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Multispacer Typing of *Rickettsia prowazekii* Enabling Epidemiological Studies of Epidemic Typhus†

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Currently, there is no tool for typing *Rickettsia prowazekii*, the causative agent of epidemic typhus, currently considered a potential bioterrorism agent, at the strain level. To test if the multispacer typing (MST) method could differentiate strains of *R. prowazekii*, we amplified and sequenced the 25 most variable intergenic spacers between the *R. prowazekii* and *R. conorii* genomes in five strains and 10 body louse amplicons of *R. prowazekii* from various geographic origins. Two intergenic spacers, i.e., *rpmE*/tRNA^{fMet} and *serS*/*virB4*, were variable among tested *R. prowazekii* isolates and allowed identification of three and two genotypes, respectively. When the genotypes obtained from the two spacers were combined, we identified four different genotypes. MST demonstrated that several *R. prowazekii* strains circulated in human body lice during an outbreak of epidemic typhus in Burundi. This may help to discriminate between natural and intentional outbreaks. Our study supports the usefulness of MST as a versatile method for rickettsial strain genotyping.

Rickettsia prowazekii, the agent of epidemic typhus, is a short, gram-negative intracellular rod that has a genome of 1,111,523 bp (2) and belongs to the alpha subgroup of *Proteobacteria*. Humans and the eastern flying squirrel, *Glaucomys volans volans*, in the United States are the only known reservoirs of *R. prowazekii* (8). Epidemic typhus occurs under conditions that lead to lack of hygiene (3) and the proliferation of *Pediculus humanus humanus*, the human body louse which is the vector of *R. prowazekii*. Numerous outbreaks have been described in the past and even in recent times, for example, in central Africa (29). Also, sporadic cases continue to occur and have been reported in Peru (28) and northern Africa (24) and also in industrialized countries such as Russia (36) and the United States, where autochthonous infections have been documented in people who were in contact with flying squirrels (8).

Present-day threats posed by *R. prowazekii* include natural exposure and, possibly, the use of laboratory-manipulated strains as agents of biological warfare (27). *R. prowazekii* has been classified on the B list of potential bioterrorism agents by the Centers for Diseases Control and Prevention (Atlanta, GA). A method of differentiating strains of *R. prowazekii* would be very useful in determining the cause of outbreaks that might occur.

Rickettsial species were first characterized by serotyping with antisera raised in mice and subsequently by protein analysis with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6) and pulsed-field gel electrophoresis (31). The latter method could differentiate *R. prowazekii* strains Breinl and Evir (14). However, since rickettsiae are strict intracellular bacteria, serotyping, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and pulsed-field gel electrophoresis are often difficult, time consuming, and expensive and lack interlaboratory reproducibility. Because rickettsiae express few remarkable phenotypic characteristics, their precise identification and phylogenic classification have mainly become dependent on the study of sequences of various genes they contain. The 16S rRNA and *gltA* genes, which were studied initially, have been demonstrated to lack intraspecies variability (32, 34). Other studies have focused on a gene family encoding cell surface-exposed proteins (*sca* genes). Among these, *ompA*, *ompB* and *sca4*, also named gene D, have proven useful to infer reliable phylogenetic relationships among *Rickettsia* spp. (18, 33, 35). However, although *sca* genes were more variable than the 16S rRNA and *gltA* genes at the interspecies level and had a certain degree of intraspecies variability, they had only limited interstrain variability (18, 33, 35). Similarly, *ompA*, which is one of the most discriminatory genes among spotted fever group rickettsiae, cannot be used for typhus group rickettsiae because it only occurs in the group as a remnant gene (25). Using DNA microarray, Ge et al. found that the genetic variation between the Breinl and Madrid E strains was only 3% (20), thus confirming the unsuitability of coding DNA for typing rickettsiae at the strain level. Recently, we developed a genotyping method for *Yersinia pestis* named multispacer typing (MST) (12). This method, based on the comparison of several intergenic spacer sequences, has proven useful among strains of *Y. pestis* (12), *R. conorii* (19), *Bartonella quintana* (16), and *Coxiella burnetii* (22).

In this report, we applied MST based on 25 intergenic spacers to a total of 15 *R. prowazekii* strains or DNA amplicons of the same organism made from human body lice. By comparing these sequences, we were able to estimate the usefulness of MST for genotyping *R. prowazekii* at the strain level.

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† In memory of Natasha Balayeva, a famous rickettsiologist and friend.

