The first detection of *Rickettsia aeschlimannii* and *Rickettsia massiliae* in *Rhipicephalus turanicus* ticks, in northwest China

Qing-Qing Wei1†, Li-Ping Guo1†, An-Dong Wang2†, Lu-Meng Mu1, Ke Zhang3, Chuang-Fu Chen2, Wan-Jiang Zhang1 and Yuan-Zhi Wang1*

**Abstract**

**Background:** *Rickettsia* spp. belonging to the spotted fever group (SFG) cause infections in humans, domestic animals and wildlife. At least five SFG rickettsial species have been reported in China, but the occurrence of *R. aeschlimannii* and *R. massiliae* in ticks has not been characterized to date.

**Findings:** A total of 114 adult ticks were collected from sheep in Yining County, Xinjiang Uygur Autonomous Region, in northwest China. The ticks were identified from morphological and molecular characteristics. All samples were examined by polymerase chain reaction (PCR), and six genetic markers were used to determine the *Rickettsia* spp. in the ticks. The ticks collected were identified as *Rhipicephalus turanicus*. Three different lineages of *Rh. turanicus* from Yining County were discovered on phylogenetic analysis of 16S rDNA and cox1. Twenty-one of the 114 samples (18.42%) were positive for rickettsial agents. Phylogenetic analysis based on six genetic sequences showed that three rickettsial species were present, namely: *R. aeschlimannii* (19.05%, 4/21), *R. massiliae* (19.05%, 4/21) and *R. sibirica* variant (61.90%, 13/21), which is clustered in the clade of *R. sibirica* subsp. *sibirica*.

**Conclusions:** This is the first description of *R. aeschlimannii* and *R. massiliae* in China. *R. massiliae*, *R. aeschlimannii* and *R. sibirica* variant co-circulate in the region of the China-Kazakhstan border, in northwest China. Rickettsial agents in ticks of the genus *Rhipicephalus* from migrant birds, transported livestock, wildlife and human beings should be investigated further in the region of the China–Central Asian border.

**Keywords:** *Rickettsia aeschlimannii*, *Rickettsia massiliae*, *Rhipicephalus turanicus* ticks, Northwest China

**Findings**

**Background**

*Rickettsia* spp. belonging to the spotted fever group (SFG) cause infections in animals and humans worldwide [1, 2]. To date, at least five validated SFG rickettsial species have been detected in ticks in China, including *R. heilongjiangii*, *R. sibirica*, *R. raoultii*, *R. slovaca* and *R. felis* [3]. Molecular evidence of the first four species was reported in northeastern and northwestern China, mainly in *Dermacentor* and *Haemaphysalis* ticks [4–6], and the last was found in *Rhipicephalus sanguineus* from Jiangsu Province [7].

Xinjiang Uygur Autonomous Region (XUAR), the largest province in China, occupies one-sixth of China, borders eight countries with a 5,600-km frontier, and there are 29 trading ports. In the present study, we assessed the occurrence of rickettsial agents in *Rh. turanicus* ticks in Yining County, the location of Yining Port, which is adjacent to Kazakhstan.

**Methods**

**Tick sampling and identification**

A total of 114 ticks were collected from sheep in Yining County (928 m above sea level, at 44°00′36″N 81°12′21″E).
All of the ticks were identified morphologically according to previous reports, and 23 representative ticks underwent molecular analysis based on partial mitochondrial (16S rDNA and cox1) gene sequences [8].

**Ethical approval**
This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2014-6).

**PCR amplification and sequence analysis**
For genetic detection of *Rickettsia* spp., the genomic DNA of all the ticks was extracted from individual specimens using the TIANamp Genomic DNA Kit (Tiangen, Beijing, China). All samples were examined by polymerase chain reaction (PCR), and six genetic markers [434-, 1332-, 1060-, 488-, 491-, and 812-bp products of the genes encoding the 17 kilodalton antigen (*17-kDa*), 16S rRNA (*rrs*), citrate synthase (*gltA*), surface cell antigen 1 (*sca1*), and outer membrane proteins A and B (*ompA* and *ompB*)] were amplified using previously described primers [3]. The amplification products were purified using the TIANgel Midi Purification Kit (TIANGEN, Beijing, China) and then cloned into the pGEM-T Easy vector and subjected to sequencing. A phylogenetic tree was constructed using the maximum likelihood and neighbor-joining algorithms with MEGA 6.0 software [9].

**Results**
The ticks were identified morphologically as *Rh. turanicus*. Sequencing data from the 23 representative ticks indicated three different lineages of *Rh. turanicus* from Yining County on the basis of phylogenetic analysis of 16S rDNA and cox1 (shown in Additional file 1). Six nucleotide sequences from our study have been deposited in the GenBank database (16S rDNA: KF547984, KF547987, and KF547989; cox1: KF188136–KF188138).

