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Emerging spotted fever group rickettsiae in ticks, northwestern China

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ABSTRACT

We report *Rickettsia conorii* subsp. *indica*, *Candidatus* R. barbariae and *R. massiliae* in *Rhipicephalus turanicus* from sheep around the Taklamakan desert, northwestern China. The topology of the phylogenetic trees produced from the maximum likelihood (ML) analyses of the *ompA-gltA-rrs-geneD-ompB* concatenated sequence data was very similar to that of the neighbor joining (NJ) tree, and with total support of 69%–100% bootstrap values for the inclusion of the rickettsiae in *Rh. turanicus* within the clade that contained *R. conorii* subsp. *indica*; *Candidatus* R. barbariae and *Rickettsia* sp. Tselentii; *R. massiliae* str. AZT80; and *R. massiliae* MTU5, respectively. Studies suggest that the co-existence of these spotted fever group rickettsiae is a threat to public health in China. Work is important in exploring novel and emerging pathogens.

1. Introduction

Infections with spotted fever group (SFG) rickettsiae have been reported in various parts of the world over the past decades. Tick bite events are common and cases are globally increasing (Jones et al., 2008). However, reports of tick-borne rickettsial disease as well as the rickettsia species involved are limited in China, especially in less developed communities. To date, in China, seven validated SFG rickettsial species have been detected in ticks: *Rickettsia heilongjiangii*, *R. sibirica*, *R. raoultii*, *R. slovaca*, *R. felis*, *R. aeschlimannii* and *R. massiliae*, and cases of human infection with rickettsiae such as *R. raoultii* have been reported in the last years (Wei et al., 2015; Jia et al., 2014). The diagnosis and treatment of the rickettsiosis are difficult in the absence of molecular analysis because the commercial serological assays do not distinguish among the SFG species (Myers et al., 2013).

As the largest province in China, Xinjiang Uygur Autonomous Region (XUAR) covers over one-sixth of the country, includes the majority of the arid areas in the country, and is abundant in tick species (Zheng et al., 2006). Tick-associated pathogens and diseases, nevertheless, are underestimated because of the tense political environment and limited research, especially around the Taklamakan desert, in the southern region of XUAR. Here, an investigation was carried out to identify the ticks and *Rickettsia* spp. in this region.

2. Materials and methods

During 2013–2014, a total of 133 adult ticks were collected from sheep from six sites around the Taklamakan desert (Table 1). All of the ticks were identified morphologically according to previous reports and were submitted to molecular analysis based on partial mitochondrial *16S rDNA* gene sequences (Dantas-Torres et al., 2013).

The genomic DNA was extracted from each individual tick using the TIANamp Genomic DNA Kit (TianGen, Beijing, China). Six PCR targets, 17 kilodalton antigen (*17-kDa*), 16S rRNA (*rrs*), citrate synthase (*gltA*), cell surface antigen 1 (*sca1*), outer membrane protein A (*ompA*) and *ompB* (named *ompB*[1] in this study) were assessed within each sample to investigate the presence of SFG rickettsiae (Anstead and Chilton, 2013a,b).

Two additional genetic markers were used to confirm the presence of the rickettsiae in ticks. Another part of the *ompB* gene (named *ompB*[2] in this study; 1063 bp) was amplified

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Table 1
Tick species and PCR results of rickettsiae from questing adult ticks in study sites, northwestern China.

Location, coordinates	Tick species	No.	*Rickettsia* species, No. (%) positive
Yecheng 37°89′N–77°42′E	*Rh. turanicus*	6	*R. conorii* 3 (50) *Candidatus* Rickettsia barbariae 2 (33.33)
Qira 36°59′N–80°48′E	*Rh. turanicus*	16	*Candidatus* Rickettsia barbariae 3 (18.75)
Tumxuk 39°51′N–79°03′E	*Rh. turanicus*	30	*R. conorii* 6 (20) *Candidatus* Rickettsia barbariae 12 (40) *R. massiliae* 1 (3.33)
	D. marginatus	3	0
Pishan 37°37′N–78°16′E	*Rh. turanicus*	62	*R. conorii* 10 (16.13) *Candidatus* Rickettsia barbariae 18 (29.03) *R. massiliae* 1 (1.61)
	D. marginatus	2	0
Kuqa 41°43′ N–82°57′E	*Rh. turanicus*	3	*R. conorii* 2 (66.67) *Candidatus* Rickettsia barbariae 1 (33.33)
	Hy. asiaticum	2	0
Atux 39°42′N–76°09′E	*D. marginatus*	9	0
Total (133)	*Rh. turanicus*	117	*R. conorii* 21 (17.95) *Candidatus* Rickettsia barbariae 36 (30.77) *R. massiliae* 2 (1.71)
	D. marginatus	14	0
	Hy. asiaticum	2	0

