Emended description of *Rickettsia felis* (Bouyer *et al.* 2001), a temperature-dependent cultured bacterium

Unité des Rickettsies, CNRS
UPRESA 6020, Faculté de
Médecine, Université de la
Méditerranée, 27 Bd Jean
Moulin, 13385 Marseille
cedex 05, France

Bernard La Scola, Sonia Meconi, Florence Fenollar, Jean-Marc Rolain,
Véronique Roux and Didier Raoult

Author for correspondence: Didier Raoult. Tel: +33 4 91 32 43 75. Fax: +33 4 91 38 77 72.
e-mail: Didier.Raoult@medecine.univ-mrs.fr

On the basis of phenotypic data obtained on the strain Marseille-URRWFXCal₂^T, isolated from the cat flea *Ctenocephalides felis*, the description of *Rickettsia felis* (Bouyer *et al.*, 2001) is emended and Marseille-URRWFXCal₂^T is proposed as the type strain of the species. On the basis of polyphasic characterization, especially the inability to grow at temperatures higher than 32 °C on Vero cells that allow growth of other *Rickettsia* to at least 35 °C, it is confirmed that this agent, although different from other recognized rickettsial species, is genotypically indistinguishable from bacteria previously detected within cat fleas and provisionally named ELB. Comparison of the phenotypic characteristics previously described for *R. felis* and those observed for the isolate in this study indicated some differences, although concurrent analysis of the two was not possible as no extant isolates of the first isolate of *R. felis* exist.

Keywords: *Rickettsia felis*, ELB agent, *rpoB* gene, actin polymerization, antibiotic susceptibility

INTRODUCTION

In 1990, whilst examining the potential of cat fleas (*Ctenocephalides felis*) to act as vectors for a peculiar form of epidemic typhus in the USA, a *Rickettsia*-like organism, named the ELB agent, was observed within midgut epithelial cells of a group of cat fleas by electron microscopy (Adams *et al.*, 1990). The taxonomic position of this agent within the genus *Rickettsia* was confirmed by sequence comparison following the successful amplification of a 17 kDa protein gene fragment from infected flea tissue using PCR incorporating genus-specific primers (Azad *et al.*, 1992). Attempts to isolate the ELB agent were made (Radulovic *et al.*, 1995a, b; Higgins *et al.*, 1996), but it now appears that the recovered culture was contaminated by *Rickettsia typhi* (Radulovic *et al.*, 1995a, 1996). Before this error was identified, the name *Rickettsia felis* was informally proposed for the organism following characterization of a suspected isolate recovered by Vero cell culture. This isolate was

not deposited in any culture collections and therefore no independent confirmatory analysis or further comparison with other *Rickettsia* species has been possible. The proposed name *R. felis* has recently been formally validated on the basis of molecular data (Bouyer *et al.*, 2001) and confirmed previous studies that underlined the unique position of this *Rickettsia* species (Adams *et al.*, 1990; Azad *et al.*, 1992; Bouyer *et al.*, 2000; Higgins *et al.*, 1996; Noden *et al.*, 1998; Radulovic *et al.*, 1995b; Raoult *et al.*, 2000; Zavala-Velazquez *et al.*, 2000).

By inoculating crushed cat fleas onto XTC-2 cells incubated at 28 °C, we isolated an intracellular bacterium genotypically indistinguishable from the ELB agent (Raoult *et al.*, 2000), but that was clearly phenotypically distinct from *R. felis*. We present herein additional data for an extended description of Marseille-URRWFXCal₂^T, the reference strain of the species, and an emended description of the species *R. felis*.

METHODS

Source of organisms. Strain Marseille-URRWFXCal₂^T was isolated from *Ctenocephalides felis* obtained from Dr Jay Georgi, Flea Data Inc., Freville, NY, USA. Isolation and

The GenBank accession numbers for the sequences of the 3' end of the *Rickettsia felis ompA* gene and the *R. felis rpoB* gene are AF231136 and AF236795, respectively.

