NOTE

***Rickettsia slovaca* sp. nov., a member of the spotted fever group rickettsiae**Zuzana Sekeyová,^{1,2} Véronique Roux,¹ Wenbin Xu,¹ Josef Řeháček² and Didier Raoult¹Author 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.fr¹ Unité des Rickettsies, Faculté de Médecine, CNRS UPRES-A 6020, 13385 Marseille, France² Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

The name *Rickettsia slovaca* sp. nov. (type strain is strain B) is proposed for a member of the spotted fever group (SFG) rickettsiae which was isolated from *Dermacentor marginatus* ticks in Slovakia in 1968, and was recently implicated in human febrile illness. This rickettsia can be phenotypically distinguished from other SFG rickettsiae by microimmunofluorescence serotyping, SDS-PAGE, Western blotting and mAbs. Genotypic differences between *R. slovaca* and the other SFG representatives can be demonstrated by PCR-RFLP analysis, pulsed-field gel electrophoresis and sequencing of 16S rRNA, *gltA* and *ompA* genes.

Keywords: *Rickettsia slovaca*, spotted fever group rickettsiae, taxonomic and phylogenetic analysis

Rickettsiae belong to the order *Rickettsiales*, whose members were described as obligately intracellular, Gram-negative micro-organisms. Traditionally, the species have been divided into two groups, namely the typhus group (TG) and the spotted fever group (SFG) on the basis of clinical presentation, immunological reactivity, DNA G + C content and intracellular position (Weiss & Moulder, 1984). Recently, serotypes of SFG rickettsiae have been isolated from patient specimens and supposedly non-pathogenic rickettsiae have been isolated from arthropods (Baird, 1992; Beati *et al.*, 1992, 1997; Burgdorfer *et al.*, 1979; Chen *et al.*, 1996; Ereemeeva *et al.*, 1995; Fan *et al.*, 1988; Goldwasser *et al.*, 1974; Kelly *et al.*, 1996; Tarasevich *et al.*, 1991; Uchida *et al.*, 1992; Werren *et al.*, 1994; Yu *et al.*, 1993). Presently, the SFG rickettsiae includes 14 validated species (Raoult & Roux, 1997; Weiss & Moulder, 1984).

Dermacentor marginatus ticks were collected in 1968 near the village of Velký Lom (Slovakia) and subjected to the haemolymph test to detect the presence of *Coxiella burnetii*. Bacteria were characterized in 14 out of 35 adult ticks, but their morphology and localization in tick haemocytes were different from those of the Q

fever agent. Isolation in guinea pigs was unsuccessful, but seroconversion with *Rickettsia conorii* as an antigen was noted. Isolation of rickettsiae was performed twice during the same year from a pool of male and female *D. marginatus* ticks collected in the surroundings of the villages of Šula and Madačka, which are in the same district as the village of Velký Lom, and from a pool of males of the same tick species collected near the village of Madačka. The two strains were named 'B' and 'D', respectively (Řeháček, 1984).

Strains B and D were identified as members of the SFG rickettsiae (Brezina *et al.*, 1969) and this relationship was confirmed by the discovery of a G + C content similar to that of *Rickettsia sibirica* and *R. conorii* (Schramek, 1974) and by complement fixation test results. This latter approach showed that these two isolates were closely related, but differed from all prototype strains of the SFG examined (Úrvölgyi & Brezina, 1978). Strains isolated from *D. marginatus* in central Slovakia and Armenia and from rodents of the genus *Apodemus* and *Microtus* in eastern Slovakia were demonstrated to be more closely related to *R. sibirica* (Makarova *et al.*, 1978). A new SFG member was identified and it was proposed that it be named *Rickettsia slovaca* (Úrvölgyi & Brezina, 1978).

Abbreviations: MIF, microimmunofluorescence; rOmp, rickettsial outer-membrane protein; PFGE, pulsed-field gel electrophoresis; SFG, spotted fever group.

The GenBank accession numbers for the *Rickettsia slovaca* strain B 16S rRNA, *gltA* and *ompA* gene sequences determined in this work are L36224, U59725, and U43808 and U83454, respectively.

