

MOLECULAR TYPING OF *RICKETTSIA AKARI*

M.E. Ereemeeva^a, M.M. Sturgeon^b, J.K. Willard^b, S.E. Karpathy^b, A. Madan^c,
G.A. Dasch^b

^a Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, USA

^b Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA

^c Covance Genomics Laboratory, Redmond, WA, USA

Abstract. *Rickettsia akari*, an obligately intracellular bacterium, is the causative agent of the cosmopolitan urban disease rickettsialpox. *R. akari* is an atypical representative of spotted fever group rickettsiae (SFG) as it is associated with rodent mites rather than ticks or fleas; however, only limited information is available about the degree of genetic variability found among isolates of *R. akari*. We examined 13 isolates of *R. akari* from humans, rodents and mites in the USA, the former Soviet Union, and the former Yugoslavia made between 1946 and 2003 for diversity in their tandem repeat regions (TR) and intergenic regions (IGR). The 1.23 Mb genome of *R. akari* strain Hartford CWPP was analyzed using Tandem Repeat Finder software (<http://tandem.bu.edu>) and 374 different TRs were identified, with size variation from 1 to 483 bp and with TR copy numbers ranging between 21 and 1.9, respectively. No size polymorphisms were detected among the 11 TR regions examined from 5 open reading frames and 6 IGR. Eighteen non-TR IGR's were amplified and sequenced for the same isolates comprising a total of 5.995 bp (0.49%) of the Hartford CWPP strain chromosome. Three single nucleotide polymorphism (SNP) sites were detected in two IGR's which permitted separation of the five *R. akari* isolates from Ukraine SSR from the other eight isolates. In conclusion, this is the first study reporting genetic heterogeneity among *R. akari* isolates of different geographic origins. Further exploration of this genetic diversity is needed to understand better the geographic distribution of *R. akari* and the epidemiology of rickettsialpox. The potential of mites as hosts for other rickettsial agents also needs further investigation.

Key words: rickettsialpox, mite, *Liponyssoides sanguineus*, *Rickettsia akari*, rickettsiae, spotted fever group rickettsiae, tandem repeats, intergenic regions, genotyping, multilocus sequence typing.

ГЕНЕТИЧЕСКОЕ ТИПИРОВАНИЕ *RICKETTSIA AKARI*

Еремеева М.Е.¹, Стуржеон М.М.², Уиллард Д.К.², Карпати С.Е.², Мадан А.³, Даш Г.А.²

¹ Колледж народного здравоохранения им. Цзянн-Пин Хсу, Университет Южной Джорджии, Стейтсборо, Джорджия, США

² Центр Контроля и Профилактики Заболеваний, Атланта, Джорджия, США

³ Кованс Геномик лаборатория, Редмонд, Вашингтон, США

Резюме. *Rickettsia akari* является облигатной внутриклеточной бактерией и вызывает осповидный риккетсиоз. *Rickettsia akari* является атипичным представителем группы риккетсиозных пятнистых лихорадок (SFG), поскольку она циркулирует в гамазовых клещах; однако в отношении степени генетического разнообразия изолятов *R. akari* имеются лишь ограниченные данные. Мы изучили 13 изолятов *R. akari*, выделенных от человека, грызунов и клещей в США, странах бывшего СССР и бывшей Югославии за период с 1946 по 2003 г. на предмет разнообразия в составе tandemных повторов (TR) и межгенных районов (IGR).

Адрес для переписки:

Еремеева Марина Евгеньевна
30458, США, Джорджия, Стейтсборо, почтовый ящик 8015,
Университет Южной Джорджии.
Тел.: +1 912 478-05-04. Факс: +1 912 478-58-11.
E-mail: meremeeva@georgiasouthern.edu

Contacts:

Marina E. Ereemeeva
Georgia Southern University PO Box 8015, Statesboro, GA 30458, USA.
Phone: +1 912 478-05-04. Fax: +1 912 478-58-11.
E-mail: meremeeva@georgiasouthern.edu

