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Genomic Study of *Rickettsia akari* by Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis of *Sma*I-, *Eag*I-, and *Bss*HII-digested DNA was used to perform restriction fragment length polymorphism analysis of *Rickettsia akari* strains isolated from humans, rodents, and mites in the United States and Ukraine. Although some differences in biological and serological characteristics were present between strains, the genomic studies demonstrated a high degree of intraspecies homogeneity of *R. akari* isolates. Our results confirm the value of pulsed-field gel electrophoresis–restriction fragment length polymorphism analysis for the identification of species of rickettsiae.

Rickettsia akari is an obligate gram-negative intracellular bacterium that causes rickettsialpox in humans (11, 19, 20). Domestic mice and gray rats are natural reservoirs of these bacteria, which are transmitted to humans by the bite of the mite *Allodermanyssus* (*Liponyssoides*) *sanguineus* (11, 19, 20). Rickettsialpox is a mild disease characterized by an eschar-like lesion at the site of the mite bite, fever, headache, lymphadenopathy with leukopenia, and a papulovesicular rash (11, 16). While outbreaks of rickettsialpox were reported in the United States (11) and Ukraine (16, 20) in the 1940s and 1950s, only sporadic cases have been reported since then. *R. akari* has been isolated from voles in Korea (12), although disease in humans has not been reported. Serological surveys have been performed to determine the prevalence of *R. akari* infections in humans in the Balkan countries, Italy, Costa Rica, and Africa (16) and indicated that *R. akari* might have a worldwide distribution. Recent reports of human rickettsialpox in Croatia (14) and 13 clinical cases from New York City (13) indicate that infections with *R. akari* are still prevalent.

R. akari strains isolated from humans, mice, rats, and mites in areas of the United States and Ukraine where the organism is endemic have been found to be identical by several classical methods of identification (16, 20). Six Ukrainian isolates and an isolate from the United States could not been differentiated by restriction fragment length polymorphism (RFLP) analysis of PCR-amplified DNA fragments with two primer pairs based on the specific sequences from the citrate synthase gene of *Rickettsia prowazekii* and the *ompB* gene of *Rickettsia rickettsii* (3).

The purpose of the study described here was to perform a genomic analysis comparing *R. akari* isolates from humans, animals, and mites in an area of Ukraine where the organism is endemic and a reference strain of *R. akari* (strain MK; American Type Culture Collection [ATCC], Rockville, Md.).

Six Ukrainian *R. akari* strains were obtained from the Collection of The Gamaleya Research Institute of Epidemiology and Microbiology (Moscow, Russia), where they had been lyophilized and stored at −20°C. The organisms studied were strain Toger, strain Askalunin, and strain CK (all isolated from humans); strain R-1 (isolated from a rat [*Rattus norvegicus*]); strain M-3 (isolated from a domestic mouse); and strain As4 (isolated from a mite [*A. sanguineus*]). The isolation of these strains has been described previously (20). The reference MK strain of *R. akari*, isolated from a patient in the United States (11), was supplied by ATCC (ATCC VR-148).

The biological and serological characteristics of the *R. akari* strains were obtained by classical tools of rickettsiology (20).

The rickettsiae were grown in Vero cells at 32°C in a 5% CO_2 atmosphere with Eagle's minimal essential medium (Seromed, Berlin, Germany) supplemented with 4% fetal calf serum (Seromed) and 1% L-glutamine. At 6 to 9 days after inoculation, heavily infected cells were harvested and the rickettsiae were purified by Renografin (Radioselectan; Schering, Lys-Lez-Lannoy, France) density gradient centrifugation (1).

For pulsed-field gel electrophoresis (PFGE), the preparation of DNA-embedded agarose blocks and endonuclease digestion were performed as described previously (3, 17). The following three restriction endonucleases were used: *Sma*I (CCCGGG), *Eag*I (CGGCCG), and *Bss*HII (GCGCGC) (New England BioLabs, Beverly, Mass.). Blocks containing digested DNA were placed on a 1% agarose gel (Sigma, St. Louis, Mo.), and migration was performed for 24 to 48 h with ramped pulse times of 3 to 5, 2 to 30, and 5 to 120 s to obtain an optimal separation of the digested fragments. Bacteriophage lambda ladder PFG marker (48.5 to 1.018 kb) and low-range PFG

TABLE 1. Biological characteristics of *R. akari* strains

Strain	Source	Infectivity for guinea pigs		Infectivity for chicken embryos	
		Duration of fever (days)	No. of animals with fever/no. of experimental animals	ID_{50}[a]	Coefficient of multiplication
MK	Human	5–8	5/5	5.0	2.7
Toger	Human	5–6	5/5	6.0	2.3
CK	Human	3–8	4/4	5.7	2.8
Askalunin	Human	0–5	3/4	5.3	2.5
R-1	Rat	5–8	4/4	5.8	2.4
M-3	Mouse	4–8	4/4	5.3	2.5
As4	Mite	4–6	2/2	5.0	2.5

[a] ID_{50}, 50% infectious dose.