Justification for a new species

R. slovaca has features in common with the other SFG rickettsiae as determined by electron microscopy and

Table 1. Reactivities of mAbs directed against *R. slovaca* with all available SFG rickettsiae

mAb/specificity*	Titre of mAb with SFG rickettsial species†:														
	Afr	Eth	Aes	Aka	AF	Aus	Bar	Bel	BJ	HA	Hel	Hon	Ind	Itt	Jpn
SV2-C21/LPS-like	4096	4096	4096	4096	4096	4096	4096	–	4096	4096	4096	4096	4096	4096	4096
SV2-C22/LPS-like	4096	4096	4096	4096	4096	4096	4096	–	4096	4096	4096	4096	4096	4096	4096
SV6-E9/rOmp	8192	8192	–	–	8192	–	1024	–	8192	8192	–	2048	8192	8192	–
SV10-D2/rOmp	128	2048	–	–	4096	–	–	–	–	–	–	–	4096	2048	–
SV10-E8/rOmp	16384	16384	–	–	–	–	–	–	16384	16384	–	–	16384	–	–
	Titre of mAb with SFG rickettsial species†:														
	Ken	Man	M-1	Mor	Sev	Mtu1	GS	Mon	Par	Rhi	Ric	S	Sib	Slo	Ttt
SV2-C21/LPS-like	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096
SV2-C22/LPS-like	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096	4096
SV6-E9/rOmp	8192	8192	8192	8192	8192	4096	4096	–	4096	2048	4096	4096	16384	2048	8192
SV10-D2/rOmp	4096	4096	512	2048	4096	4096	4096	–	–	–	–	–	–	16384	–
SV10-E8/rOmp	16384	16384	16384	16384	16384	–	–	–	16384	–	–	8192	16384	32768	–

* rOmp, rickettsial Omp; LPS-like, LPS-like antigen.

† The rickettsial species studied were *R. africae* Z9-Hu (Afr), *R. africae* Ethiopian (Eth), *R. aeschlimannii* MC16 (Aes), *R. akari* Kaplan (Aka), Astrakhan fever rickettsia A-167 (AF), *R. australis* Phillips (Aus), Strain Bar29 (Bar), *R. bellii* 369L42-1 (Bel), Strain BJ90 (BJ), '*R. mongolotimonae*' HA91 (HA), *R. helvetica* C9P9 (Hel), *R. honei* RB (Hon), *R. conorii* Indian tick typhus rickettsia (Ind), Israeli tick typhus rickettsia ISTT CDC 1 (Itt), *R. japonica* YH (Jpn), *R. conorii* Kenya tick typhus rickettsia (Ken), *R. conorii* Manuel (Man), *R. conorii* M-1 (M-1), *R. conorii* Moroccan (Mor), *R. conorii* Seven (Sev), *R. massiliae* Mtu1 (Mtu1), *R. massiliae* GS (GS), *R. montanensis* Tick strain (Mon), *R. parkeri* Maculatum 20 (Par), *R. rhipicephali* 3-7-6 (Rhi), *R. rickettsii* Sheila Smith (Ric), Strain S (S), *R. sibirica* 246 (Sib), '*R. slovaca*' 13-B (Slo) and Thai tick typhus rickettsia TT-118 (Ttt). –, no reactivity of rickettsia with mAb diluted 1:4 in hybridoma culture supernatant.

serological identification. However, microimmunofluorescence (MIF), mAbs, SDS-PAGE, pulsed-field gel electrophoresis (PFGE), PCR-RFLP and sequencing of the genes encoding citrate synthase (*glhA*), 16S rRNA and rOmpA (*ompA*) indicate that this rickettsia is different from all other SFG rickettsiae.

(i) **MIF serotyping.** Mouse immunization and MIF serotyping were performed (Beati *et al.*, 1992, 1994; Ereemeeva *et al.*, 1995) and specificity differences (SPDs) were calculated using previously described methods (Philip *et al.*, 1978). Calculated SPDs between *R. slovaca* and the other considered rickettsiae were variable and illustrated that MIF data are not completely reliable for definitive speciation with SFG rickettsiae.

(ii) **mAbs.** mAbs against *R. slovaca* were a gift from D. H. Walker (University of Texas, USA). These mAbs have cross-reacted in MIF with other SFG rickettsiae (Table 1; extracted from Xu & Raoult, 1997). Two mAbs with specificity to LPS surface structure (SV2-C21 and SV2-C22) cross-reacted with all available SFG rickettsiae, except with the strain *Rickettsia bellii*. Different results were obtained using mAbs SV6-E9, SV10-D2 and SV10-E8, which are directed against rickettsial outer-membrane proteins (rOmps). All three reacted with *Rickettsia africae*, Indian tick typhus rickettsia, Kenya tick typhus rickettsia and *R. conorii*, but gave the highest titres with homologous *R. slovaca*. The anti-rOmp mAbs did not react with *Rickettsia aeschlimannii*, *Rickettsia*

akari, *Rickettsia australis*, *R. bellii*, *Rickettsia helvetica*, *Rickettsia japonica* and '*Rickettsia mongolotimonae*'. However, particular positive or negative reactions with each of tested mAbs, suggesting absence of the epitope on the surface of the rickettsiae, were also recorded.

mAbs raised against the other SFG rickettsial species, such as *R. africae*, *R. conorii* and *R. sibirica*, have been shown to cross-react with *R. slovaca* suggesting that this strain shares a number of common epitopes with other SFG rickettsial species. However, when screened with a large number of various mAbs, *R. slovaca* exhibited specific reactivity profiles and could be distinguished from other SFG rickettsial species, thus indicating the strain's antigenic divergence (Xu *et al.*, 1997; Xu & Raoult, 1997, 1998).