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При помощи программы Tandem Repeat Finder software (<http://tandem.bu.edu>) по поиску тандемных повторов Tandem Repeat Finder (<http://tandem.bu.edu>) был проанализирован геном длиной 1,23 Мб штамма *R. akari* Hartford CWPP; обнаружены 374 различных TR размером от 1 до 483 п.н. и количестве копий TR колеблющихся от 21 до 1,9 соответственно. Среди 11 TR из 5 открытых рамок считывания и 6 IGR не обнаружено размерного полиморфизма. Восемнадцать не-TR IGR были амплифицированы и секвенированы из всех изолятов, составляющих в целом 5995 п.н. (0,49%) хромосомы штамма Hartford CWPP. Три точечные мутации (SNP) были обнаружены в двух IGR, что позволило отделить пять изолятов *R. akari* из Украинской ССР от восьми других изолятов. В заключение следует отметить, что настоящее исследование является первым описанием генетического разнообразия среди изолятов *R. akari* из различных географических регионов. Дальнейшее изучение генетического разнообразия необходимо для более точного понимания географического распространения *R. akari* и эпидемиологии осповидного риккетсиоза. Оценка потенциала гамазовых клещей в качестве хозяев для возбудителей других риккетсиозов также требует дополнительных исследований.

Ключевые слова: осповидный риккетсиоз, гамазовые клещи, *Liponyssoides sanguineus*, *Rickettsia akari*, риккетсиу, генотипирование, тандемные повторы, межгенные фрагменты, мультилокусное генотипирование.

Introduction

Rickettsia akari is an obligate intracellular gram-negative bacterium which causes rickettsialpox in humans [2, 21]. The etiological agent is thought to have a worldwide distribution [31]. *R. akari* circulates in natural reservoirs of rats and house mice, *Mus musculus*, and is transmitted to humans by the bite of the mouse mite, *Liponyssoides sanguineus* [2, 22]. The transmission typically occurs in situations associated with disturbances of peridomestic structures infested by the rodents, rodent-infested and crowded conditions, or when the rodent population is reduced substantially or eliminated by rodent control or illnesses [1, 26, 31, 44]. Rickettsialpox is one of the few rickettsioses which is characterized as an urban disease. Clinically, it manifests as a mild disease accompanied by the classic triad of the eschar lesion at a site of the mite bite, fever, and vesicular rash, as well as headache and lymphadenopathy and leukopenia [2, 3, 21, 31].

At the time of its discovery, large scale outbreaks of rickettsialpox were identified in the United States and Ukraine SSR of the former Soviet Union (hereafter Ukraine) [2, 3, 21], and for the subsequent decade rickettsialpox received substantial attention from the public health and medical communities [31]. Serological surveys determined the prevalence of *R. akari* infections in several European, Central American and African countries [31]; however, disease reporting quickly diminished after that and only sporadic reports appeared in the peer-reviewed literature [31, 35]. Rickettsialpox received renewed attention at the beginning of the 21st century due to the need for differential diagnostic assessment for cutaneous lesions since its vesicular rash and later scab could lead to misidentification as chickenpox, herpes, and/or anthrax [5, 25, 31, 33, 46]. More recently clinically and laboratory confirmed cases of rickettsialpox have been reported from the USA, Ukraine, the former Yugoslavia (Croatia), Turkey, Netherlands and Mexico [29, 31, 35, 38, 46].

Rickettsia akari strains which were isolated from patients, mice, rats and mites from areas in the USA and Ukraine exhibit identical features when compared with classical identification methods, by using restriction fragment polymorphism analysis of the *gltA* and *ompB* amplicons, and with whole genome restriction profiles using pulsed-field gel electrophoresis [11, 12]. A Croatian isolate from the former Yugoslavia also could not be distinguished from reference USA *R. akari* strains using serological methods and monoclonal antibody typing [35], and similar conclusions were derived for *R. akari* responsible for rickettsialpox in Turkey [29] and Mexico but the latter samples were not typed [46]. These observations suggest that a single ancestral strain of *R. akari* was widely disseminated around the globe with rodents and their mites. The purpose of this study was to perform deeper genetic analysis of the available *R. akari* isolates from various geographic locations; we compared their variability in some of the tandem repeat sites and intergenic regions that could be identified from the complete genome sequence of *R. akari* Hartford CWPP strain (NCBI accession # NC_009881).