using primers ompB-out1 (5′-ACAGCTACCATAGTAGCCAG-3′) and ompB-out2 (5′-TGCAGTATAGTTACCACCG-3′) for the first phase, and ompB-inter1 (5′-TGCTGCGGCTTCTACATT-3′) and ompB-inter2 (5′-ACCGCCAGCGTTCCCTAT-3′) for the second phase. The PCR conditions consisted of an initial 5-min denaturation at 95 °C, followed by 35 cycles at 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 2 min 30 s, with a final extension at 72 °C for 5 min (first phase), and an initial 5-min denaturation at 95 °C, followed by 35 cycles at 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min 10 s, with a final extension at 72 °C for 5 min. Part of the PS120-protein-encoding gene (*gene D*; 920 bp) fragment was amplified using primers gene D-F (5′-CGGTAACCTAGATACAAGTGA-3′) and gene D-R (5′-TATAAGCTATTGCGTCATCTC-3′) according to the sequences available in GenBank, and samples were amplified as recommended (Sekeyova et al., 2001). The PCR conditions consisted of an initial 5-min denaturation at 95 °C, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 8 min *R. aeschlimannii* from *Rhipicephalus turanicus* and double distilled water were used, respectively, as positive and negative controls (Wei et al., 2015). The amplification products were purified using the TIANgel Midi Purification Kit, cloned into the pGEM-T Easy vector (TianGen) and then subjected to sequencing (BGI, Shenzhen, China). Phylogenetic trees were constructed using the maximum likelihood (ML) and neighbor joining (NJ) algorithms with MEGA 6.0 (Tamura et al., 2013). Bootstrap analyses (500 replicates for ML analyses and 1000 replicates for the NJ analyses) were conducted to determine the relative support for clades in the consensus trees (Pattengale et al., 2010; Anstead and Chilton, 2013a,b).

3. Results

A total of 117 *Rh. turanicus* ticks (80 males, 37 females), 14 *Dermacentor marginatus* (9 males, 5 females) and two *Hyalomma asiaticum* (females) were collected in questing areas. Phylogenetic analyses based on the partial mitochondrial *16S rDNA* gene sequences are shown in Technical Appendix A in Supplementary material.

The ticks were screened first by *ompA* PCR, and positive samples were analyzed for all targeted genes. The results showed that 59 (44.36%) of the 133 ticks analyzed were positive for *Rickettsia* spp. Of these, 36 (30.76%) were confirmed as *Candidatus* R. barbariae, 21 (17.95%) as *R. conorii* subsp. *indica*, and two (1.71%) as *R. massiliae* in 117 *Rh. turanicus* ticks (Table 1). The *R. conorii* subsp. *indica* showed 100%, 100%, 100%, 99.60%, and 99.84% pairwise nucleotide sequence identity to *R. conorii* subsp. *indica rrs* (L36107), *gltA* (U59730), *ompA* (U43794), *ompB* (AF123726) and *R. conorii* subsp. *conorii sca1*(AE006914), respectively. The *R. massiliae* species had 99.74%–100% pairwise nucleotide sequence identity to genome sequences of the reference strains *R. massiliae* MTU5 (accession no. CP000683) for all six genes analyzed. For *Candidatus* R. barbariae, however, the amplified regions of the *gltA* and *ompB*[1] genes in this study were different from the existing sequences and no sequences of the *sca1* gene were available in GenBank, it showed 99.54%, 99.47% and 99.52% similarity with *R. sibirica* subsp. *sibirica* (accession no. KM288711), *R. parkeri* str. Portsmouth (accession no. CP003341) and *R. africae* ESF-5 (CP001612) for the *gltA*, *ompB*[1] and *sca1* genes, respectively. The rest of the genes of *Candidatus* R. barbariae had 100% pairwise nucleotide sequence identity to the reference sequences (EU272186, JF803896 and EU272189 for *ompA*, *17-kDa* and *rrs*, respectively; Table 2). For the *ompB*[2] (1063 bp) and *gene D* (920 bp) fragments, the BLAST results showed that the *R. conorii* subsp. *indica* identified in this study had 100% pairwise nucleotide sequence identity while the *Candidatus* R. barbariae had 99.89%–100% similarity with reference sequences availiable in GenBank (Table 2).

None of the ticks belonging to the species *D. marginatus* and *Hy. asiaticum* was positive for rickettsiae. All the sequences obtained in this study were deposited in GenBank under accession nos. KU364354–KU364359, KU364361–KU364367, KU364369–KU364378, and KU757300–KU757306. The topology of the phylogenetic trees produced from the ML analyses of the *ompA*, *gltA*, *gene D*, and *ompB*[2] gene sequences, as well as the *ompA-gltA-rrs-geneD-ompB* concatenated sequence data, were very similar to the NJ trees, and with total support of 69%–100% bootstrap values for the inclusion of the rickettsiae in *Rh. turanicus* within the clade that contained *R. conorii* subsp. *indica*; *Candidatus* R. barbariae and *rickettsia* sp. Tselentii; *R. massiliae* str. AZT80 and *R. massiliae* MTU5, respectively (Technical Appendix A in Supplementary material, Fig. 1).

4. Discussion

We report the presence of *R. conorii* subsp. *indica* in *Rh. turanicus* ticks for the first time, and identify *Candidatus* R. barbariae and *R. massiliae* in *Rh. turanicus* from sheep in China. The co-circulation of these rickettsial species around the Taklamakan desert increases the known range of vectors and reservoirs for SFG rickettsiae and provides a basis for assessing the risk of infection in humans.