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TABLE 1. *Rickettsia prowazekii* strains and louse amplicons used in our study

Strain or louse amplicon (reference)	Source (clinical specimen)	Geographical origin	Supplier or source[b]
Strains			
Madrid E (9)[a]	Passaged mutant on animals from a human isolate (blood)	Spain	Naval Medical Research Center
Breinl, ATCC VR142 (38)	Human (blood)	Poland	ATCC
BatnaRp22 (7)	Human (blood)	Algeria	Our laboratory
Evir (4)	Passaged mutant on animals from a human isolate (blood)	Russia	Gamaleya Institute
Kuzina (13)	Human (blood)	Russia	Gamaleya Institute
Louse amplicons			
Rw26860 (17)	Human	Rwanda	
Rw26862 (17)	Human	Rwanda	
Rw26875 (17)	Human	Rwanda	
Rw26877 (17)	Human	Rwanda	
Rw26879 (17)	Human	Rwanda	
Bur12726 (17)	Human	Burundi	
Bur12727 (17)	Human	Burundi	
Bur12728 (17)	Human	Burundi	
Bur12729 (17)	Human	Burundi	
Bur12749 (17)	Human	Burundi	

[a] For *R. prowazekii* strain Madrid E, we obtained DNA from the Naval Medical Research Center.
[b] ATCC, American Type Culture Collection.

MATERIALS AND METHODS

Study design. In order to identify intergenic spacers that exhibit variable sequences among *R. prowazekii* strains, we aligned the genome sequences of *R. conorii* and *R. prowazekii* and identified the 25 most variable intergenic spacers. These spacers were then amplified and sequenced in five *R. prowazekii* strains and amplicons from 10 body lice from various geographic origins.

***Rickettsia prowazekii* strains and amplicons.** In our study, we used cultures of four *R. prowazekii* strains (Table 1) and compared their sequences to those obtained from the DNA, extracted as described below, from the Madrid E strain and provided by Patrick Rozmajzl (Naval Medical Research Center, Silver Spring, MD). Rickettsial strains were propagated onto L929 cell monolayers (ATCC CCL NCTC clone 929) at 35°C in Eagle's minimal essential medium (Seromed, Berlin, Germany) supplemented with 4% fetal bovine serum (Seromed) and 2 mM glutamine. When Gimenez-stained cells (21) were heavily infected (after 3 to 5 days of culture), they were harvested, centrifuged (12,000 $\times$ *g* for 10 min), resuspended in minimal essential medium, and stored at −70°C until processed further.

We also used amplicons (see below) of *R. prowazekii* that we previously identified by *gltA* PCR amplification and sequencing in 10 body lice collected in a refugee camp in Burundi and in a jail in Rwanda in 2001 (17).

Selection of target sequences. We aligned the genome sequences of *R. conorii* (GenBank accession number NC_003103) and *R. prowazekii* (accession number NC_000963) using BLAST software (1) and identified conserved or degraded fragments within intergenic sequences. The 25 most variable intergenic spacers were selected from 100- to 500-bp sequences separating two consecutive genes in both genomes which had a BLASTN value of <75 between both genomes.

DNA extraction and PCR-based sequencing method. Genomic DNA was extracted from rickettsial cultures using the QIAamp Tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

The PCR primers used in this study were obtained from Eurogentec (Seraing, Belgium) and are described in Table 2. Their specificity was verified using BLAST software (1). PCRs were carried out in a PTC-200 automated thermal cycler (MJ Research, Waltham, MA). Two microliters of the DNA preparation was amplified in a 50-μl reaction mixture containing 50 pM of each primer; 200 μM (each) dATP, dCTP, dGTP, and dTTP (Invitrogen); 1 U eLONGase polymerase (Invitrogen, Gaithersburg, MD), 2 μl of eLONGase buffer A, and 8 μl of eLONGase buffer B. The following conditions were used for amplification: an initial 3 min of denaturation at 94°C was followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at various temperatures indicated in Table 1, and extension for 1 min at 68°C. Amplification was completed by holding the reaction mixture for 3 min at 68°C to allow complete extension of the PCR products. PCR products were purified using a QIAquick Spin PCR purification kit (QIAGEN) as described by the manufacturer. Sequencing reactions were carried out using the d-Rhodamine Terminator cycle sequencing ready reaction kit with Amplitaq Polymerase FS (Perkin-Elmer, Coignieres, France) as described by the manufacturer. For all PCR products, sequences from both DNA strands were determined twice. Sequencing products were resolved using an ABI 3100 automated sequencer (Perkin-Elmer). Sequence analysis was performed using the software package ABI Prism DNA Sequencing Analysis Software version 3.0 (Perkin-Elmer). Sterile water was used as a negative control in each assay.