Materials and Methods

***R. akari* isolates and DNA extraction.** Fourteen stocks of strains of *R. akari* that were isolated from humans, rodents and mites in the USA, the former Soviet Union, and former Yugoslavia between 1946 and 2003 were analyzed. The five Ukrainian strains were from the Reference Collection of the Gamaleya Research Institute of Epidemiology in Moscow. The isolates from the U.S. consisted of the Kaplan (MK) reference strain isolated in 1946 from a patient in New York City, five isolates from patients in various locations in New York during 2001 to 2003 [32], and two stocks of Hartford strain: CWPP (plaque-purified by Charles Wisseman, Jr., from the University of Maryland School of Medicine) and an earlier unpurified passage Hartford H5564 (from the Harvard

University School of Public Health Collection) which was isolated from house mouse mites collected in West Hartford, Connecticut in 1952 (J. Spielman personal communication and [15]). The *R. akari* Croatian strain was isolated from a human from Yugoslavia in 1991 [35], and was obtained from David H. Walker (University of Texas Medical Branch, Galveston, TX). All strains were propagated in embryonated chicken eggs and passaged in Vero E6 cells and their whole cell DNAs (Vero cell DNA and *Rickettsia* DNA) were extracted using QIAamp protocol according to the manufacturer's instructions (Qiagen, Valencia, CA). Extracted DNAs were stored at +4°C before analysis.

Tandem repeat site identification and analysis. The 1.23 Mb genome of *R. akari* strain Hartford CWPP (NCBI accession # NC_009881) obtained from DNA from Vero cell propagated and Renografin purified rickettsiae at the CDC was analyzed for the presence and distribution of tandem repeats using Tandem Repeat (TR) Finder software version 3.21 with advanced parameters setting (<http://tandem.bu.edu>) [4]. The predicted tandem repeat sites were catalogued according to their size and their individual characteristics (fig. 1). Primers were designed for selected TR sites containing identical repeats with a period size of 6 to 17 nucleotides (tabl. 1) whose

variable periodicity would be distinguishable by agarose gel electrophoresis. All amplifications were conducted using Taq polymerase (Qiagen, Valencia, CA) and 20 pmol of each forward and reverse primer, and cycling conditions consisted of 40 cycles of 1 min at 95°C, 30 sec at 50°C, and 1 min at 68°C. Resulting amplicons were analyzed by electrophoresis in 1% agarose gels in 0.5X Tris-Borate-EDTA buffer (pH 8.0) for 30 min at 80 V and stained with ethidium bromide.

Analysis of intergenic regions. To identify intergenic region (IGR) spacers that might exhibit variable sequences in different isolates, homologous sequences of pairs of adjacent genes and their IGR were aligned for *R. akari* Hartford CWPP and *R. rickettsii* Sheila Smith (NCBI accession number NC_009882). Of these, 18 of 28 sites evaluated were selected for further analysis based on the level of intraspecies variations found in *R. rickettsii*, *R. prowazekii* and *R. conorii* [17, 23, 49]. The primers used to amplify each IGR and predicted sizes for each amplicon are listed in table 2. All amplifications were conducted using Taq polymerase (Qiagen) according to the PCR protocol described previously [23]. Resulting amplicons from different isolates were analyzed for size variability by electrophoresis in the same 1% agarose gels run for 30 min at 80 V as described above.