Table 1. Oligonucleotide primers used for *rpoB* and 3' end of *ompA* PCR amplifications and sequencing reactions

Gene	Primer	Nucleotide sequence (5'–3')	Gene position relative to ORF	Reference
<i>ompA</i>	OMPAPUL3519*	GGTATAATAACTGACAATAC	3519–3538	This manuscript
	190-5238*	ACTATTAAAGGCTAGGCTATT	5238–5218	Fournier <i>et al.</i> (1998)
	OMPAPUL4905*	TCAGGCTCTGAAGTTAAACT	4905–4924	This manuscript
	190-5831*	GTGTCGCTAGGTTTTACAAC	5831–5812	Fournier <i>et al.</i> (1998)
	190-5768*	CACCGCTACAGGAAGCAGAT	5768–5787	Fournier <i>et al.</i> (1998)
	190-6808*	CACGAACCTTTCACACTACC	6808–6790	Fournier <i>et al.</i> (1998)
	OMPAPUL3775†	GCTAAGAACGCTGATGTTGA	3775–3794	This manuscript
	OMPAPUL3794†	TCAACATCAGCGTTCTTAGC	3794–3775	This manuscript
	OMPAPUL4320†	TTATCGTACCGTTACCGGCTG	4320–4300	This manuscript
	OMPAPUL4495†	CTGAGATATTTGCTAACGA	4495–4513	This manuscript
	OMPAPUL4924†	AGTTTAACTTCAGAGCCTGA	4924–4905	This manuscript
	OMPAPUL5569†	GCTGAAGATATAGCTGAAGA	5569–5588	This manuscript
	OMPAPUL5588†	TCTTCAGCTATATCTTCAGC	5588–5569	This manuscript
	OMPAPUL6249†	CGGCACTAGAGACTGTCCGGC	6249–6268	This manuscript
	OMPAPUL6322†	CATTAAGTACCTGTATAGC	6322–6303	This manuscript
<i>rpoB</i>	RPOPULM25*	GTAATTTTATCAGTYAGGAG	M25–M6	This manuscript
	RPOPUL539*	TGGACTTTTCCTTCATCATG	539–520	This manuscript
	Bap355D*	GAGCAAGAAGTATATATGGG	400–419	Drancourt <i>et al.</i> (1999)
	RPOPUL2237*	TCTACCTGCTCAACAATACC	2237–2218	This manuscript
	RPOPUL1939*	CAAGGAGAGTTYATTAATTGCCG	1939–1961	This manuscript
	Bap3850r*	GCCCAACATTCCATTTTCRCC	3927–3908	Drancourt <i>et al.</i> (1999)
	RPOPUL965†	CTGCAAGCGATGAAGTATTA	965–984	This manuscript
	RPOPUL984†	TAATACTTCATCGCTTGCGAG	984–965	This manuscript
	RPOPUL1586†	CCCAATTTATGGATCAAACAAA	1586–1607	This manuscript
	RPOPUL1607†	TTGTTTGATCCATAAATTGGG	1607–1586	This manuscript
	RPOPUL2630†	AAGCGTTGCGTCATCTTGAT	2630–2650	This manuscript
	RPOPUL2650†	ATCAAGATGACGCAACGCTT	2650–2630	This manuscript
	RPOPUL3096†	GGAAGATGCAAATGTAATGAATG	3096–3118	This manuscript
	RPOPUL3118†	CATTCATTACATTTGCATCTTCC	3118–3096	This manuscript
	RPOPUL3538†	CTTGAGCTATACGGAGAGAA	3538–3557	This manuscript
	RPOPUL3557†	TTCTCTCCGTATAGCTCAAG	3557–3538	This manuscript

* Oligonucleotide primer used for PCR amplification and sequencing reaction.

† Oligonucleotide primer used only for sequencing reaction.

maintenance of this isolate in XTC-2 cells has been detailed elsewhere (Raoult *et al.*, 2000). The isolate was carefully checked for lack of *R. typhi* contamination (Raoult *et al.*, 2000). Other strains studied were *R. typhi* (Wilmington strain), *Rickettsia prowazekii* (Brein 1 strain), *Rickettsia conorii* (Seven strain) and *Rickettsia akarii* (MK Kaplan strain). Routine culture of these bacteria in HEL cells has been described previously (La Scola & Raoult, 1996).