(iii) **SDS-PAGE and Western blot immunoassay.** SDS-PAGE protein analysis performed for the identification of rickettsial isolates has revealed that *R. slovaca* profiles differed from those of other members of the SFG (Balayeva *et al.*, 1993; Beati *et al.*, 1993; Ereemeeva *et al.*, 1993, 1995) in the high molecular mass range. Western blot immunoassay with mouse serum anti-*R. conorii* or anti-*R. slovaca* revealed two reactive zones: specific high-molecular-mass proteins (two bands) and LPS antigen (Beati *et al.*, 1993; Ereemeeva *et al.*, 1995).

(iv) **PCR-RFLP analysis of specific genes.** An electrophoresis migration pattern of the PCR-amplified DNA of *R. slovaca* with the Rp CS.877p and Rp CS.1258n

Table 2. Description of the rickettsial strains and the nucleotide accession numbers for the sequences used to construct the dendrogram in Fig. 1

Rickettsia	Strain	Accession no. for <i>gltA</i>	Accession no. for <i>ompA</i>	References
<i>R. sibirica</i>	246, ATCC VR-151 ^T		U43807, U83455	Roux <i>et al.</i> (1996); Fournier <i>et al.</i> (1998)
' <i>R. mongolotimonae</i> '	HA-91		U43796, U83439	
<i>R. parkeri</i>	Maculatum 20		U43802, U83449	
<i>R. africae</i>	ESF-5		U43790, U83436	
Strain S	Strain S		U43805, U83452	
<i>R. conorii</i>	Seven, ATCC VR-613 ^T		U43806, U83448	
<i>R. conorii</i>	Indian tick typhus rickettsia, ATCC VR-597 ^T		U43794, U83440	
Astrakhan fever rickettsia	A-167		U43791, U83437	
Israeli tick typhus rickettsia	ISTT CDC1		U43797, U83441	
<i>R. slovaca</i>	B		U43808, U83454	
Thai tick typhus rickettsia	TT-118, ATCC VR-599 ^T		U43809, U83456	Roux <i>et al.</i> (1996,1997); Fournier <i>et al.</i> (1998)
<i>R. rickettsii</i>	R (Bitterroot), ATCC VR-891 ^T		U43804, U83451	
<i>R. japonica</i>	YM	U59724	U43795, U83442	
Bar 29	Bar 29	U59720	U43792, U83438	
<i>R. massiliae</i>	Mtu1	U59719	U43799, U83445	
<i>R. rhipicephali</i>	3-6-7	U59721	U43803, U83450	
<i>R. aeschlimannii</i>	MC16	U59522	U43800, U83446	
<i>R. montanensis</i>	M/5-6	U74756	U43801, U83447	
<i>R. helvetica</i>	C9P9	U59723		
<i>R. australis</i>	Phillips	U59718		
<i>R. akari</i>	MK (Kaplan), ATCC VR-148 ^T	U59717		Roux <i>et al.</i> (1997)
<i>R. typhi</i>	Wilmington, ATCC VR-144 ^T	U59714		
<i>R. prowazekii</i>	Breinl, ATCC VR-142 ^T	M17149		
AB bacterium		U59712		
<i>R. canadensis</i>	2678, ATCC VR-610 ^T	U59713		
<i>R. bellii</i>	369L42-1	U59716		R. A. Heinzen and others, GenBank
<i>Coxiella burnetii</i>	Hamilton	M36338		

primer pair (chosen from the sequence of the *gltA* gene of *Rickettsia prowazekii*) digested with *AluI* restriction endonuclease revealed the profile found in most of the SFG rickettsiae (Eremeeva *et al.*, 1994; Regnery *et al.*, 1991). In an extension of the previous work of Regnery *et al.* (1991) and Eremeeva *et al.* (1994), Roux *et al.* (1996) amplified by PCR and sequenced a fragment of the gene encoding the protein rOmpA from 21 serotypes of the SFG. The authors considered that *R. slovaca* presented a specific profile after enzymic digestion with *PstI* and showed the specificity of the sequence. By PCR-RFLP of a fragment of the gene encoding the membrane protein rOmpB (PCR was performed with the primers BG1-21 and BG2-20 and enzymic digestion was performed with *RsaI*), Eremeeva *et al.* (1994) found a specific profile for *R. slovaca* compared with the 12 other SFG rickettsiae studied.