Sequence analysis. Percentages of similarity among these sequences were determined using the MEGA 2.1 software package (23). We also determined the percentages of similarity of the intergenic spacers that were variable among *R. prowazekii* strains with the homologous spacers in the other five available *Rickettsia* genomes, i.e., *R. typhi* (GenBank accession number NC_006142), *R. conorii* (accession number NC_003103), *R. rickettsii* (accession number AADJ01000001), *R. sibirica* (accession number NZ_AABW01000001), and *R. akari* (accession number NZ_AAFE01000001). Phylogenetic relationships between *R. prowazekii* strains and louse amplicons were obtained from multispacer sequence alignment using MEGA (23) by comparison with the same intergenic spacers from the typhus group member *R. typhi* (GenBank accession number NC_006142). Distance matrices were determined under the assumptions of Kimura using complete deletion analysis and were used to infer a dendrogram by the neighbor-joining method. Phylogenetic relationships among studied *Rickettsia* species were also inferred using the maximum parsimony method available in MEGA (23) and the maximum likelihood within the Phylip environment (15).

Nucleotide sequence accession numbers. Sequences determined in this study were deposited in GenBank under the following accession numbers: AY695448, AY695447, and AY695449 for the *rpmE*/tRNA^{fMet} genotypes A, B, and C, respectively; and AY695454 and AY695452 for the *serS/virB4* genotypes A and B, respectively.

RESULTS

PCR amplification of the 25 tested intergenic spacers from the four *R. prowazekii* strains and 10 louse amplicons yielded products of the expected sizes (Table 2). The one exception was the *rpmE*/tRNA^{fMet} PCR product of Bur12749, which was 343 bp long instead of 262 bp, which was found with all other strains and louse amplicons. Unambiguous sequences were obtained from all strains and louse amplicons for all tested spacers. For 2 of the 25 intergenic spacers tested (*rpmE*/tRNA^{fMet} and *serS/virB4*), there were nucleotide sequence differences among tested samples. The details of nucleotide sub-

TABLE 2. Sequences of primers, amplicon sizes, and annealing temperatures used in this study[b]

DNA target name of variable spacers	Forward primer (5′–3′)	Reverse primer (5′–3′)	Size (bp)	Annealing temp (°C)
nusG/*rplK*	CAGTTGCAATATTGGTAAAGCA	CAGCAGCTGGAATTATCAAGTT	127	54
rpoB/*rpoC*	CAGGCATTCCTGAATCATTT	TCCGTAAAAATTTACTACGCTCA	265	54
yqiX/*gatB*	TAGGACACATATATTCTTCATC	GCAGATTTACGTTCTATTCAAGAGC	164	54
Rrf/*pyrH*	GAGCTTTCTCCATCTTTTCTTG	AAAGGGGAATATACGACAATTGAG	203	54
RP192[a]/RP193[a]	GCTCAAGTTGTGGTGTTTCTT	AAAATCCCTGTGACTGCAAAA	256	54
rne/*coxW*	CGTCTTGTGGATAGCATTTTGG	CCATCTTGTAATTAAGTTTTGATGC	607	54
asmA/RP348	ATAAGTGAGTATTTTAAAGCTTTAGGC	TTTTAAATTATCAAACAAAAGTGTGAG	661	54
murG/RP413[a]	GAAGAAAAGAAGGGCATAAGCTA	CAAGCTGAAAGTAAAAACATTCC	231	54
lig/*tgt*	TATACATCGCTATTTTATCAGCG	CAAAATCTTATGAGTCGTATTAGAACA	139	54
folC/*nuoN*1	CTTGATTTTGCCAGGTAGGCAGCGG	GGCAAAACATTGCCCTAAAA	1,955	54
pth/*rplY*	TCTTGTGTATTGATACTCTGTGCC	TTCGCTAAAATCATCGCAAG	229	54
ntrY/*rpsU*	AGCTGCTGTTGCTAAAGTAAAAA	CAAGAAGCAGCAAGAAGACAGA	227	54
23S rRNA/5 rRNA	ACCACCACGTTGATAGGTC	GGGATCGTGTGTTTCACTCA	259	54
tmk/*proP*4	TCCCTCAAAGGTAATAAACTTGC	TGGAAAAATCCCTTTTGCTT	180	54
dksA/*xerC*	TAGGACACATATATTCTTCATC	GCAGATTTACGTTCTATTCAAGAGC	92	54
serS/*virB*4	CGGATGTCTTGATAAATTACATGG	TCAAATTTTCGTAAACCACTAAACA	254	54
PbpA2/RP0856[a]	AAATAACCATTAATAATCG	TGGCGTTACAAAAGAATTATGA	120	54
spo0J/*abcT*1	TAACAATAGACAATTGTCGCTTAGG	TTTTGTTTCCTTATTATTTTACACTGC	204	54
RP072/RP073[a]	GCGATAAGCGATTTATTAGGC	GAAAGCCTAAAGCCTCCACA	27	54
$tRNA^{fMet}$/RP102[a]	GGTCGTTGGTTCAAATCCAG	AAGTCGTCATTGCGAGAAGG	299	54
mppA/*purC*	GCAATTATCGGTCCGAATG	TTTCATTTATTTGTCTCAAAATTCA	327	54
$tRNA^{Gly}$/$tRNA^{Tyr}$	AGCTTGGAAGGCTGGAACTC	ATCCTTCTCCCTCCACCACT	163	54
rpmE/$tRNA^{fMet}$	TTCCGGAAATGTAGTAAATCAATC	TCAGGTTATGAGCCTGACGA	262	54
fabZ/*lpxD*	TGTTAGGATCGATTTTAAGTACTCTATCT	TGGATTGGCATAGACAATCTATTA	190	54
fusA/$tRNA^{Trp}$	GTATGATATTCTCACATTATG	AGGAGCGACAGGAATCGAAC	142	54