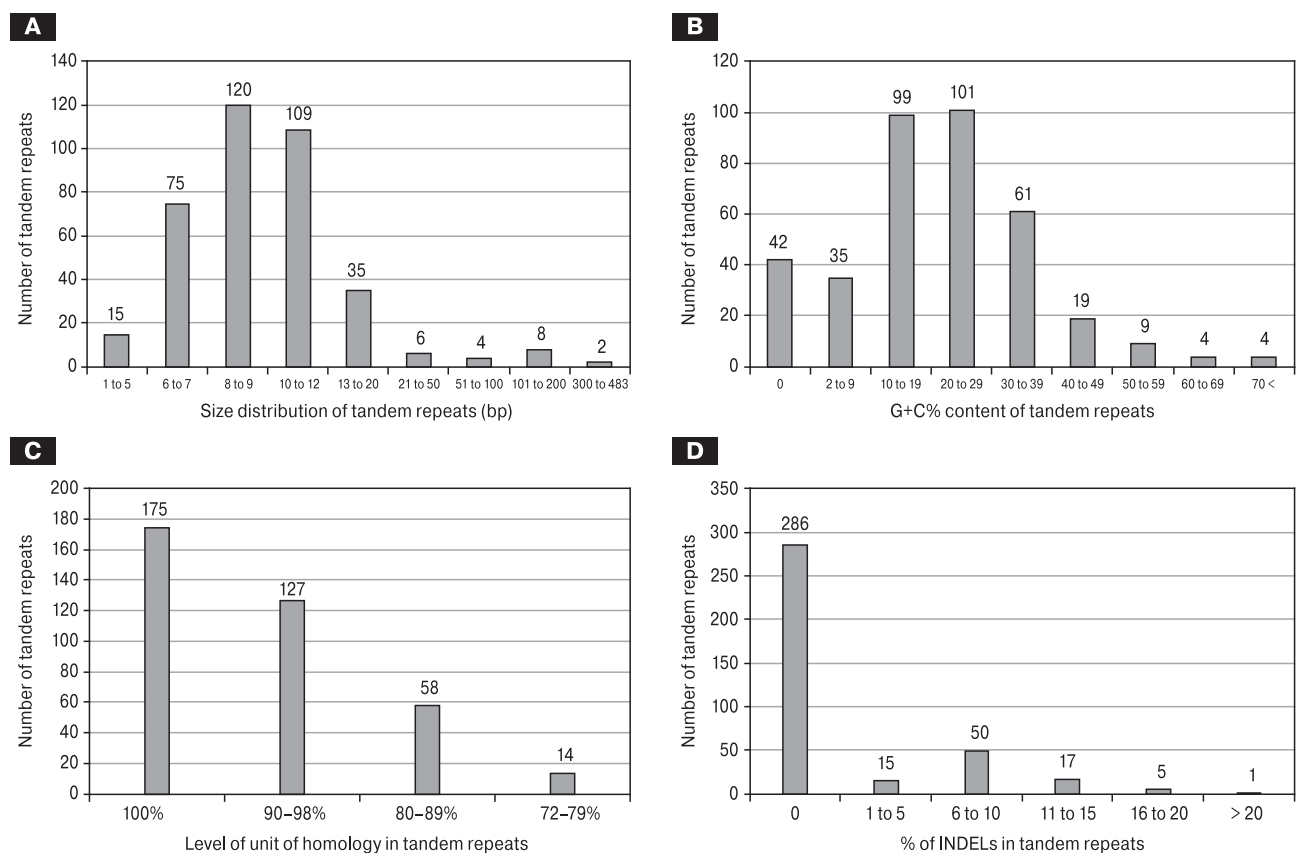


Figure 1. Properties of tandem repeat regions identified in *R. akari* Hartford CWPP

A) Size distribution of tandem repeats; B) G+C% content of tandem repeats; C) Level of unit homology in tandem repeats; D) % of INDEL in tandem repeats

Sequencing and sequence analysis. Amplicons were purified using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI). Purified PCR fragments were sequenced in both directions using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's directions, using an ABI 3100 genetic analyzer (Applied Biosystems). Sequencing reads were assembled using the SeqMerge program of the GCG software package (Accelrys, San Diego, CA). Identity of each sequence was confirmed using BLAST and sequence comparisons were done using the ClustalW alignment program as formulated in MEGA3 [27]. New sequences generated as a part of this study were submitted to the NCBI GenBank under the following accession numbers: MN524593-MN524601.