The authors determined the sequence of the 590 bp used for SFG rickettsiae identification as described by

Roux *et al.* (1996) and confirmed the identification of strain D as *R. slovaca*.

(v) **PFGE.** Roux & Raoult (1993) studied the chromosomes of SFG rickettsiae using PFGE. The mean size of the *R. slovaca* genome was estimated to be 1248 kb. This value is consistent with the genome size of other SFG rickettsiae. Specific profiles were obtained after total DNA digestion with low-frequency restriction endonucleases but the same patterns were obtained for different isolates of *R. slovaca* (Eremeeva *et al.*, 1993). A genetic relationship dendrogram established from PFGE patterns shows that the estimated levels of genetic divergence between *R. slovaca* and other rickettsiae studied are as follows: 32% with the closest relative, Thai tick typhus rickettsia; 36% with *R. sibirica* and *R. conorii*; and 36–52% with the other SFG rickettsiae tested.

(vi) **Phylogenetic analyses inferred from sequence comparisons.** Several studies have investigated the phylo-

Fig. 1. Dendrogram representing phylogenetic relationships between *Rickettsia* species. The tree includes data determined from analysis of the *gltA* and *ompA* genes. Sequences extracted from GenBank were aligned with the multisequence alignment program CLUSTAL, which is part of the BISCANCE software package. Phylogenetic relationships were inferred with version 3.4 of the PHYLIP software package. The evolutionary distance values were determined by the Jukes & Cantor method. These values were used to construct a dendrogram by the neighbour-joining method. Nucleotide accession numbers for sequences used to construct this dendrogram are listed in Table 2.

Fig. 2. Geographical distribution of the *D. marginatus* tick (dark shading). White circles indicate the locations where *R. slovaca* was isolated.

genetic organization of the bacteria of the genus *Rickettsia*. Sequence analysis has shown that *R. slovaca* has 16S rRNA, *gltA* and *ompA* gene sequences that differ from those of all the other SFG rickettsiae

(Fig. 1). For the 16S rRNA gene, the level of similarity ranges from 98.1% with AB bacterium to 99.8% with *R. conorii* (Roux & Raoult, 1995). For *gltA* and *ompA*, the levels of similarity range from 86.6% with *R. bellii* to 99.8% with *R. sibirica* and from 95.3% with *Rickettsia montanensis* to 98.4% with *Rickettsia rickettsii*, respectively (Fournier *et al.*, 1998; Roux *et al.*, 1997). The position of *R. slovaca* was best established when phylogenetic analysis was inferred from *ompA* sequence comparisons. The species did not specifically cluster with any other rickettsia species but branched alone between the *Rickettsia massiliae* group and the large cluster including the *R. conorii* complex and the *R. sibirica* group (Fournier *et al.*, 1998).

On the basis of the distinctive clinical, epidemiological, phenotypic and genotypic features described above, we propose that *R. slovaca* should be considered a separate taxonomic species.

Description of *Rickettsia slovaca* sp. nov.

Rickettsia slovaca (slo.va.ca'. L. gen. n. *slovaca* from Slovakia, the country where the organism was first isolated).

Obligately intracellular Gram-negative bacteria. Most of the strains were isolated from *Dermacentor marginatus*.

Fig. 3. Electron micrograph of *R. slovaca* cultivated on Vero cells. Bar, 1 μ m.

tus ticks (Řeháček *et al.*, 1972). However, rickettsial strains closely related to *R. slovaca* were detected and isolated from *Ixodes ricinus* (Úrvölgyi & Brezina, 1978; Řeháček, 1984), *Haemaphysalis punctata* (Řeháček *et al.*, 1972), *Haemaphysalis inermis* (Řeháček, 1984), *Ixodes hexagonus* (Sixl *et al.*, 1973) and *Argas persicus* (Řeháček *et al.*, 1977b). Distribution of *R. slovaca* widely exceeds the borders of the Slovak and Czech Republics. It has since been isolated in Armenia (Balayeva *et al.*, 1994; Makarova *et al.*, 1978; Řeháček *et al.*, 1977b; Tarasevich *et al.*, 1976), Crimea (Balayeva *et al.*, 1993), The Ukraine (Balayeva *et al.*, 1993; Úrvölgyi & Brezina, 1978), France (Beati *et al.*, 1993; Raoult *et al.*, 1997), Switzerland (Beati *et al.*, 1994), Yugoslavia (Manor *et al.*, 1992; Sixl *et al.*, 1976) and Portugal (Bacellar *et al.*, 1995). Positive serology was reported from the Lithuanian Republic (Tarasevich *et al.*, 1981), Germany (Řeháček *et al.*, 1977a) and Austria (Sixl *et al.*, 1973). It therefore seems to closely follow the distribution of its main host tick, *D. marginatus*, which can be found throughout Europe and as far as the western border of the People's Republic of China (Eremeeva *et al.*, 1993) (Fig. 2). *R. slovaca* exhibits features characteristic of those of the SFG rickettsiae. It is a typical rod-shaped rickettsia,