Analysis of major proteins using SDS-PAGE and Western-blotting. Rickettsial strains were renografin-purified as described previously (Raoult *et al.*, 2000). Renografin-purified preparations of Marseille-URRWFXCal₂^T, *R. typhi*, *R. prowazekii*, *R. conorii* and *R. akarii* proteins were resuspended in SDS-PAGE sample buffer (0.625 M Tris/HCl, pH 8.0; 2%, w/v, SDS; 5%, v/v, 2-mercaptoethanol; 10%, v/v, glycerol; 0.002%, w/v, bromophenol blue). An aliquot of each was then heated for 10 min at 100 °C, then heated and unheated aliquots were loaded onto on 9–16% linear gradient polyacrylamide gels (18 cm × 20 cm × 1.5 mm). Proteins were electrophoretically resolved at

40 mA for 5 h at 10 °C (Laemmli, 1970). Resolved proteins were stained with silver stain. Major immunogenic proteins were studied by Western blotting using purified unheated antigens as previously reported (Laemmli, 1970; Teyssie *et al.*, 1992) and anti-Marseille-URRWFXCal₂^T rabbit polyclonal antibodies (Raoult *et al.*, 2000).

Detection of actin polymerization. Adherent XTC-2 cells were cultivated in glass Lab-Tek chamber slides (Miles) under the conditions described above. Marseille-URRWFXCal₂^T cells were deposited on adherent cells for 1 or 2 h incubation at room temperature. Extracellular bacteria were removed by extensive washing of the cells. After 48 h incubation at 28 °C, cells were fixed with 1% formaldehyde for 20 min and washed. Actin filaments and bacteria were stained by incubation with PBS containing 10 U bodipy phalloidin ml⁻¹ (Molecular Probes), 1% bovine serum albumin, 100 µg lysophosphatidylcholine ml⁻¹ (Sigma) and specific mouse antibodies directed against Marseille-URRWFXCal₂^T at 1:400 dilution for 30 min. After washing, rhodamine-conjugated F(ab')₂ antimouse

IgG was added to the cell preparation for 30 min. Cells were then examined with a laser confocal fluorescence microscope (Leica) equipped with a $\times 100$ (NA 1.4) oil immersion lens. As a control, *R. conorii* and *R. typhi* were processed in the same manner, but using specific polyclonal antibodies for detection of bacteria.

Antibiotic susceptibility. The activity of erythromycin against Marseille-URRWFXCal₂^T was determined in Vero cells by a colorimetric assay using Gimenez staining. Vero cells in culture in 48-well microtitre plates were infected with 2000 p.f.u. rickettsiae for 1 h at room temperature. Erythromycin was added at different concentrations in different rows. Drug-free rows infected with 2000, 200, 20 or 0 p.f.u. served as controls. After 9 days incubation of the plates at 32 °C, cell culture monolayers were stained using Gimenez dye to reveal the presence of infected foci (clusters of rickettsiae) in 25 randomly chosen fields for each well. The minimal antibiotic concentration allowing complete inhibition of foci formation as compared to drug-free control was recorded as the MIC. For doxycycline and rifampicin, a single concentration of 4 $\mu\text{g ml}^{-1}$ was tested. Experiments were performed in duplicate to confirm the results. The incubation temperature was lowered to 32 °C to allow growth of Marseille-URRWFXCal₂^T. As controls, *R. conorii* and *R. typhi* were processed in the same manner.