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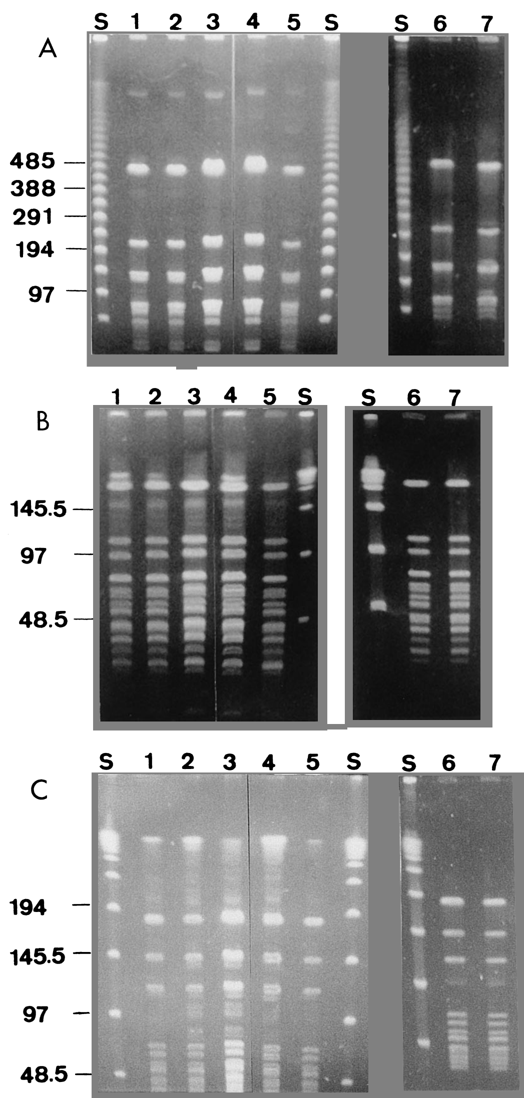

FIG. 1. PFGE profiles of DNA from *R. akari* performed at 14°C in a 1% agarose gel in 0.5× TBE (Tris-borate-EDTA) buffer. (A) Separation of *Bss*HII restriction endonuclease fragments (45 h of migration at 150 V and ramped pulse times of 5 to 120 s); (B) separation of *Sma*I restriction endonuclease fragments (24 h of migration at 190 V and ramped pulse time of 3 to 10 s); (C) separation of *Eag*I restriction endonuclease fragments (35 h of migration at 180 V and ramped pulse time of 5 to 20 s). Lanes: 1, strain MK; 2, strain Toger; 3, strain Askalunin; 4, strain CK; 5, strain As4; 6, strain R-1; 7, strain M-3; S, bacteriophage lambda ladder PFG marker, with sizes (in kilobases) indicated on the left.

marker (0.1 to 200 kb) (New England BioLabs) were used to determine digestion fragment sizes. After migration the gels were stained with 0.5% aqueous ethidium bromide for 30 min and were analyzed by computer with the Imager documentation system (Appligene, SA, Illkirch, France). Calculations of DNA fragment size were performed with the QGel-D program (Quantigel Corp., Madison, Wis.).

The biological characteristics of the *R. akari* strains are provided in Table 1. All of the Ukrainian strains were pathogenic in guinea pigs, resulting in temperatures of 39 to 40.0°C for 3 to 8 days. Three strains, Toger, R-1, and M-3, caused periorchitis, as did the reference strain MK. Infection with strain Askalunin resulted in an inapparent infection in one guinea pig and milder febrile responses in all infected animals.

Results of infection of white mice by intraperitoneal inoculation of a concentrated suspension of strain Toger grown in yolk sacs was found to depend on the weights of the animals. While the mortality rate was 100% within 10 days of infection in mice weighing 6.0 to 8.0 g, mortality was 50% within 5 days in mice weighing 10.0 to 12.0 g, and inapparent infections occurred in mice weighing 16.0 to 18.0 g. Growth of all strains in the yolk sacs of 5-day-old chicken embryos was moderate or slow (Table 1).

The *R. akari* strains had similar serologic properties when they were examined by the complement fixation test (Table 2). Titers determined in reactions with mouse antisera to either a homologous or a heterologous strain ranged from 1:80 to 1:320 for strain MK and five Ukrainian strains. Strain Askalunin seemed to be different from the other strains because the titer (1:40) with antisera to all strains by the complement fixation test was lower.