with dimensions of approximately $0.37\text{--}0.45 \times 0.8\text{--}1.2 \mu\text{m}$ (Řeháček, 1984). *R. slovaca* can be propagated on chicken embryo, HEL, Vero, L929 and tick cells, although no cytopathologic changes in any of the cell cultures inoculated (despite the high multiplicity of infection) have been noted (Beati *et al.*, 1993; Řeháček, 1984; Řeháček *et al.*, 1977a). Infected cells stained by the Gimenez method revealed rickettsiae either scattered in host cytoplasm, in clusters or occasionally within the nuclei of cells. Electron microscopical studies showed that the bacteria were free in the cytoplasm and were surrounded by a slime layer (Řeháček, 1984) (Fig. 3).

The quantitative distribution of *R. slovaca* in organs and haemolymph of infected adult *D. marginatus* collected in a natural focus of this rickettsia in central Slovakia has been demonstrated (Župančičová & Kováčová, 1972). The majority of rickettsiae were seen in the haemolymph, gut, Malpighian tubes and nerve ganglia; rickettsiae were less frequent in the connective tissues, salivary glands and testes (Župančičová & Kováčová, 1972).

Intraperitoneal injection of rickettsiae does not kill white mice. Pathological changes in mice are limited to

a slightly enlarged spleen. Guinea pigs react only occasionally to intraperitoneal injection of rickettsiae, by demonstrating a brief and mild fever. *Lepus europaeus* infected subcutaneously with *R. slovaca* suffer a asymptomatic infection accompanied by irregular rickettsaemia and distribution of rickettsiae to different organs (Brezina *et al.*, 1969; Řeháček, 1984). The course of infection with rickettsiae in hamsters after intraperitoneal inoculation is asymptomatic (Jablonskaja, 1976).

Human illness caused by *R. slovaca* was not described until recently. This agent has been suspected to be responsible for meningoencephalitis, accompanied by a prolonged persistence of neurasthenic disorders (Mittmayer *et al.*, 1980; Řeháček, 1984; Řeháček & Tarasevich, 1988). The most recent reported case in France confirmed its pathologic role (Raoult *et al.*, 1997). The symptoms of the patient's illness were severe headache, a necrotic eschar on the occiput surrounded by a reddish halo and four enlarged occipital lymph nodes, and a general weakness and tiredness that persisted for 2 months. A patient with meningoradiculitis following a tick bite was observed in south-west France (D. Raoult, unpublished data). Cases of patients bitten by *D. marginatus* ticks who developed the same clinical symptoms as those presented by this French patient were described in France as early as 1955. A rickettsial origin of these tick-transmitted diseases was suspected, but the aetiological agent was not identified (Giroud *et al.*, 1965). In 1981, again in France, the pathogenicity of *R. slovaca* was hypothesized by Edlinger in the aetiology of erythema chronicum migrans (Edlinger, 1981). Raoult *et al.* (1997) suggested that *Borrelia*-seronegative patients with manifestations resembling those of Lyme disease (a local erythema accompanied by meningoencephalitis) should perhaps be tested for *R. slovaca*. This might also apply in the case of patients from Hungary whose clinical symptoms – a benign infection with positive serology to Lyme borreliosis – detected in only some of the patients raised questions about the aetiology of the ongoing disease (Lakos, 1997).

In vitro susceptibility of *R. slovaca* to various antibiotics has been previously tested; the MIC for doxycycline was found to be $0.125 \mu\text{g ml}^{-1}$ and the MIC for rifampicin was $0.5 \mu\text{g ml}^{-1}$. Doxycycline is the recommended treatment, and fluoroquinolones can be used as alternatives (MICs of $1 \mu\text{g ml}^{-1}$). MICs for thiamphenicol, amoxicillin, cotrimoxazole are relatively high ($1\text{--}128 \mu\text{g ml}^{-1}$), whereas macrolide compounds are also effective, and can therefore be considered as alternative treatments during childhood and pregnancy (Rolain *et al.*, 1998).