[a] Open reading frames encoding putative proteins of unknown function are numbered with reference to the genome sequence of *R. prowazekii* strain Madrid E (GenBank accession number NC_000963).
[b] Intergenic spacers are named after the names of the 5′ and 3′ open reading frames.

stitutions are given in Table 3. Sequences from the other 23 spacers were identical among studied strains and DNA amplicons. Within the *rpmE*/$tRNA^{fMet}$ spacer, the presence of a single nucleotide substitution at position 111 enabled the strains and louse amplicons to be classified into two genotypes: type A, including the Breinl strain and louse amplicons Rw26860, Rw26862, Rw26877, Bur12727, Bur12728, and Bur12729; and type B, made of strains Madrid E, Evir, BatnaRp22, and Kuzina and of louse amplicons Rw26875, Rw26879, and Bur12726. In addition, the presence of an 81-bp repeated fragment at the 5′ extremity of the spacer classified the Bur12749 louse amplicon into genotype C (Table 3). One nucleotide substitution within the *serS*/*virB4* spacer at position 70 enabled *R. prowazekii* strains to be classified into two genotypes: genotype A, including the Rw26860 louse amplicon, and genotype B, including all five strains and the other nine louse amplicons.

TABLE 3. Classification of *R. prowazekii* and louse amplicons with MST genotyping

Strain or louse amplicon	*rpmE*/$tRNA^{fMet}$			*serS*/*virB*4		MST type
	Nucleotide substitution, position 111[a]	81-bp repeat	Type	Nucleotide substitution, position 70[a]	Type	
Breinl	T	−	A	A	B	1
Rw26862	T	−	A	A	B	1
Rw26877	T	−	A	A	B	1
Bur12727	T	−	A	A	B	1
Bur12728	T	−	A	A	B	1
Bur12729	T	−	A	A	B	1
Madrid E	C	−	B	A	B	2
Evir	C	−	B	A	B	2
BatnaRp22	C	−	B	A	B	2
Kuzina	C	−	B	A	B	2
Rw26875	C	−	B	A	B	2
Rw26879	C	−	B	A	B	2
Bur12726	C	−	B	A	B	2
Rw26860	T	−	A	T	A	3
Bur12749	T	+	C	A	B	4

[a] Nucleotide substitutions are numbered with reference to the genome of *R. prowazekii* strain Madrid E (NC_000963).