Twenty-one of the 114 samples (18.42%) were positive by PCR for products of six rickettsial genetic markers. Out of the 21 positive samples, four were confirmed as *R. aeschlimanni*, four were identified as *R. massiliae*, and the remaining thirteen were *R. sibirica* variant based on phylogenetic tree of the representative makers (*ompA* gene and *gltA* gene) and the 17-kDa-ompA-gltA-rrs-sca1-ompB concatenated sequence (shown in Additional file 2; Fig. 1). There were no differences in the DNA sequences of six responding genetic markers for *R.
aeschlimannii, with sequence similarities of 99.74% (1,169bp/1,172bp), 100% (1,048bp/1,048bp), 98.49% (458bp/465bp), 98.77% (722bp/731bp) and 99.33% (593bp/597bp) for the rrs, gltA, ompA, ompB and sca1 genes, respectively, and 99.19% (366 bp/369bp) to R. raoultii strain Alashankou-99 for the 17k-Da gene (KT261761). Except the sca1 gene, which has two different sequences with sequence similarities of 99.13% (573bp/578bp) and 99.48% (576bp/579bp) to R. massiliae MTU5 (CP000683), and the ompB gene, which has two different sequences with sequence similarities of 100% (765bp/765bp) and 98.56% (754bp/765bp) to R. massiliae MTU5 (CP000683), the DNA sequences of four genetic markers for R. massiliae were the same, with sequence similarities of 100% (383bp/383bp), 100% (1,162bp/1,162bp), 99.90% (1,022bp/1,023bp), 100% (434bp/434bp) for the 17k-Da, rrs, gltA, ompA genes, respectively. However, for the R. sibirica variant, except the gltA gene, which has two different sequences with sequence similarities of 99.54% (1,075bp/1,080bp) and 99.63% (1,076bp/1,080bp) to R. sibirica subsp. sibirica (KM288781), respectively, the sequences of the other five responding genetic markers have different levels of divergences, with sequence similarities of 100% (385bp/385bp) to R. raoultii strain Alashankou-131 (KT261760) for the 17k-Da gene, 99.82% (1,121bp/1,123bp) to R. raoultii isolate BL029-2 (KJ410261) for the rrs gene, 99.58% (469bp/471bp) to Rickettsia sp. Tselentii (EU194445) for the ompA gene, 99.48% (772bp/776bp) to R. parkeri str. Portsmouth (CP003341) for the ompB gene and 99.34 (598/602) to R. aferica ESF-5 (CP001612) for the sca1 gene. The similarities and divergences of the sequences in this study are shown in Additional file 3: Table S1. All the sequences obtained from our study have been deposited in the GenBank database [17 kDa: KT318742, KT588057, KT588065; rrs: KT318741, KT588056, KT588064; gltA: KT318743, KT588058, KT588066, KT588070; sca1: KT318746, KT588061, KT588063, KT588069; ompA: KT318744, KT588059, KT588067; ompB: KT318745, KT588060, KT588062, KT588068].

Discussion

R. massiliae, R. rhipicephali and R. aeschlimannii are grouped phylogenetically into a clade in the family Rickettsiaceae [10]. R. massiliae was first isolated in 1990 from a Rh. turanicus tick in an area near Marseille, France [11]. Since then, this pathogen has been identified from other Rhipicephalus ticks in regions of Europe, North and Central Africa, and the United States [12]. Furthermore, cases showed that it can cause human infection. R. aeschlimannii was first described from Hyalomma marginatum in Morocco in 1997 [13]. The presence of R. aeschlimannii has been demonstrated in Hyalomma ticks from Europe (e.g. France, Croatia, Spain, Italy), Asia (e.g. Israel, Turkey) and Africa (e.g. Mali, Algeria, Egypt) [14–16] and from Haemaphysalis ticks in Spain and Kazakhstan [17]. Furthermore, Ixodes ricinus, H. punctata, Rh. bursa, and Rh. sanguineus isolated from human Spanish patients were shown to contain DNA from R. aeschlimannii [14], and there is a report of R. aeschlimannii from Rh. turanicus infecting a man in Greece in 2013 [18]. In this study, we report the first molecular evidence that R. aeschlimannii and R. massiliae are present in Rh. turanicus from sheep in the region of the China-Kazakhstan border, in the northwest of China.