rpoB and ompA 3' end PCR amplification and sequencing. Genomic DNA extractions, PCR amplifications and sequencing reactions were performed as described previously (Raoult *et al.*, 2000). PCR amplification of the *ompA* 3' end was obtained using primer pair 190-5768 and 190-6808. An improved PCR method for walking in uncloned genomic DNA, using the Universal Genome Walker kit (Clontech) had to be used for the determination of the 5' end of the sequence. Primers 190-5831, OMPAPUL5588, OMPAPUL5238, OMPAPUL4924, OMPAPUL4320 and OMPAPUL3794 were used. *ompA* was then amplified in three fragments using primer pairs OMPAPUL3519 and 190-5238, OMPAPUL4905 and 190-5831, and 190-5768 and 190-6808, and sequences were confirmed using the primers listed in Table 1. For *rpoB* amplification, the primers were designed following identification of suitable hybridization sites within conserved regions of the gene after alignment of the sequence of Bar 29 (AF076436), *Rickettsia massiliae* (AF76433), *R. conorii* Seven (AF076434), *R. prowazekii* (AF034531) and *R. typhi* (AF083622), or were described by Drancourt & Raoult (1999). *rpoB* was amplified in three fragments using primer pairs RPOPULM25 and RPOPUL539, Bap355D and RPOPUL2237, and RPOPUL1939 and Bap3850r. Sequencing reactions were performed using the primers described in Table 1. The *ompA* and *rpoB* sequences were translated into protein sequences using PC/GENE software (IntelliGenetics). The percentages of similarity were determined using the same software package. The 5' and 3' ends of the *ompA* gene were joined and phylogenetic analyses were performed as described previously (Raoult *et al.*, 2000).

RESULTS AND DISCUSSION

The SDS-PAGE profile of Marseille-URRWFXCal₂^T was clearly different from those derived from *R. typhi*, *R. prowazekii*, *R. conorii* and *R. akari* (Fig. 1). On unheated samples, Marseille-URRWFXCal₂^T expressed a high-molecular-mass protein of over 150 kDa which was not shared with other species. On

Fig. 1. Silver-stained SDS-PAGE of whole-cell protein preparations of Marseille-URRWFXCal₂^T, *R. conorii*, *R. typhi*, *R. prowazekii* and *R. akari*. Lanes: 1, 6, Marseille-URRWFXCal₂^T; 2, 7, *R. conorii*; 3, 8, *R. typhi*; 4, 8, *R. prowazekii*; 5, 10, *R. akari*. Lanes: 1–5, unheated samples; 6–10, heated samples.

heated samples, all species shared a high-molecular-mass protein of about 120 kDa. Some other minor differences were also observed, thus the SDS-PAGE protein profile of our strain is markedly different to that of the first description of *R. felis* which was previously found to closely resemble that of *R. typhi*. This resemblance was also reflected by Western blotting (Radulovic *et al.*, 1995b; Higgins *et al.*, 1996; Azad *et al.*, 1997), whereas we recently demonstrated that the immunogenic antigens of Marseille-URRWFXCal₂^T were distinguishable from those of *R. typhi* with both a human serum reacting specifically to Marseille-URRWFXCal₂^T and a mouse antiserum raised against the purified isolate (Raoult *et al.*, 2000), specifically because of a reaction against the 30 kDa antigen. These findings were confirmed in this study using rabbit polyclonal antibodies (Fig. 2).

Fluorescence and double staining of actin and bacteria was performed to evaluate capability of Marseille-URRWFXCal₂^T to polymerize actin intracellularly. Polar polymerization of actin was not observed within cells infected by Marseille-URRWFXCal₂^T or *R. typhi* contrary to those infected by *R. conorii* as described previously (Fig. 3). Actin polymerization seems to be associated with a capability to grow in an intranuclear location, a feature common to the spotted fever group rickettsia studied to date, but absent in the typhus

Fig. 2. Western blot of rickettsial proteins probed with anti-Marseille-URRWFXCal₂^T rabbit polyclonal serum. Lanes: 1, Marseille-URRWFXCal₂^T; 2, *R. conorii*; 3, *R. typhi*; 4, *R. prowazekii*; 5, *R. akari*.

group members and Marseille-URRWFXCal₂^T (Heinzen *et al.*, 1993; Teyssie *et al.*, 1992; Burgdorfer *et al.*, 1968; Van Kirk *et al.*, 2000).

Analysis of the antibiotic susceptibilities of Marseille-URRWFXCal₂^T, revealed an MIC for erythromycin of 32 µg ml⁻¹, and that a concentration of 4 µg doxycycline or rifampicin ml⁻¹ was inhibitory. Results obtained for controls were identical to those obtained previously (Rolain *et al.*, 1998). Susceptibility to doxycycline and rifampicin is thus common to all species tested, whereas susceptibility to erythromycin is a feature of the typhus group (Rolain *et al.*, 1998) and of the first description of *R. felis* (Radulovic *et al.*, 1995a, 1996).