Results

Analysis of the *R. akari* Hartford CWPP tandem repeat regions. The characteristics of the tandem repeats (TR) present in the *R. akari* Hartford CWPP genome are summarized in Figure 1. The TR analysis identified 374 different loci containing tandem repeats, with repeat size variation from 1 to 483 bp and with copy numbers ranging between 1.9 and 21. Eleven selected primer pairs produced a single band of the predicted size when using DNA of Hartford CWPP strain (tabl. 1). Homologous amplicon frag-

ments were obtained for each *R. akari* isolate DNA available; the yield of PCR amplicons for each TR region was comparable for each set of primers thus permitting direct comparisons of their sizes. No electrophoretic polymorphisms were detected among the 11 TR regions examined with a repeat motif of 6 bp to 17 bp; these regions were amplified from 5 open reading frames and 6 intergenic regions.

Analysis of the intergenic regions. Of the twenty-one sets of IGR primers tested, 18 sets clearly amplified fragments of IGRs as expected with Hartford CWPP DNA; these were located in all regions of the chromosome of *R. akari* Hartford CWPP (tabl. 2). There was no overlap between TR identified in IGR of the *R. akari* genome (tabl. 1) and the IGR sites tested in this portion of the study. The eighteen pairs all amplified a single fragment of the same size from the genomic DNAs for each of the 14 isolate DNA tested. Lack of detectable differences in electrophoretic mobility suggested that INDELS > 6 bp were not present among the homologous fragments analyzed. Indeed, when sequenced, 16 of 18 amplicons had identical nucleotide sequences for all 14 DNAs to the genome sequence of Hartford CWPP isolate. Only two IGRs contained single nucleotide polymorphisms (SNP): one SNP was found within the *lig-tgt* IGR amplicon, and two SNPs were identified within the *tRNA^{Leu}-mgtE* IGR amplicon (tabl. 2). Thus, the tested IGR genome regions are highly conserved among the available isolates of *R. akari*.

Table 1. Tandem repeat sites and primers used to analysis these regions

Target site and its location	Primer (5'-3')	Amplicon size (bp) based on the reference genome	Repeat motif in the reference genome	Size (number) of repeats in the reference genome
AKTR 27720–27727 (C)*	F: AAATCGTACGGCTAGTACAA R: CTAATCTCTTTCCGATGCTT	225	TTAAAAAAT	9 bp (2)
AKTR 63635–63650 (C)	F: TCTGAATCACTCACCGGTTT R: ACGGGTTTTTCAGGACTTAAT	242	GATTTGAT	8 bp (2)
AKTR 109301–109324 (C)	F: AGCTAATTCGCATCTT R: AGTTTATTGCGATAGTCAAGG	296	GGTGGC	6 bp (4)
AKTR 117808–117823 (IGR)	F: GCCCTCTCCAATATCAGAT R: TTACGAAGTTTCTGCCATAGC	227	ATTTTTGT	8 bp (2)
AKTR 150931–150964 (IGR)	F: GCTATGGCTCCGGGTAGAAT R: TTACGCTGTGAATCCGGTGAA	846	ATCGTCATTGCAAGCAG	17 bp (2)
AKTR 204937–204954 (IGR)	F: GAAGTCCTGTGGTTCCGATT R: TATAGGCTTAGCGGTTCTAC	239	TAAATCATC	9 bp (2)
AKTR 239619–239634 (IGR)	F: CCCAGGGCAGAAGTATCATT R: AATAAGTATTGCGTATGGCTT	278	CATAATTT	8 bp (2)
AKTR 261657–261672 (C)	F: AAATAGTTGGGAAGTTAATGA R: AATATGGCAATGCTTACC	328	GCTATATT	8 bp (2)
AKTR 288108–288135 (IGR)	F: TTAGGTGGTACGGCAGA R: CTTATCGCAGCGTATTACGTC	421	TCATAAGAAATAAA	14 bp (2)
AKTR 347653–347670 (C)	F: AAGCGGCTGATGATATTG R: TTTAGTGCCGTTTCTTCGATA	775	CAATATAAA	9 bp (2)
AKTR 417521–417540 (IGR)	F: ACAACGCCGATTATATCAA R: GCAACTGCCGAATATCAACC	185	AATAATAGCA	10 bp (2)

