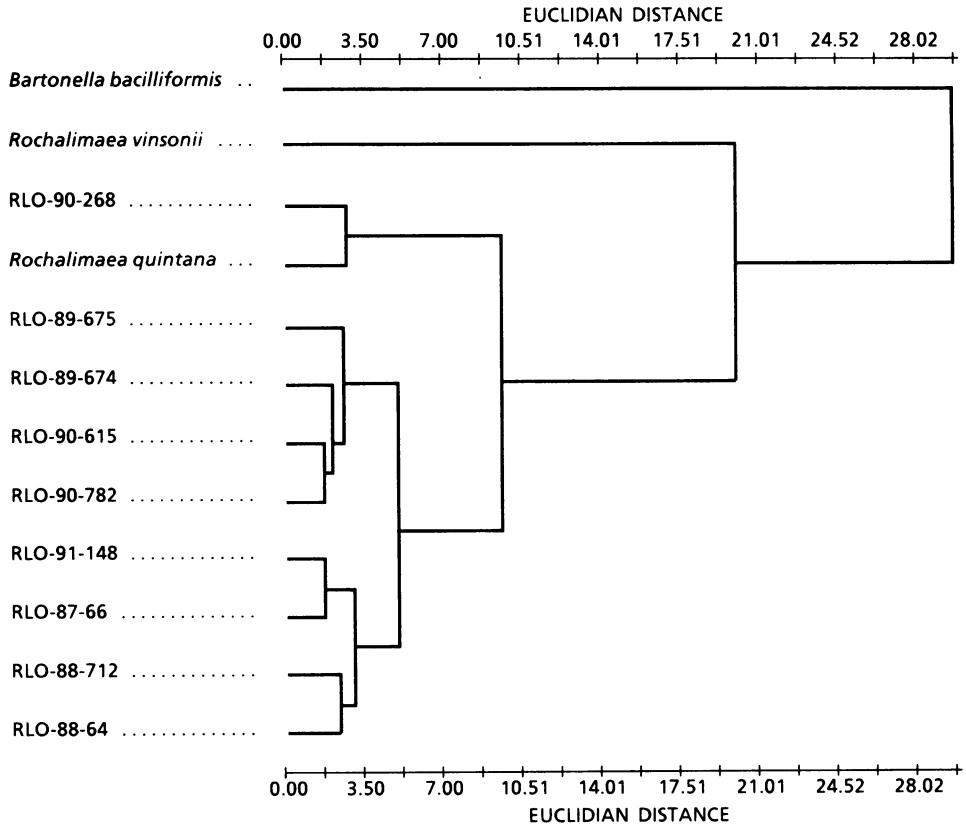

FIG. 1. Dendrogram based on cellular fatty acid compositions of *B. bacilliformis* and *Rochalimaea* spp.

TABLE 4. DNA interrelatedness of RLOs and their relatedness to *Rochalimaea* spp., *B. bacilliformis*, and *A. felis*

Source of unlabeled DNA	% Relatedness to labeled DNA from:								
	RLO 87-66			*R. quintana*			*R. vinsonii*		
	55°C	D[a]	70°C	55°C	D	70°C	55°C	D	70°C
RLO									
87-66	100	0.0	100	67	10.5	35	64	11.5	33
88-64	99	0.0	100	69	10.5	39	63	11.5	29
88-712	92	1.0	99	67	11.5	36	58	12.0	30
89-674	100	1.0	100	66	10.0	38			
89-675	98	0.5	100	68	11.0	33			
90-268	66	6.5	44	100	1.5	99			
90-615	100	0.0	100	68	11.0	37			
90-782	100	1.0	100	71	10.0	43			
91-148	92	0.5	91	62	11.0	32			
R. vinsonii	55	10.0	29	62	11.5	32	100	0.0	100
R. quintana	56	8.5	38	100	0.0	100	55	11.0	31
B. bacilliformis KC583	43	9.5	35	41	14.5	15			
B. bacilliformis KC584				45	13.5	21			
A. felis	1			1					

[a] D, divergence, calculated to the nearest 0.5%.

1966 for the recovery of *R. quintana* from the blood during trench fever. He recommended direct plating of blood on blood agar, followed by incubation at 37°C for 12 to 14 days in 5% CO_2. A reported effect of hemoglobin, compared with that of whole blood, on the growth of *R. quintana* is also consistent with our findings of better growth on rabbit or sheep blood agars than on chocolate agar (29). Freshly prepared blood medium was used by Vinson (34) and also in a later study by Varela and colleagues (33), but the latter investigators noted that for growth of some strains of *R. quintana*, heat inactivation of complement was required. As with *R. quintana* (20, 21), erythrocytes or an erythrocyte-associated factor promotes optimal growth of *R. henselae*. Weaker growth on media such as *Haemophilus* test medium suggests that the blood requirement may be partially replaced by hemin or starch.