The *ompA* and *rpoB* genes were sequenced from base 3539 to 6722 and from base 1 to 3866, respectively. For *rpoB*, sequence similarities ranged from 96 (*R. massiliae*, Bar 29, *R. conorii*) to 87% for nucleotide sequences. Sequence similarity was 92% between *R. felis* and *R. prowazekii*. For amino acid sequences, similarities ranged from 98 (*R. massiliae*, Bar 29, *R. conorii*) to 83% (*R. typhi*). Sequence similarity was 96% between *R. felis* and *R. prowazekii*. The dendrograms obtained from *ompA* with the three different tree-building methods used showed similar phylogenetic positions for *R. felis* which is clearly separated from other *Rickettsia* species (bootstrap value of 100%; Fig. 4). These data are in accordance with those reported Bouyer *et al.* (2001). Interestingly, in their original description of *R. felis*, the authors

Fig. 3. Double staining of actin and Marseille-URRWFXCal₂^T (a), *R. conorii* (b) and *R. typhi* (c). Note the polar actin polymerization within cells infected by *R. conorii* (arrows).

speculated that the rOmpA protein, which is specific to the spotted fever group rickettsia, was absent from their isolate (Radulovic *et al.*, 1995b). However, studies have subsequently demonstrated that the *ompA* gene in this bacterium contains several premature stops that would result in a truncated rOmpA protein (Bouyer *et al.*, 2000, 2001).

A main observation that differentiates Marseille-

Fig. 4. Phylogenetic tree of the bacteria belonging to the genus *Rickettsia*, inferred from comparison of *ompA* sequences. The evolutionary distance values were determined by the neighbour-joining method. The numbers at nodes are proportions of 100 bootstrap resamplings that support the topology shown.

URRWFXCal₂^T from previous descriptions of *R. felis* is its growth temperature. Marseille-URRWFXCal₂^T did not grow at either 35 or 37 °C and grew weakly at 32 °C in Vero cells, whereas it grew well in XTC-2 and Vero cells at 28 °C (Raoult *et al.*, 2000). As XTC-2 cells degenerate rapidly at temperatures above 28 °C, we could not test the growth of Marseille-URRWFXCal₂^T in these cells at higher temperatures. *R. felis* has been reported to induce a cytopathic effect leading to the formation of late, tiny plaques when grown at 34 °C in either Vero cells, HUVECs or L929 cells (Radulovic *et al.*, 1995b). Furthermore, at 32 and 28 °C, Marseille-URRWFXCal₂^T did not grow on L929 cells (Raoult *et al.*, 2000). These results demonstrate that the previously isolated *R. felis* strain is not the same organism as that which we repeatedly recovered from infected cat fleas obtained from the El Laboratory colony (Raoult *et al.*, 2000). Although genotypic data derived from PCR-based analysis of the ELB agent in flea extracts concur with those derived from examination of the Marseille-URRWFXCal₂^T isolate (Adams *et al.*, 1990; Azad *et al.*, 1992; Bouyer *et al.*, 2000, 2001; Higgins *et al.*, 1996; Noden *et al.*, 1998; Radulovic *et al.*, 1995b; Raoult *et al.*, 2000; Zavala-Velazquez *et al.*, 2000), there is clearly a discrepancy between phenotypic data derived from analysis of the first proposed isolate of the ELB agent, named *R. felis*, and those of Marseille-URRWFXCal₂^T. Our inability to address this dis-

crepancy resulted directly from the unavailability of extant *R. felis* isolates. Indeed, further doubt over the credibility of data derived from examination of *R. felis* isolates resulted following the isolation of only *R. typhi* from stocks of *R. felis* supplied to our lab by the authors of the original description of this organism (Higgins *et al.*, 1996).