*C, coding sequence, IGR, intergenic regions

The IGR *lig-tgt* SNP at nucleotide 369 is shared among all the USA isolates and the isolate from Croatia but is different from the homologous nucleotide in all five of the isolates from the Ukraine (tbl. 2). The USA and Croatian isolates have a C|T substitution as compared with the Ukrainian isolates. The annotation of the *R. akari* Hartford CWPP genome contains two small open reading frames on the reverse strand of the 458-bp *lig-tgt* intergenic region, one of 195 nt encoding a 65-aa hypothetical protein and another of 228-nt encoding a 75-aa protein which belongs to a family of toxin-antitoxin proteins. The C|T substitution results in a corresponding methionine-to-valine change in the amino acid sequence of the latter protein. The *tRNA^{Leu}-mgtE* IGR, contained two SNPs at nucleotides 173 and 215 of the amplicon. These SNPs could also be linked with the same two groups of geographic locations found

in the *lig-tgt* region: the Croatian and all the USA isolates have a G|A substitution at nucleotide 173 and an A|T substitution at nucleotide 215 when compared to the Ukrainian isolates.

Discussion

The most recent classifications based on conserved genetic attributes places *R. akari* into a so-called transitional group that also includes flea-borne *Rickettsia felis* and related organisms, *Rickettsia australis*, and *Rickettsia hoogstraalii* [19]. Genetic analysis of *R. akari* isolates of various geographic origins conducted in this study revealed a substantial level of genome conservation in the 0.5% of the genome we sampled by sequencing IGR regions that vary significantly from homologous regions in other SFGR. This suggests that a single ancestral strain

Table 2. Primers used to analyze intergenic regions*

Target region	Primers (5'-3')	Size (<i>R. akari</i>)	Size (<i>R. rickettsii</i>)
SpoOJ-abcT1	F: AAAGATTTGGAAGAATTAGACTTGAT R: TTTGCTTAAACCAACCATTTC	227 bp	272 bp
RC1137-tlc5	F: CGGGATAACGCCGAGTAATA R: ATGCCGCTCTGAATTTGTTT	377 bp	316 bp
Pal-RC1201	F: TGCAAGCACACATAATGCAA R: TCAAAATCGATTCTCTTTTCC	253 bp	247 bp
RC1027-XthA2	F: GGTATGTAAATGAGCCTTATCAACT R: TCAGTAGTATAAGTAGCTCCTGCTGTC	151 bp	135 bp
<i>nusG-rplK</i>	F: CAGTTGCAATATTGGTAAAGCA R: CAGCAGCTGGAATTATCAAGTT	289 bp	344 bp
<i>dskA-xerC</i>	F: TCCCATAGGTAATTTAGGTGTTTC R: TACTACCGCATATCCAATTAATA	108 bp	177 bp
<i>cspA-tRNA^{Leu}</i>	F: CGCCATTGCTCTGTTCAATA R: TCCGTTATGTCTACCATTCCA	460 bp	459 bp
PCNB3	F: AATTCGGCTTTCTCACA R: CTTGGCGTTTGCTTGGTCT	255 bp	255 bp
<i>rpoB-rpoC</i>	F: CAGGCATTCCTGAATCATT R: TCCGTAATAATTTACTACGCTCA	385 bp	324 bp
23S-5S rRNA	F: ACCACCACGTTGATAGGTC R: GGGATCGTGTGTTTCACTCA	345 bp	351 bp
<i>yqiX-gatB</i>	F: CTGCGGCAGTACCGACTATT R: ATCCGACGCTTGTAATCAG	305 bp	358 bp
<i>rne-CoxW</i>	F: CGGAAAAGAATGCAGAGTCTTG R: CCATTTTGAATTAACCTTTTCTGC	121 bp	241 bp
RC0241-0242	F: AGCTCAAATTGTGGTGTTC R: GGGATCCCTATTACAGCAAAA	349 bp	394 bp
<i>dnaN-RC0584</i>	F: TCGTCATGCCTGTTAAGGTG R: TTGGATAATCACCCGCTAAGA	351 bp	417 bp
RC0102-0103	F: GCGATAAGCGATTTATTAGGC R: GAAAGCCTAAAGCCTCCACA	359 bp	344 bp
<i>lig-tgt</i>	F: TTTTGTGCTTCTCTTCAGAT R: CCAAAATCTCATGAGCCGTA	524 bp	395 bp
<i>tmk-ProP4</i>	F: TTCCCCTCCCCTCAAATGTAA R: CGGAGCAAGAAACCCATAAA	361 bp	359 bp
<i>tRNA^{Leu}-mgtE</i>	F: AGCATTGAGGGTGCTGTTCT R: TTCAGCAAATGATCGTGATG	452 bp	342 bp