To date, R. sibirica is known to contain two subspecies [19], R. sibirica subsp. sibirica and R. sibirica subsp. mongolotimonae. The former was first isolated in Russia but it has subsequently been found in northern China [5]. In contrast, R. sibirica subsp. mongolotimonae was first isolated in Inner Mongolia and then found in Europe and Africa [20, 21]. Here, the R. sibirica variant found in the region of the China–Kazakhstan border appeared divergent in the ompA, ompB and sca1, used to differentiate Rickettsia species, although it was closest to R. sibirica subsp. sibirica, on the basis of the gltA gene and the phylogenetic tree of the 17-kDa-ompA-gltA-rrs-sca1-ompB concatenated sequence. Further genomic analysis should be carried out to confirm the classification of the R. sibirica variant found in this study.

The Rh. turanicus tick is widely distributed throughout the Mediterranean subregion, Africa, and Asia, including China, especially in XUAR [22], and it has been implicated as a vector of several human and veterinary pathogens, including Rickettsia spp. [18]. Here, R. massiliae, R. aeschlimannii and R. sibirica variant were found in the same area, Yining County, which suggests that several SFG Rickettsia spp. co-circulate in Rh. turanicus as a potential vector near the China-Kazakhstan border.

In 2004, Shpynov et al detected R. aeschlimannii in the Alma-Ata region, east of Kazakhstan [17]. Here we found that Rh. turanicus in the region of the China-Kazakhstan border showed genetic divergence in the loci of 16S rDNA and coxI, which indicates that these ticks collected from sheep may come from different lineages. At present, it is unknown whether these ticks are imported from the Chinese hinterland or abroad through migrant birds, or with internationally transported livestock. This topic needs to be further investigated.

Conclusions

This is the first report of the molecular analysis of R. aeschlimannii and R. massiliae in China. The findings of the study suggest that R. massiliae, R. aeschlimannii and R. sibirica variant co-circulate in Rh. turanicus in the
region of the China–Kazakhstan border, in northwest China. The origin of the *Rhipicephalus* genus (such as migrant birds, transported livestock, wildlife and human beings) and the epidemiology of tick-borne pathogens should be further investigated in the region of the China–Central Asian border.

**Additional files**

Additional file 1: The photo of *Rhipicephalus turanicus* and Phylogenetic tree of *Rhipicephalus turanicus* 16S rDNA and CO1 gene. (DOC 198 kb)

Additional file 2: The single gene Phylogenetic tree of *Rickettsia* spp. (DOCX 863 kb)

Additional file 3: Closest relative sequences to the partial 17-kDa, 16S, gltA, ompA, omp8 and sca1 genes, sequences of the *Rickettsia aeschlimannii* (Table S1A), *Rickettsia massiliae* (Table S1B) and *Rickettsia sibirica* (Table S1C) detected in the *Rhipicephalus turanicus* ticks, Northwest of China. (DOCK 22 kb)

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

YZW conceived and designed the study, and critically revised the manuscript. QQW, LPG, ADW and YZW performed the experiments, analyzed the data and drafted the manuscript. LMM, YZW CFC and WJZ performed the data and drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

This research was supported in part by grants from the National Natural Science Foundation of China (Granted No. 81560338), the National Science & Technology Pillar Program (No. 2013BAI05B05) and Co-innovation Center for the High Incidence of Zoonotic Disease Prevention and Control in Western China (No. 2013-179).

**Author details**

1. School of Medicine, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832000, People’s Republic of China.
2. School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832000, People’s Republic of China.
3. Pingdingshan University, Pingdingshan, Henan 467000, People’s Republic of China. (Present address: Chinese Spotted Fever Group Rickettsiae, J Clin Microbiol. 2021;59:2210–2218)

**Received:** 3 September 2015  **Accepted:** 3 December 2015

**Published online:** 10 December 2015

**References**

1. Maina AN, Jiang J, Ornulo SA, Cutler SJ, Ade F, Ogola E, et al. High prevalence of *Rickettsia africae* variants in *Amblyomma vaingatun* ticks from domestic mammals in rural western Kenya: implications for human health. Vector Borne Zoonotic Dis. 2014;14(10):693–702.

2. Lopez-Velez R, Palomar AM, Otoo JA, Norman FF, Pérez-Molina JA, Portillo A. Novel *Candidatus rickettsia* species detected in rostril tick from human, Gabon, 2014. Emerg Infect Dis. 2015;21(2):325–7.

3. Guo LP, Mu LM, Xu J, Jiang SH, Wang AD, Chen CF, et al. *Rickettsia rauvali* in Haemaphysalis elinacea from marbled molecats. China-Kazakhstan border. Parasit Vectors. 2015;8:461.