In their recent official description of *R. felis*, Bouyer *et al.* (2001) justify the formal description of *R. felis* without isolation and description of any type species, as this species is considered as non-cultivable, like *Rickettsia peacockii* that was also described on the basis of molecular data only (Niebylski *et al.*, 1997). The same approach was also used for the initial description of *Tropheryma whipplei*, the agent of Whipple's disease (Relman *et al.*, 1992). In the last 2 years, these three micro-organisms have been successfully isolated using different strategies, allowing conventional formal description (Raoult *et al.*, 2000; Simser *et al.*, 2001; La Scola *et al.*, 2001) and demonstrating that 'uncultivable' micro-organisms are mostly 'not yet cultured'. To date there are no human pathogens that cannot be propagated by mean of axenic culture, cell culture or inoculation in animals. Thus when a new potential human pathogen is identified by means of molecular techniques, efforts must be made to propagate it. Moreover, the definition of a species based on genotypic data only, such as 16S

Table 2. Comparison of *R. felis* with other rickettsiae

Data from previous studies (Raoult *et al.*, 2000; Radulovic *et al.*, 1995a, b, 1996; Higgins *et al.*, 1996). NA, Not available.

Character	<i>R. conorii</i>	<i>R. typhi</i>	<i>R. felis</i> Marseille- URRWFXCal ₂ ^T	' <i>R. felis</i> '
Growth at:				
28 °C (XTC-2)	+	+	+	NA
32 °C (Vero)	+	+	+ (weak)	NA
34 °C (Vero)	+	+	—	+
Growth on:				
XTC-2 cells	+	+	+	NA
Vero cells	+	+	+	+
L-929 cells	+	+	—	+
Observation within:				
Cytoplasm	+	+	+	+
Nucleus	+	—	—	—
Actin polymerization in XTC-2 cells	+	—	—	NA
Inhibition in Vero cells by 4 µg erythromycin ml ⁻¹	—	+	—	+
Proteins on SDS-PAGE:				
150 kDa	+	—	+	—
Heat-labile 30 kDa	—	—	+	—
Detectable genes:				
<i>ompA</i>	+	—	+	—
<i>ompB</i>	+	+	+	+

rDNA, may not be sufficient for defining species, as observed for other species such as *Bacillus* spp. or *Afpia* spp. (Ash *et al.*, 1991; Fox *et al.*, 1992; La Scola *et al.*, 2002). To assist the recording of new putative taxa of prokaryotes based on limited genotypic data, it was proposed that such micro-organisms should be named as *Candidatus* until sufficient data became available to give an official name (Murray & Schleifer, 1994). Following these recommendations, *R. felis* and *R. peacockii* should have been named '*Candidatus R. felis*' and '*Candidatus R. peacockii*', awaiting successful cultivation, including stable subcultures, to be proposed for a formal description. Following these recommendations would avoid any confusion such as now observed for *R. felis* for which discrepant phenotypic data will exist in the scientific literature. To cite Murray & Schleifer (1994), 'it must be hoped that the candidate names used should be validated, but this cannot be guaranteed, and other names might then be more appropriate'. To avoid any confusion which could result from the introduction of a new name after two successive descriptions of *R. felis* (Higgins *et al.*, 1996; Bouyer *et al.*, 2001) we propose to retain the name *Rickettsia felis* and that strain Marseille-URRWFXCal₂^T become the reference strain as the cultivable isolate of the ELB agent. The characters that allow differentiation of *R. felis* from other rickettsial species are summarized in Table 2. Contrary to genotypic data, the phenotypic data about *R. felis*

previously reported must now be considered unreliable (Radulovic *et al.*, 1995a, b, 1996; Higgins *et al.*, 1996).