*Primer designation and their sequences are defined according to previously published information [16].

Table 3. Origin of *R. akari* isolates and position of SNPs in intergenic regions

Isolate	Geographic origin	Year	Source	lig-tgt (nt 377 of amplicon)*	tRNA ^{Leu} -mgtE (nt 193 of amplicon)*	tRNA ^{Leu} -mgtE (nt 235 of amplicon)*
Hartford CWPP	USA	1952	Mouse mite	C	G	A
Hartford H5564	USA	1952	Mouse mite	C	G	A
Kaplan	USA	1946	Human	C	G	A
Columbia1	USA	2001	Human	C	G	A
Columbia2	USA	2002	Human	C	G	A
Columbia3	USA	2002	Human	C	G	A
Bronx	USA	2002	Human	C	G	A
Manhattan	USA	2003	Human	C	G	A
Croatian	Yugoslavia	1991	Human	C	G	A
As4	Ukraine	1950	Mite	T	A	T
Aschkalunin	Ukraine	1950	Human	T	A	T
M3	Ukraine	1950	Mouse	T	A	T
R1	Ukraine	1950	Rat	T	ND	ND
Toger	Ukraine	1950	Human	T	A	T

*Nucleotide position of the sequenced portion of the amplicon is indicated.

of *R. akari* has spread globally with their rodent and rodent mite hosts. We found three stable SNPs that permitted identification of two geographic variant types of *R. akari*. These findings underscore the need for further research and evaluation of this neglected species of pathogenic *Rickettsia*. Known examples with diversity of *R. bellii* organisms and an expanding list of near relatives of *R. felis* [6, 18, 25, 28, 48] suggest our sampling of relatives of *R. akari* and possibly other variants of *R. akari* may be far from complete.

In clinical diagnostic practice molecular tools typically target well characterized and conserved portions of the genomes, particularly coding sequences, which enable accurate and reliable detection of specific pathogens including epidemiological circumstances when infection due to *R. akari* is suspected [10]. Our study augments the number of regions which can be used for identification of *R. akari*.

Previous studies demonstrated that diversity in the number of variable tandem repeats (VNTR), and occurrence and distributions of insertion and deletions (INDEL) permit reliable identification of different species and even isolates of *Rickettsia* [13, 14, 41, 42, 45]. Several VNTR loci were informative to distinguish geographic variants of *R. rickettsii* and confirming that *R. rickettsii* circulating in Arizona consists of unique genetic lineage [13, 14, 45]. VNTR typing targeting locus Rc-65, (in *dksA-xerC* intergenic region) was used for accurate and rapid differentiation between the Portuguese *R. conorii* Malish-like and Israeli tick typhus strains circulating in the same geographic locales [41], and this typing system was applied directly to ticks and clinical samples [42]. Further examination of other TR sites may find useful variable tandem repeat loci in *R. akari*. This low technology approach does not require DNA sequencing.