Antisera to *Rickettsia conorii* and *Rickettsia sibirica* had high titers when they were reacted with the homologous antigens (1:160 to 1:320) but low titers when they were reacted with the *R. akari* strains (1:20 or less). In contrast, the titers of guinea pig antisera raised against the strains of *R. akari* were generally similar when the antisera were reacted with strains of *R. akari*, *R. sibirica*, or *R. conorii*. Antisera to strain Askalunin and strain R-1, however, reacted at lower titers with *R. sibirica*, while titers in antisera against *R. conorii* were similar to those in antisera against the homologous strains (Table 2).

The results of genomic analysis by PFGE after digestion with low-frequency-cutting endonucleases are presented in Fig. 1 and Table 3. The *Sma*I restriction endonuclease profile of reference strain MK had 18 fragments of 209 to 10 kb, while that of *Eag*I had 15 fragments of 199 to 21 kb and that of *Bss*HII had 12 fragments of 477 to 8 kb. These results were similar to those published previously (17). The six Ukrainian

TABLE 2. Serological characteristics of *R. akari* strains

Strain	Titers to the indicated strains in[a]:													
	Mouse serum							Guinea pig serum						
	MK	Toger	CK	Askalunin	R-1	M-3	As4	MK	Toger	CK	Askalunin	R-1	M-3	As4
MK	80	160	160	160	80	80	80	320	320	320	160	320	160	320
Toger	80	160	160	80	80	80	80	—	—	—	—	—	—	—
CK	160	160	320	160	160	160	80	—	—	—	—	—	—	—
Askalunin	40	40	40	40	40	40	40	—	—	—	—	—	—	—
R-1	160	160	160	160	80	160	160	—	—	—	—	—		
M-3	160	80	160	80	160	80	80	—	—	—	—	—	—	—
As4	160	80	160	160	80	160	160	—	—	—	—	—	—	—
R. sibirica K-1	<10	10	10	<10	<10	—	—	160	160	160	40	40	80	160
R. conorii strain M-1	20	20	20	<10	20	—	—	160	320	160	80	160	160	160

[a] The data were obtained in a macrocomplement fixation test performed with 2 to 4 U of whole soluble antigen. —, the reaction was not performed.

TABLE 3. Restriction endonuclease fragments of *R. akari* DNA separated by PFGE

No. of fragments	Size of fragment (kb)		
	*Bss*HII	*Eag*I	*Sma*I
1	479	199	209
2	220	148	110
3	141	120	98
4	129	74	82
5	70	66	73
6	64	56	68
7	55	49	60
8	50	44	57
9	45	40	54
10	36	36	45
11	15	33	43
12	8	30	40
13		28	36
14		25	34
15		21	27
16			19
17			15
18			10

strains all had similar restriction endonuclease profiles which were identical to that of the reference strain MK.

PFGE has previously been reported to be reliable method for the identification of rickettsial isolates at the species level (5, 17). It can clearly differentiate rickettsiae of the typhus and spotted fever groups and demonstrates a high degree of intraspecies homogeneity for isolates of *R. typhi* (5), *R. conorii* (3, 17), *R. sibirica*, and *Rickettsia slovaca* (3) and Astrakhan fever rickettsiae (4). These results suggest a high degree of genomic conservation and stability within *Rickettsia* species. Genomic stability has also been determined in *R. sibirica* isolates by using high-frequency restriction endonuclease analysis (2) and in *R. rickettsii* and *Rickettsia montana* strains by RFLP analysis in combination with DNA probe hybridization and DNA sequence comparisons (7, 15). Numerical analysis has shown that 93% nucleotide variability occurs in the form of interspecies variations, because the differences between the species examined were all in the range of 1 to 4% (7, 15). The minor strain differences or the absence of strain differences and the good species differentiation between rickettsial isolates have been related to the recent divergence of *Rickettsia* species, each of which represents an independently evolving lineage (6).

The genomic stability and low rate of divergence can be explained by the biological features of rickettsiae, which are fastidious intracellular bacteria with a long generation time (19).

Point mutations of chromosomal DNA have been shown to make little contribution to RFLP analysis for typing strain of different bacteria (9). In contrast, the plasmids, insertion elements, and repetitive sequences were found to be responsible for DNA rearrangements (6, 9, 10). Plasmids and insertion elements have not been found in *R. akari* or other *Rickettsia* species to date. Repetitive sequences have been detected in the *ompA* gene of spotted fever group rickettsiae (8), and their strain-specific variability has been shown among strains of *R. rickettsii* (8) and the *R. conorii* complex (18). Sequencing of outer membrane protein genes or a portion of these genes is probably the best technique for differentiating these strains. However, whether differences in *ompA* sequences exist among *R. akari* isolates and their value in phylogenetic and epidemiologic studies require further investigation.

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