Identification and Genetic Diversity Analysis of *Rickettsia* in *Dermacentor Nuttalli* Within Inner Mongolia, China

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Abstract

Background

The pathogen genus *Rickettsia* contains the lineages *spotted fever group*, *typhus group*, *transitional group*, and the *ancestral group*, of which the *spotted fever group Rickettsia* (SFGR) is transmitted by ticks. *Dermacentor nuttalli* is considered the main vector carrying SFGR. Studying the genetic diversity and population structure of *Rickettsia* is essential for developing effective control strategies and predicting evolutionary trends of the pathogens.

Methods

We collected 408 *Dermacentor nuttalli* in the Inner Mongolia Autonomous region in 2019, detected *Rickettsia* infection, and characterized the haplotypes. The extracted *Rickettsia* DNA of the *gltA* and *ompA* genes were amplified and sequenced.

Result

In this study, 10 haplotypes of the *gltA* gene and 22 haplotypes of the *ompA* gene were obtained. In the two resulting phylogenetic trees, the haplotypes G1-G7 and G9 of the *gltA* gene clustered with *Rickettsia raoultii*, while G8 and G10 clustered with *Rickettsia sibirica*. Haplotypes O1-O15, O18 and O20-O22 of the *ompA* gene clustered with *Rickettsia raoultii*, while O16 and O19 clustered with *Rickettsia sibirica*. The average haplotype diversity was 0.3 for *gltA* and 0.7 for *ompA*, while the average nucleotide diversity was greater than 0.05. Neutrality tests were insignificant for Tajima's D results and Fu's F_s results. The fixation index values (F_{ST}) showed that the degree of genetic differentiation between most sampled populations was small ($F_{ST} < 0.05$), while others were medium ($F_{ST} > 0.05$) and large ($F_{ST} > 0.15$). Analysis of molecular variance (AMOVA) revealed that the variation within populations was greater than that between populations. The mismatch analysis of *Rickettsia* showed double peaks.

Conclusion

We found two genotypes of *Rickettsia*: *Rickettsia raoultii* and *Rickettsia sibirica*. The high genetic diversity of *Rickettsia* allows for easy adaption to different environments; furthermore, genetic differentiation between populations is small and *Rickettsia* populations do not show a pedigree geographical structure. The high rates of retention and infestation of *Rickettsia* in *Dermacentor nuttalli* together with the animal husbandry exchange in China gradually lead to the genetic characteristics of *Rickettsia* harmonizing across various regions. Overall, the significant genetic diversity and geographic structure of *Rickettsia* in *Dermacentor nuttalli* are critical for SFGR control.

Background

Rickettsia is an important zoonotic pathogen. It brings great harm to the health of humans and animals [1, 2]. According to the phylogeny of *Rickettsia*, its species belong to four groups: spotted fever group (SFG), typhus group (TG), transitional group (TRG), and ancestral group (AG). SFG is transmitted by ticks [3, 4], while TG is transmitted by lice and fleas [5]. The vast territory and diverse habitats of Inner Mongolia greatly benefit the survival of ticks. *Dermacentor nuttalli*, is the dominant tick species, and is considered to be the main vector carrying SFGR in Inner Mongolia [6–8]. SFGR infections occur around the world and may cause serious diseases in humans. In China *Rickettsia heilongjiang* Fournier et al., 2003, *Rickettsia raoultii* Mediannikov et al. 2008, *Rickettsia slovaca* Sekeyová et al. 1998, *Rickettsia sibirica* Zdrodovskii, 1948, *Rickettsia mongolotimonae* Roux et al. 1997, *Rickettsia monacensis* Simser et al. 2019, and *Candidatus Rickettsia hebei* Zou et al. 2011 and *Candidatus Rickettsia jingxinensis* Liu et al. 2016 have been detected in ticks. In recent years, an increasing number of potentially novel SFGR have been detected in ticks, emphasizing that SFGR pose a threat to public health in China [9–12].

Molecular biological techniques allow for the identification of *Rickettsia* species. Although 16S rRNA can accurately identify a pathogen as belonging to *Rickettsia*, it is hard to distinguish the species due to the fact that in almost all prokaryotes this is a highly conserved region. The *gltA* and *ompA* genes are used for species identification in *Rickettsia* [13–16]. The *gltA* gene encodes a citrate lyase, the sequence of which allows for a highly reliable identification of the evolutionary distances among *Rickettsia*. The *ompA* gene is an outer membrane protein gene and is considered as the ‘gold-standard’ for species identification in *Rickettsia*, owing to the highly specific 5’end [17].

Previous studies demonstrated that the genetic diversity of *Dermacentor nuttalli* is rich and allows for its existence in different geographical environments [18]. Therefore, in our investigation we increased the number of tick species, exploring the *Rickettsia* infection and genotype distribution, furthering to analyze the genetic diversity of *Rickettsia*. Studying the genetic diversity of *Rickettsia* is essential for developing effective control strategies and predicting pathogen evolutionary trends.