Intergenic spacer region analysis has permitted investigators to examine strain diversity among

and within various species of *Rickettsia* including *R. prowazekii*, *R. conorii*, *R. sibirica*, and *R. rickettsii* [7, 16, 23, 47, 49]. This approach can be applied to both laboratory grown isolated strains and to field and clinical samples [7, 39] and even to fixed pathology specimens [30]. Furthermore, multispace intergenic typing based on three regions, the *dksA-xerC*, *mppA-purC*, and *rpmE-tRNA^{Met}* spacer sequences, identified 61 genotypes among *Rickettsia* species, allowing the differentiation of each species by at least one distinct genotype [17]. In our study, no variations were detected within *dksA-xerC* fragment sequenced for 14 isolates of *R. akari*; however, we detected three SNPs in two other intergenic regions, namely *ligA-tgt* and *tRNA^{Leu}-mgtE* spacers.

Because of current views regarding the low impact and low morbidity of rickettsialpox, the specific mechanisms underlying pathogenesis of *R. akari* infection are not well examined and not fully understood beyond just a few fragmentary findings implicating the roles of macrophages and TLR2 and TLR4 receptors in those interactions [34, 43]. In this study, we detected for the first time SNP-level diversity among geographically separated isolates of *R. akari*. One mutation we identified is responsible for a change in the amino acid sequence of a small peptide belonging to the antitoxin of a toxin-antitoxin stability system and specifically to a family of bacterial proteins which prevents the death of the host cells. Since at this point this toxin-antitoxin system is only partially characterized in rickettsiae [40], whether this mutations affects the pathogenicity of *R. akari* and the mechanisms and outcomes of their interactions with endothelial cells and macrophages is unknown. This is specifically important because recent reports of rickettsialpox from Mexico indicate unusual morbidity and severe clinical manifestations of the infection in pediatric and adult patients [46]. Our data sug-

gests that only limited genetic diversity exists among isolates of *R. akari* associated with previous outbreaks but only a small portion of the genome has been sampled. Consequently, one useful step would be to evaluate the genome sequence of a representative strain from Ukraine and to compare its properties to the prototype US strains that have received more study. Another benefit of such analysis would be to identify additional genetic markers permitting more discriminating surveillance, tracking and studying of the vector and the host associations of these isolates.

Molecular surveys of various collections of ectoparasites besides *L. sanguineus* should be performed to determine the existence of mite-associated rickettsiae and their related organisms. Korean and Chinese reports have indicated that other mites may be infected with *R. akari* or *R. akari*-like organisms [8, 9, 20]. Reeves et al. also found other rickettsial agents in mites from the USA and Egypt [36, 37]. If associations of *R. akari* and its near relatives can be established with other ectoparasites, it will be impor-

tant to determine if there are other wildlife reservoirs for *R. akari* or closely-related pathogens, and if disturbances in these interactions can cause rickettsial-pox-like illnesses in other ecological settings.

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Авторы:

Еремеева М.Е., д.б.н., профессор, зав. лабораторией, Колледж народного здравоохранения им. Цзянь-Пин Хсу, Университет Южной Джорджии, Джорджия, Стейтсборо, США;
Стуржеон М.М., магистр общественного здравоохранения, стажер, Центр контроля и профилактики заболеваний, Атланта, Джорджия, США;
Уиллард Д.К., бакалавр биологических наук, стажер, Центр контроля и профилактики заболеваний, Атланта, Джорджия, США;
Карпати С.Е., д.б.н., микробиолог, Центр контроля и профилактики заболеваний, Атланта, Джорджия, США;
Мадан А., д.б.н., старший научный сотрудник, Кованс Геномик лаборатория, Редмонд, Вашингтон, США;
Даш Г.А., д.б.н., ведущий микробиолог и руководитель группы, Центр контроля и профилактики заболеваний, Атланта, Джорджия, США.

Authors:

Eremeeva M.E., PhD, MD (Biochemistry), Professor, Laboratory Director, Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, USA;
Sturgeon M.M., MPH, Training Fellow, Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA;
Willard J.K., BS, Training Fellow, Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA;
Karpathy S.E., PhD, Microbiologist, Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA;
Madan A., PhD, Senior Research Scientist, Covance Genomics Laboratory, Redmond, WA, USA;
Dasch G.A., PhD, Leading Microbiologist and Group Leader, Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA.

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