Emendation of the description of *Rickettsia felis* (Bouyer *et al.* 2001)

Rickettsia felis is an obligate intracellular, Gram-negative bacterium in the order *Rickettsiales*. The reference strain, Marseille-URRWFXCal₂^T, is available in our official collection and in the French National Culture Collection (Institut Pasteur, Paris, France) under the accession number I-2363. This strain is available upon request from the authors or from the Institut National de la Propriété Industrielle (Paris, France). It was isolated from cat fleas, *Ctenocephalides felis*, by the shell vial cell culture procedure using XTC-2 cells at 28 °C. *R. felis* can be grown in XTC-2 cells at 28 °C and Vero cells at 28 and 32 °C, but not on L-929 and MRC-5 cells or at a higher incubation temperature than 34 °C. *R. felis* induces cytopathic foci and plaque formation in XTC-2 and Vero cells at 9 and 18 days, respectively. The bacterium is present in the cytoplasm of infected cells and not in the nuclei; it is unable to polymerize actin within the cytoplasm of infected cells. *R. felis* is susceptible to doxycycline and rifampicin and resistant to erythromycin. *R. felis* can be detected in infected cells by Gimenez staining and by immunofluorescence with mouse polyclonal anti-*R. felis* antibody. *R. felis* DNA can be amplified with rickettsial

specific primer for the 17 kDa protein, 16S rRNA, *gltA*, *ompA*, *ompB* and *rpoB* genes. *R. felis* DNA has been amplified from the blood of a Texas patient suspected of having *R. typhi* infection and from a Brazilian and two Mexican patients with undiagnosed febrile exanthematous illness.

ACKNOWLEDGEMENTS

The authors are indebted to R. J. Birtles for reviewing the manuscript.