Type strain. The type strain of *R. slovaca* is strain B, an isolate obtained from *D. marginatus* ticks; this strain has been deposited in the Collection of the World Health Organization Collaborative Center for Rickettsial Reference, Marseille, France and in the American Type Culture Collection.

Acknowledgements

The authors would like to thank Philippe Brouqui for the electron micrograph, David Walker for providing the mAbs against *R. slovaca* and Emma Birtles for checking the manuscript.

References

- Bacellar, F., Regnery, R. L., Nuncio, M. S. & Filipe, A. R. (1995). Genotypic evaluation of rickettsial isolates recovered from various species of ticks in Portugal. *Epidemiol Infect* **144**, 169–178.
- Baird, R. W., Lloyd, M., Stenos, J., Ross, B. C., Stewart, R. S. & Dwyer, B. (1992). Characterization and comparison of Australian human spotted fever group rickettsiae. *J Clin Microbiol* **30**, 2896–2902.
- Balayeva, N. M., Demkin, V. V., Rydkina, E. B., Ignatovich, V. F., Artemiev, M. I., Lichoded, L. Ya & Genig, V. A. (1993). Genotypic and biological characteristics of non-identified strain of spotted fever group rickettsiae isolated in Crimea. *Acta Virol* **37**, 475–483.
- Balayeva, N. M., Ereemeeva, M. E. & Raoult, D. (1994). Genomic identification of *Rickettsia slovaca* among spotted fever group rickettsia isolates from *Dermacentor marginatus* in Armenia. *Acta Virol* **38**, 321–325.
- Beati, L., Finidori, J. P., Gilot, B. & Raoult, D. (1992). Comparison of serologic typing, sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein analysis, and genetic restriction fragment length polymorphism analysis for identification of rickettsiae: characterization of two new rickettsial strains. *J Clin Microbiol* **30**, 1922–1930.
- Beati, L., Finidori, J. P. & Raoult, D. (1993). First isolation of *Rickettsia slovaca* from *Dermacentor marginatus* in France. *Am J Trop Med Hyg* **48**, 257–268.
- Beati, L., Humair, P. F., Aeschlimann, A. & Raoult, D. (1994). Identification of spotted fever group rickettsiae isolated from *Dermacentor marginatus* and *Ixodes ricinus* ticks collected in Switzerland. *Am J Trop Med Hyg* **51**, 138–148.
- Beati, L., Meskini, M., Thiers, B. & Raoult, D. (1997). *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. *Int J Syst Bacteriol* **47**, 548–554.
- Brezina, R., Řeháček, J. & Majerská, M. (1969). Two strains of rickettsiae of Rocky Mountains spotted fever group recovered from *Dermacentor marginatus* ticks in Czechoslovakia. Results of preliminary serological identification. *Acta Virol* **13**, 142–145.
- Burgdorfer, W., Aeschlimann, A., Peter, O., Hayes, S. F. & Philip, R. N. (1979). *Ixodes ricinus*: vector of an hitherto undescribed spotted fever group agent in Switzerland. *Acta Trop* **36**, 357–367.
- Chen, D. Q., Campbell, B. C. & Purcell, A. H. (1996). A new rickettsia from a herbivorous insect, the pea aphid *Acyrtosiphon pisum* (Harris). *Curr Microbiol* **33**, 123–128.
- Edlinger, E. (1981). Piqûres de tiques et réactions sérologiques paraspécifiques à *Rickettsia conorii*. *Arch Inst Pasteur Tunis* **58**, 345–353.
- Ereemeeva, M. E., Balayeva, N. M., Ignatovich, V. F. & Raoult, D. (1993). Proteinic and genomic identification of spotted fever group rickettsiae isolated in the former USSR. *J Clin Microbiol* **31**, 2625–2633.
- Ereemeeva, M. E., Yu, X. J. & Raoult, D. (1994). Differentiation among spotted fever group rickettsiae species by analysis of

restriction fragment length polymorphism of PCR-amplified DNA. *J Clin Microbiol* **32**, 803–810.

Eremeeva, M. E., Balayeva, N. M., Roux, V., Ignatovich, V., Kotsinjan, M. & Raoult, D. (1995). Genomic and proteinic characterization of strain S, a rickettsia isolated from *Rhipicephalus sanguineus* ticks in Armenia. *J Clin Microbiol* **33**, 2738–2744.

Fan, M. Y., Yu, X. J. & Walker, D. H. (1988). Antigenic analysis of Chinese strains of spotted fever group rickettsiae by protein immunoblotting. *Am J Trop Med Hyg* **39**, 497–501.

Fournier, P.-E., Roux, V. & Raoult, D. (1998) Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *Int J Syst Bacteriol* **48**, 839–849.