4. Zhang JZ, Fan MY, Wu YM, Fournier PE, Roux V, Raoult D. Genetic Classification of *Rickettsia helongjiangensis* and *Rickettsia hulinii*, two Chinese Spotted Fever Group Rickettsiae. J Clin Microbiol. 2000;38:3498–501.

5. Yu X, Jin Y, Fan M, Xu G, Liu Q, Raoult D. Genotypic and antigenic identification of two new strains of spotted fever group *rickettsiae* isolated from China. J Clin Microbiol. 1993;31(1):183–8.

6. Tian ZC, Liu QY, Shen H, Xie JR, Luo J, Tian MY. First report on the occurrence of *Rickettsia slovaca* and *Rickettsia rauvali* in Dermacentor silvarum in China. Parasit Vectors. 2012;5:19:1–4.

7. Zhang J, Lu G, Kelly P, Zhang Z, Wei L, Yu D, et al. First report of *Rickettsia felis* in China. BMC Infect Dis. 2014;14:682.

8. Dantas-Torres F, Latrofa MS, Annciosa G, Gannelli A, Parisi A, Otranto D. Morphological and genetic diversity of *Rickettsiae sanguineus senso lato* from the New and Old Worlds. Parasit Vectors. 2013;6:213.

9. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731–9.

10. Vitale G, Mansuelo S, Rolain JM, Raoult D. *Rickettsia massiliae* human isolation. Emerg Infect Dis. 2006;12(1):174–5.

11. Beati L, Raoult D. *Rickettsia massiliae* sp. nov., a new spotted fever group *Rickettsia* Int J Syst Bacteriol. 1993;43(4):889–90.

12. Fernández de Mera IG, Zivkovic Z, Bolaños M, Carranza C, Pérez-Arellano JL, Gutiérrez C, et al. *Rickettsia massiliae* in the Canary Islands. Emerg Infect Dis. 2009;15(11):1869–70.

13. Sarif M, Soccolovitschi C, Boudoubeh N, Hassar M, Raoult D, Parola P. Spotted fever group *rickettsiae* in ticks, Morocco. Emerg Infect Dis. 2008;14:1067–73.

14. Fernandez-Soto P, Encinas-Grandes A, Perez-Sanchez R. *Rickettsia aeschlimannii* in Spain: molecular evidence in *Hyalomma marginatum* and five other tick species that feed on humans. Emerg Infect Dis. 2003;9(8):889–90.

15. Gabriela K, Gad B, Kosta Y. Molecular Detection of *Rickettsia africae*, *Rickettsia aeschlimannii*, and *Rickettsia sibirica mongolotimonae* in Carabids and *Hyalomma spp.* Ticks from Israel. Vector-borne and Zoonotic Dis. 2013;13:851–6.

16. Orkun Ö, Karaer Z, Çakmak A, Nalbantoglu S. Identification of tick-borne pathogens in ticks feeding on humans in Turkey. PLoS Negl Trop Dis. 2014;8(8):1–11.

17. Shpynov S, Fournier PE, Rudakov N, Tankibaev M, Tarasevich I, Raoult D. Detection of a *Rickettsia* closely related to *Rickettsia aeschlimannii*, *Rickettsia helongjiangensis*, *Rickettsia sp.* strain RpA4, and *Ehrlichia mursi* in ticks collected in Russia and Kazakhstan. J Clin Microbiol. 2004;42:2221–3.

18. Germanakis A, Chochlakis D, Angelakis E, Tselentis Y, Psaroulaki A. *Rickettsia aeschlimannii* infection in a man, Greece. Emerg Infect Dis. 2013;19:1176–7.

19. Fournier PE, Zhu Y, Yu X, Raoult D. Proposal to create subspecies of *Rickettsia sibirica* and an emended description of *Rickettsia sibirica*. Ann N Y Acad Sci. 2006;1078:597–606.

20. Fournier PE, Gouriet F, Lucht F, Raoult D. Lymphangitis-associated rickettsiosis, a new rickettsiosis caused by *Rickettsia sibirica* mongolotimonae, seven new cases and review of the literature. Clin Infect Dis. 2005;40(10):1435–44.

21. Ramos JM, Iado I, Padilla S, Masía M, Anda P, Gutiérrez F. Human infection with *Rickettsia sibirica mongolotimonae*, Spain, 2007–2011. Emerg Infect Dis. 2013;19(2):267–9.

22. Chochlakis D, Ioannou I, Papadopoulos B, Tselentis Y, Psaroulaki A. *Rhipicephalus turanicus*: from low numbers to complete establishment in Cyprus. Its possible role as a bridge-vector. Parasit Vectors. 2014;7:11.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at **www.biomedcentral.com/submit**