REFERENCES

- Adams, J. R., Schmidtman, E. T. & Azad, A. F. (1990). Infection of colonized cat fleas, *Ctenocephalides felis* (Bouché), with a rickettsia-like microorganism. *Am J Trop Med Hyg* **43**, 400–409.
- Ash, C., Farrow, J. A., Dorsch, M., Stackebrandt, E. & Collins, M. D. (1991). Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int J Syst Bacteriol* **41**, 343–346.
- Azad, A. F., Sacchi, J. B., Nelson, W. M., Dasch, G. A., Schmidtman, E. T. & Carl, M. (1992). Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. *Proc Natl Acad Sci U S A* **89**, 43–46.
- Azad, A. F., Radulovic, S., Higgins, J. A., Noden, B. H. & Troyer, J. M. (1997). Flea-borne rickettsioses: ecologic considerations. *Emerg Infect Dis* **3**, 319–327.
- Bouyer, D. H., Crocquet-Valdes, P. A. & Walker, D. H. (2000). Expression and size determination of the rOmpA protein of *Rickettsia felis*. In *American Society for Rickettsiology – 15th Meeting*, p. 61. American Society for Rickettsiology and Rickettsial Diseases.
- Bouyer, D. H., Stenos, J., Crocquet-Valdes, P. A. & 7 other authors (2001). *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. *Int J Syst Evol Microbiol* **51**, 339–347.
- Burgdorfer, W., Anacker, R. L., Bird, R. G. & Bertram, D. S. (1968). Intranuclear growth of *Rickettsia rickettsii*. *J Bacteriol* **96**, 1415–1418.
- Drancourt, M. & Raoult, D. (1999). Characterization of mutations in *rpoB* gene in naturally rifampin-resistant *Rickettsia* species. *Antimicrob Agents Chemother* **43**, 2400–2403.
- Fournier, P. E., Roux, V. & Raoult, D. (1998). Phylogenetic analysis of spotted fever group rickettsiae by study of the surface protein rOmpA. *Int J Syst Bacteriol* **48**, 839–849.
- Fox, G. E., Wisotzkey, J. D. & Jurtshuk, P. J., Jr (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol* **42**, 166–170.
- Heinzen, R. A., Hayes, S. F., Peacock, M. G. & Hackstad, T. (1993). Directional actin polymerization associated with spotted fever group rickettsia infection of Vero cells. *Infect Immun* **61**, 1926–1935.
- Higgins, J. A., Radulovic, S., Schrieffer, M. E. & Azad, A. F. (1996). *Rickettsia felis*: a new species of pathogenic rickettsia isolated from cat fleas. *J Clin Microbiol* **34**, 671–674.
- La Scola, B. & Raoult, D. (1996). Diagnosis of Mediterranean spotted fever by cultivation of *Rickettsia conorii* from blood and skin samples using the centrifugation-shell vial technique and by detection of *R. conorii* in circulating endothelial cells: a 6 year follow-up. *J Clin Microbiol* **34**, 2722–2727.
- La Scola, B., Fenollar, F., Fournier, P. E., Altwegg, M., Mallet, N. & Raoult, D. (2001). Description of *Tropheryma whipplei* gen. nov. sp. nov., the Whipple's disease bacillus. *Int J Syst Evol Microbiol* **51**, 1471–1479.
- La Scola, B., Mallet, N. M., Grimont, P. & Raoult, D. (2002). Description of *Afipia birgiae* sp. nov., *Afipia massiliae* sp. nov. and recognition of *Afipia felis* genospecies A. *Int J Syst Evol Microbiol* **52**, 1773–1782.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Murray, R. G. E. & Schleifer, K. H. (1994). Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. *Int J Syst Bacteriol* **44**, 174–176.
- Niebylski, M. L., Schrupf, M. E., Burgdorfer, W., Fisher, E. R., Gage, K. L. & Schwan, T. G. (1997). *Rickettsia peacockii* sp. nov., a new species infecting wood ticks, *Dermacentor andersoni*, in Western Montana. *Int J Syst Bacteriol* **47**, 446–452.
- Noden, B. H., Radulovic, S., Higgins, J. A. & Azad, A. F. (1998). Molecular identification of *Rickettsia typhi* and *R. felis* in co-infected *Ctenocephalides felis* (Siphonaptera: Pulicidae). *J Med Entomol* **35**, 410–414.
- Radulovic, S., Higgins, J. A., Jaworski, D. C. & Azad, A. F. (1995a). *In vitro* and *in vivo* antibiotic susceptibilities of ELB rickettsiae. *Antimicrob Agents Chemother* **39**, 2564–2566.
- Radulovic, S., Higgins, J. A., Jaworski, D. C., Dasch, G. A. & Azad, A. F. (1995b). Isolation, cultivation, and partial characterization of the ELB agent associated with cat fleas. *Infect Immun* **63**, 4826–4829.
- Radulovic, S., Higgins, J. A., Jaworski, D. C. & Azad, A. F. (1996). *In vitro* and *in vivo* antibiotic susceptibilities of ELB rickettsiae (erratum). *Antimicrob Agents Chemother* **40**, 2912.
- Raoult, D., La Scola, B., Enea, M., Fournier, P. E., Roux, V., Fenollar, F., Galvao, M. A. M. & De Lamballerie, X. (2000). A flea associated *Rickettsia* pathogenic for humans. *Emerg Infect Dis* **7**, 73–81.
- Relman, D. A., Schmidt, T. M., MacDermott, R. P. & Falkow, S. (1992). Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* **327**, 293–301.
- Rolain, J. M., Maurin, M., Vestris, G. & Raoult, D. (1998). *In vitro* susceptibilities of 27 Rickettsiae to 13 antimicrobials. *Antimicrob Agents Chemother* **42**, 1537–1541.
- Simser, J. A., Palmer, A. T., Munderloh, U. G. & Kurtti, T. J. (2001). Isolation of a spotted fever Rickettsia *Rickettsia peacockii* in a rocky mountain wood tick *Dermacentor andersoni* cell line. *Appl Environ Microbiol* **67**, 546–552.
- Teyssie, N., Chiche-Portiche, C. & Raoult, D. (1992). Intracellular movements of *Rickettsia conorii* and *R. typhi* based on actin polymerization. *Res Microbiol* **143**, 821–829.
- Van Kirk, L. S., Hayes, S. F. & Heizen, R. A. (2000). Ultrastructure of *Rickettsia rickettsii* actin tails and localization of cytoskeletal proteins. *Infect Immun* **68**, 4706–4713.
- Wedincamp, J., Jr & Foil, L. D. (2000). Infection and seroconversion of cats exposed to cats fleas (*Ctenocephalides felis* Bouche) infected with *Rickettsia felis*. *J Vector Ecol* **25**, 123–126.
- Zavala-Velazquez, J. E., Ruiz-Sosa, J. A., Sanchez-Elias, R. A., Becerra-Carmona, G. & Walker, D. H. (2000). *Rickettsia felis* rickettsiosis in Yucatan. *Lancet* **356**, 1079–1080.