Giroud, P., Capponi, M., Dumas, N. & Rageau, J. (1965). Epidémiologie rurale. Résultats concernant *Dermacentor marginatus* et *reticulatus* prélevés dans différentes régions de France et leur contamination avec des rickettsies ou des éléments proches. *C R Acad Sci Paris* **250**, 5419–5421.

Goldwasser, R. A., Steiman, Y., Klinberg, W., Swartz, T. A. & Klingberg, M. A. (1974). The isolation of strains of rickettsiae of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. *Scand J Infect Dis* **6**, 53–62.

Jablonskaja, V. A. (1976). Characteristics of culture, pathogenic and immunogenic properties of rickettsiae isolated in Czechoslovakia. *Z Mikrobiol (Moscow)* **53**, 133–134.

Kelly, P. J., Beati, L., Mason, P. R., Matthewman, L. A., Roux, V. & Raoult, D. (1996). *Rickettsia africae* sp. nov., the aetiological agent of African tick bite fever. *Int J Syst Bacteriol* **46**, 611–614.

Lakos, A. (1997). Tick-borne lymphadenopathy – a new rickettsial disease? *Lancet* **350**, 1006.

Makarova, V. A., Tarasevich, I. V. & Plotnikova, L. F. (1978). Antigenic structure of rickettsiae isolated in Czechoslovakia and the USSR and their position in the spotted fever group. *Proceedings of the 2nd International Symposium on Rickettsiae and Rickettsial Diseases, Smolenice, June 21–25 1976*, pp. 293–298. Edited by J. Kazar, R. M. Ormsbee & I. V. Tarasevich. Bratislava: Veda, Slovak Academy of Science.

Manor, E., Novakovic, S., Walker, D. H. & Azad, A. F. (1992). Differentiation of spotted fever group rickettsiae from Israel and Slovenia by RFPL/PRR analysis. *Abstracts of the 10th Sesqui-annual Meeting of the American Society Rickettsiae and Rickettsial Diseases, Hamilton, Montana, USA*.

Mittmayer, T., Brezina, R. & Úrvölgyi, J. (1980). First report of an infection with *Rickettsia slovaca*. *Folia Parasitol* **27**, 373–376.

Philip, R. N., Casper, E. A., Burgdorfer, W., Gerloff, R. K., Hugues, L. E. & Bell, E. J. (1978). Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. *J Immunol* **121**, 1961–1968.

Raoult, D. & Roux, V. (1997). Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev* **10**, 694–719.

Raoult, D., Berbis, Ph., Roux, V., Xu, W. & Maurin, M. (1997). A new tick-transmitted disease due to *Rickettsia slovaca*. *Lancet* **350**, 112–113.

Regnery, R. L., Spruill, C. L. & Plicaytis, B. D. (1991). Genomic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* **173**, 1576–1589.

Řeháček, J. (1984). *Rickettsia slovaca*, the organism and its ecology. *Acta Sci Nat Acad Sci Bohemoslov Brno* **18**, 1–50.

Řeháček, J. & Tarasevich, I. V. (1988). *Akari-borne rickettsiae and rickettsioses in Eurasia*. Bratislava: Veda.

Řeháček, J., Brezina, R., Ac, P., Župančičová, M. & Kováčová, E. (1972). Contribution to the natural focality of *Rickettsiae* belonging to the Rocky Mountain Spotted Fever (RMSF) group in Slovakia. *Folia Parasitol* **19**, 41–52.

Řeháček, J., Liebis, A., Úrvölgyi, J. & Kováčová, E. (1977a). Rickettsiae of the Spotted fever isolated from *Dermacentor marginatus* ticks in South Germany. *Zentbl Bakteriell Mikrobiol Hyg I Abt Orig A Med Mikrobiol Infektkrankh Parasitol* **239**, 275–281.

Řeháček, J., Úrvölgyi, J. & Kováčová, E. (1977b). Massive occurrence of rickettsiae of the spotted fever group in Fowl Tampan, *Argas persicus*, in the Armenian S S R. *Acta Virol* **21**, 431–438.

Rolain, J. M., Maurin, M., Vestris, G. & Raoult, D. (1998). Antibiotic susceptibilities of 26 rickettsiae to 13 antibiotics. *Antimicrob Agents Chemother* **42**, 1537–1541.

Roux, V. & Raoult, D. (1993). Genotypic identification and phylogenetic analysis of the spotted fever group rickettsiae by pulsed-field gel electrophoresis. *J Bacteriol* **175**, 4895–4904.

Roux, V. & Raoult, D. (1995). Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. *Res Microbiol* **146**, 385–396.