When the spacer sequences we obtained from strain Madrid E were compared to the genome sequence from *R. prowazekii* strain Madrid E available in GenBank (accession number NC_000963), we observed seven nucleotide differences within the spacers: at positions 9 (T in our sequence versus C in the genome) and 16 (C versus T) of the *serS*/*virB4* spacer, at positions 17 (A versus C) and 257 (A versus T) of the *rpoB*/*rpoC* spacer, and at positions 9 (T versus C), 17 (C versus T), and 236 (A versus G) of the *nusG*/*rplK* spacer.

When the sequences from both spacers from *R. prowazekii* were compared to those of other rickettsial genomes, the degree of nucleotide sequence similarity ranged from 89.6% by comparison with *R. akari* to 91.4% with *R. typhi* for the *rpmE*/$tRNA^{fMet}$ spacer, compared with 99.6 to 100% among *R. prowazekii* strains. For the *serS*/*virB4* spacer, the degree of nucleotide sequence similarity ranged from 88.1% by comparison with *R. typhi* to 92.4% with *R. akari*, compared with 99.6 to 100% among *R. prowazekii* strains.

Multispacer typing. Combining the results obtained from the analysis of the two variable spacers enabled us to identify four genotypes among the *R. prowazekii* strains and louse amplicons we studied (Table 3). Genotype 1 contained the Breinl strain and the Rw26862, Rw26877, Bur12727, Bur12728, and

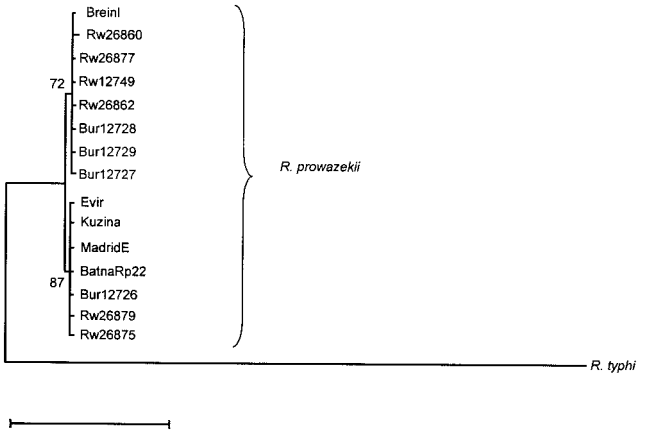

FIG. 1. Phylogenetic relationships between 15 *R. prowazekii* strains and louse amplicons inferred by comparison of the sequences of the *rpmE*/tRNA^{fMet} and *serS*/*virB4* intergenic spacers using the neighbor-joining method. The scale bar represents a 2.5% nucleotide sequence divergence. Bootstrap values are indicated at the nodes.

Bur12729 louse amplicons; genotype 2 was made of strains Madrid E, Evir, BatnaRp22, and Kuzina and of the Rw26875, Rw26879, and Bur12726 louse amplicons; genotype 3 contained only the Rw26860 louse amplicon; and genotype 4 included only the Bur12749 louse amplicon.

When sequences from the *rpmE*/tRNA^{fMet} and *serS*/*virB4* spacers were concatenated, the dendrograms obtained with the three different tree-building analysis methods used showed similar organization. All *R. prowazekii* strains and amplicons were classified within two clusters. The first cluster contained members of genotypes 1, 3, and 4, with representatives from genotypes 3 and 4 being issued from genotype 1. Representatives of genotype 2 clustered into a second group. *R. typhi* was positioned as the outermost taxon of studied species and strains (Fig. 1).

DISCUSSION

Herein, we showed that our multispacer typing method is suitable for genotyping *R. prowazekii* at the strain level. We confirmed that variable spacers at the interspecies level are also suitable targets at the intraspecies level.