Because of the highly fastidious nature of *R. henselae*, the efficiency of laboratory diagnosis by culture is unknown. Therefore, studies to determine the prevalence of this pathogen will be complex to conduct and will probably need to include antigen and antibody detection. Further analysis of the 16S rRNA gene (11, 25) or other regions of the genome may yield results suggesting primers and sequence information that could be useful for the development of probes. In the meantime, cultures for *R. henselae* should be attempted when patients have syndromes involving fevers of unknown origin or unexplained visceral or cutaneous vascular proliferative foci, especially if such persons are immunocompromised.

On initial isolation, *R. henselae* is most likely suspected to be a *Mycobacterium* sp. on the basis of growth rate or a *Campylobacter* sp. on the basis of microscopic morphology. In our laboratory, the order of frequency of pathogens isolated from blood cultures beyond 4 days of incubation are *M. avium* complex, *H. capsulatum*, and *R. henselae*. Therefore, presumptive identification of *R. henselae* is made by ruling out acid-fast bacilli and yeasts. Campylobacteremia occurs rarely, but the morphology of *R. henselae* in primary cultures is highly suggestive of *Campylobacter* spp. Another possible consideration among first impressions of *R. henselae* is *Helicobacter* spp., since both share the properties of curved morphology and relatively slow growth. Of interest is a recent report of *Campylobactercinaedi* [sic] bacteremia in a woman with transfusion-associated AIDS and "aggressive" Kaposi's sarcoma (1). Methodologic details were not given for the identification of *Campylobactercinaedi* [sic], i.e., *Campylobacter "cinaedi,"* which is now assigned to the genus *Helicobacter* (32).

Confirmatory identification of *R. henselae* is not aided by conventional or rapid test kits for biochemical characterization. The isolates are unreactive in the commonly applied tests (29). Special requirements for the demonstration of decarboxylase activities of other *Rochalimaea* spp. have been reported (35). Our results and those of Clarridge et al. (9) suggest that testing of isolates by use of substrates for preformed enzymes may be useful. Screening for additional enzyme specificities should be done, because the substrates used in the present study did not allow the differentiation of three strains from *R. quintana*. While very similar in CFA composition to *R. quintana*, it appears that *R. henselae* can apparently be identified on the basis of the amounts of $C_{18:1}$, $C_{16:0}$, and $C_{18:0}$. This would be the most practical approach to identify *R. henselae* for laboratories that have access to gas-liquid chromatography for CFA analysis. We also have developed polyclonal antiserum that is reactive in immunofluorescence assays with *R. henselae* and that is unreactive with other organisms, including *R. quintana* (31), and thus allows an early presumptive identification to be made.

DNA hybridization leaves no doubt that all of the RLOs are members of the genus *Rochalimaea*, with strain 90-268 being *R. quintana* and the other eight strains representing a previously unrecognized *Rochalimaea* species, which in all probability includes the etiologic agent of BA and parenchymal bacillary peliosis. By mutual agreement with Regnery et al. (24), the name *R. henselae* sp. nov. is proposed for this new species.

On the basis of the previously reported differences in genome size, guanine-plus-cytosine content, lack of DNA relatedness, and low level of 16S rRNA sequence homology between *A. felis* (cat scratch disease bacillus) and *R. quintana*, it is not surprising that *R. henselae* and *A. felis* DNAs are essentially unrelated (Table 4) (6, 8, 22).

The 16S rRNA sequence homology of *B. bacilliformis* and *R. quintana* is 91.7%; that between *R. quintana* and *Brucella melitensis* biotype abortus (*Brucella abortus*) is 92.5%, and that between *B. bacilliformis* and *B. melitensis* biotype abortus is 91.3% (22). Those sequence homology values are consistent with each of these species being in a different genus. However, the greater than 40% DNA relatedness by hybridization between *B. bacilliformis* and *Rochalimaea* species argues that all of these species should be in the same genus.

Bartonella is classified in the family *Bartonellaceae*, whereas *Rochalimaea* is classified in the family *Rickettsiaceae*; however, the 16S rRNA sequence homology between these genera is much greater than that between *Rochalimaea* and other genera (*Rickettsia*, *Ehrlichia*) in the family *Rickettsiaceae* (8). Furthermore, the 16S rRNA sequence homologies of both *Bartonella* and *Rochalimaea* species to *B. melitensis* biotype abortus, which is not considered to be a rickettsia, is substantially higher than their relatedness to members of the genus *Rickettsia* (8). These inconsistencies should be addressed in a systematic study of rickettsial classification.

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