Roux, V., Fournier, P. E. & Raoult, D. (1996). Differentiation of Spotted Fever Group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. *J Clin Microbiol* **34**, 2058–2065.

Roux, V., Rydkina, E., Eremeeva, M. & Raoult, D. (1997). Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. *Int J Syst Bacteriol* **47**, 252–261.

Schramek, S. (1974). Deoxyribonucleic acid base composition of rickettsiae belonging to the Rocky Mountain Spotted Fever group isolated in Czechoslovakia. *Acta Virol* **18**, 173–174.

Sixl, W., Úrvölgyi, J., Stünzner, D., Řeháček, J., Župančičová, M., Brezina, R. & Kováčová, E. (1973). Rickettsien in Österreich. 1. Untersuchungen bei Wildtieren, Haustieren und Zecken. In *Wissenschaftliche Arbeiten aus dem Burgenland. 1. Arbeitskolloquium über Naturherde von Infektionskrankheiten in Zentraleuropa*, 17–19 April 1973, Graz, Austria **1**, pp. 80–86. Eisenstadt, Österreich: Burgenländisches Landesmuseum.

Sixl, W., Stünzner, D., Kaaserer, G., Withalm, H., Sixl-Voigt, B., Beller, M., Troger, H. & Köck, M. (1976). Serologische Untersuchungen auf Rickettsien bei Kleinsäugern. In *2nd Intern. Arbeitskolloquium über 'Naturherde von Infektionskrankheiten in Zentraleuropa'*, 25–28 February 1976, Graz, Austria, pp. 359–360. Eisenstadt, Österreich: Burgenländisches Landesmuseum.

Tarasevich, I. V., Plotnikova, L. P., Fetisova, N. F. & 9 other authors (1976). Rickettsioses studies. 1. Natural foci of rickettsioses in the Armenian Soviet Socialist Republic. *Bull W H O* **53**, 25–30.

Tarasevich, I. V., Moteyunas, L. I., Řeháček, J. & 7 other authors (1981). Occurrence of rickettsioses in the Lithuanian Soviet Socialistic Republic. *Folia Parasitol* **28**, 169–177.

Tarasevich, I. V., Makarova, V., Fetisova, N. F., Stepanov, A., Miskarova, E., Balayeva, N. & Raoult, D. (1991). Astrakhan fever: a spotted-fever rickettsiosis. *Lancet* **337**, 172–173.

Uchida, T., Uchiyama, T., Kumano, K. & Walker, D. H. (1992).

Rickettsia japonica sp. nov., the aetiological agent of spotted fever group rickettsiosis in Japan. *Int J Syst Bacteriol* **42**, 303–305.

Úrvölgyi, J. & Brezina, R. (1978). *Rickettsia slovaca*: a new member of spotted fever group rickettsiae. In *Proceedings of the 2nd International Symposium on Rickettsiae and Rickettsial Diseases*, Veda, Bratislava, pp. 299–305. Edited by J. Kazar, R. M. Ormsbee & I. V. Tarasevich. Bratislava: Veda, Slovak Academy of Science.

Weiss, E. & Moulder, J. W. (1984). Order 1. *Rickettsiales* Gieszczykiewicz 1939, 25^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 687–701. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Werren, J. H., Hurst, G. D., Zhang, W., Breeuver, J. A. J., Stouthamer, R. & Majerus, M. E. N. (1994). Rickettsial relative associated with male killing in the Ladybird Beetle (*Adalia bipunctata*). *J Bacteriol* **176**, 388–394.

Xu, W. & Raoult, D. (1997). Distribution of immunogenic

epitopes on two major immunodominant proteins (rOmpA and rOmpB) of *Rickettsia conorii* among the other spotted fever group rickettsiae. *Clin Diagn Lab Immunol* **4**, 753–763.

Xu, W. & Raoult, D. (1998). Taxonomic relationships among the other spotted fever group rickettsiae as revealed by antigenic analysis with monoclonal antibodies. *J Clin Microbiol* **36**, 887–896.

Xu, W., Beati, L. & Raoult, D. (1997). Characterization of an application of monoclonal antibodies against *Rickettsia africae*, a newly recognized species of spotted fever group rickettsiae. *J Clin Microbiol* **35**, 64–70.

Yu, X., Jin, Y., Fan, M., Xu, G., Liu, Q. & Raoult, D. (1993). Genotypic and antigenic identification of two new strains of spotted fever group rickettsiae isolated from China. *J Clin Microbiol* **31**, 83–88.

Župančičová, M. & Kováčová, E. (1972). Contribution to localization of rickettsiae in ticks collected in nature. *Wiad Parazytol* **18**, 565–569.