The bioterrorism risk has recently reemerged, which highlighted the need for unambiguous, discriminatory strain characterization schemes. Prior to our study, there was no genotyping method described for *R. prowazekii* at the strain level. The development of a typing method for rickettsial strains has become crucial with the classification of *R. prowazekii* as a potential agent of bioterrorism. The recent finding by Ge et al. that limited variations occurred between coding sequences of two *R. prowazekii* strains (20) confirmed that there was little intraspecies variability in various gene sequences (18, 32, 33, 35). However, about 24% of the *R. prowazekii* genome consists of noncoding DNA, a very high percentage compared to that in other microbes sequenced so far (25). It has been suggested that intergenic spacer sequences are an important source of bacterial genome variability because they do not undergo selection pressure (11). For rickettsiae, it has been suggested that most of the intergenic sequences of *R. prowazekii* and *R. conorii* consist of decayed genes that are no longer active but have not yet been totally eliminated from the genome (2, 25). Recently, we demonstrated that the MST method, based on the comparison of intergenic spacer sequences, was a rational method for genotyping *Y. pestis* (12), *R. conorii* (19), *B. quintana* (16), and *C. burnetii* (22) at the strain level. In the present study, the comparison of the *R. conorii* and *R. prowazekii* genomes, which exhibit a high degree of colinearity, enabled us to select the 25 intergenic sequences exhibiting the highest interspecies variability. Two of these also had interstrain variability in the *R. prowazekii* strains and louse amplicons we studied. Sequences from the two variable spacers from *R. prowazekii* were clearly different from, and thus could not be confounded with, those of other *Rickettsia* species. When variations in these two variable spacers were considered, we could identify four genotypes among 15 *R. prowazekii* strains and louse amplicons. Although the number of strains or louse amplicons we studied may seem small, these include strains from three of the four current endemic foci of louse-borne typhus. *R. prowazekii* strains were classified within two genotypes, with genotype 1 incorporating the BreinI strain isolated during a large typhus outbreak in Poland following World War I (38) and genotype 2, including three European strains and one Algerian strain, isolated during World War II and later (4, 7, 9, 13). The body louse amplicons we studied were classified within four genotypes. The amplicons of the lice from Burundi could be separated into two genotypes, as could those made from the lice from Rwanda. As these lice were collected in a refugee camp in Burundi and in a jail in Rwanda during the same period in 2001, our data show that more than one strain can circulate in lice, and thus, louse-infested populations may be threatened by several strains of *R. prowazekii* at one time. These findings support our strategy of selecting intergenic sequences with the greatest interspecies variability as targets for strain typing and emphasize the usefulness of a typing technique as sensitive as ours to trace epidemic strains and differentiate natural from intentional outbreaks. The phylogenic organization of studied strains matched their genotypic classification (Fig. 1). Genotypes 3 and 4 were likely to be issued from the same lineage as members of genotype 1.

Initially, rather than amplifying and sequencing intergenic spacers from strain Madrid E, we used spacer sequences available in the genome sequence from *R. prowazekii* strain Madrid E (GenBank accession number NC_000963). We were surprised to find nucleotide differences between the genome sequence and strain Evir at seven positions within three spacers (see Results). These differences classified Madrid E within a unique MST type (data not shown). Strains Madrid E and Evir were described as having a common origin (4). Madrid E, an attenuated mutant of *R. prowazekii* obtained by passage through yolk sacs, was first described in 1943 (10) and proposed as a vaccine (26). In 1972, Balayeva and Nikolskaya reported that the virulence of the Madrid E strain was enhanced following passage through mice or guinea pigs (4, 5), and the revertant, virulent strain was named Evir. However, Wisseman suspected that a laboratory contamination of the Madrid E strain by a virulent *R. prowazekii* strain was the source of the emergence of the Evir strain rather than a spontaneous reversion to a virulent state (37). As the instability of attenuation of Madrid E raised questions about its suitability as a live vaccine antigen against epidemic typhus (30), it is an

important issue to determine whether Evir and Madrid E have a common origin. Because of this polemic, the sequence differences we observed, and the fact that we have previously demonstrated that cell culture passages do not alter MST genotypes in *R. conorii* (19), we considered it possible that Madrid E and Evir were issued from different lineages. However, an anonymous reviewer suggested that these sequence differences could be explained by errors in the genome sequence. In consequence, we asked Patrick Rozmajzl to provide us with DNA from *R. prowazekii* strain Madrid E and determined the sequences of the three spacers discordant between Evir and the Madrid E genome sequence. The spacer sequences we obtained from strains Madrid E and Evir were carefully checked nucleotide by nucleotide in both directions and were found to be identical between both strains. Therefore, we are confident that Madrid E and Evir belong to the same MST genotype and speculate that the mutations observed in the *R. prowazekii* genome are the result of sequencing errors.

The combined use of variable spacer sequences, which we have named multispacer typing (MST), could identify four genotypes among 15 *R. prowazekii* strains and body louse amplicons. This technique, which is easy to use, may be applied for tracking strains and may even be applied directly to clinical specimens or body lice, which have been demonstrated to be useful field specimens for diagnosis and surveillance of epidemic typhus outbreaks (17). Our use of MST enabled us to show that several strains of *R. prowazekii* were involved in the outbreak of epidemic typhus in Africa in 2001.

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