Methods

Sample collection

In this study, a total of 408 ticks were collected from sheep at four sampling spots in Inner Mongolia, China, namely Chengchuan Town, early Banner of Etoke Banner Ordos; Siziwang Banner; Hohhot; the Bayan WenduSumu area Arukorqin Banner Chifeng and Xinbarhu right Banner, Hulun Buir. The locations of the sampling areas are shown in Supplementary Table1.

DNA extraction, amplification and sequencing

Ticks were identified as *Dermacentor nuttalli*, all *Dermacentor nuttalli* samples were individually extracted using TIANGEN Tissue and Blood Kit (TIANGEN, Beijing, China) [19]. For identification of *Rickettsia*

infections, *Rickettsia* gltA and ompA genes were amplified by PCR following the protocols described by Bermúdez et al [20].

Specific primers targeting gltA and ompA genes of *Rickettsia* from *Dermacentor nuttalli* were synthesized by Shanghai Sangon. DNA was amplified using a system of 40μL, each including Taq PCR Master Mix (Sangon), 2 μL of DNA from each sample, 1 μL of each reverse and forward primers, filled up with sterile water. The PCR primers, amplification sizes (bp), and annealing temperatures are presented in Table 2. Sterile double distilled water was used as the negative control in each PCR reaction. After checking the PCR products with a 1.5% agarose gel electrophoresis stained with GoldeView, PCR products were directly sent for sequencing if the result presented a single band. Otherwise, the PCR product was purified using the Gel DNA Recovery Kit (TIANGEN, Beijing, China) and cloned using the p-GMT Easy vector system.

Table 1
Sample information of *Dermacentor nuttalli* populations

Location	Collection time	Area code	Longitude	Latitude	Samples
ChiFeng	2019.4	CF	121°64'	43°46'	219
Siziwang Banner	2019.5	SZWQ	111°63'	40°81'	30
Hulun Buir	2019.8	HLBE	116°82'	48°67'	74
Ordos	2019.7	EEDS	108°32'	37°70'	85

Table 2
Primers of PCR and amplification conditions

Gene target	Primer sequence(5'~3')	Amplification size(bp)	Annealing temperature(°C)
gltAF	CGATTGCTTTACTTACGACCC	1090	53
gltAR	GAGCATTTCTTTCCATTGTGCC		
ompAF	ACTAGGTGCGAATATAGACCC	712	56
ompAR	CGTACCTTTTGCGTTAACT		

Table3 *Rickettsia* infection in *Dermacentor nuttalli*

Data analysis

Sequences were edited in Seqman 7.1 and identified by comparative analysis with sequences deposited in GenBank, using the BLAST search engine. Multiple sequence alignment and sequence similarity calculations were done using DNAMAN 7.0. Phylogenetic trees were reconstructed with the neighbor-joining algorithm using MEGA7.0 with 1000 Bootstrap replicates to assess tree stability [21–23]. Sequences were analyzed in DNAsp 5.10 and Arequin 3.5 for calculating polymorphic sites, nucleotide differences, the number of haplotypes, both haplotype and nucleotide diversity, the distribution pattern of DNA variation haplotype, and genetic variation parameters [24, 25]. The extent of molecular genetic

differentiation among *Rickettsia* populations analyzed from Inner Mongolia was estimated by analysis of molecular variance (AMOVA) and F_{ST} values [26]. To determine whether genetic differentiation and population structures of *Rickettsia* varied between the four sampling localities in Inner Mongolia. Neutral tests were analyzed using Tajima's D and Fu's F_s tests using Arlequin 3.5 and DNAsp 5.10. Popart 1.7 was used to evaluate the relationships between haplotypes by constructing TCS haplotype network maps.

Results

Rickettsia infection

We collected and tested a total of 408 *D. nuttalli* in 2019 from four sites in Inner Mongolia: Chifeng (n=219), Orods (n=85), Siziwang Banner (n=30), and Hulunbeier (n=74) (Table3). Positive amplification products of the *gltA* and *ompA* genes were regarded as the infection rate of *Rickettsia* in *Dermacentor nuttalli* [27]. Across the four regions, the average infection rate of *Rickettsia* was 50.74%, with is the highest value found in the Hulunbeier region (85.14%).

Rickettsia identification

PCR amplicons from positive samples were cloned and sequenced. We detected 10 haplotypes of *gltA* sequences (Genbank Accession Numbers OK638141-OK638150) and 22 haplotypes of *ompA* sequences (Genbank Accession Numbers OL304270-OL304271, OL348251-OL348270). The sequences had the highest similarities with *Rickettsia raoultii* and *Rickettsia sibirica*, as registered in Genbank, with 98% and 99% identity respectively. In the *gltA* phylogenetic trees, haplotypes G1-G7 and G9 were clustered with *Rickettsia raoultii*, while G8 and G10 were clustered with *Rickettsia sibirica*. Furthermore, *Rickettsia* in Inner Mongolia is closely related to *Candidatus Rickettsia uralica* Igolkina et al. 2015 with 98% identity and distantly related to *Rickettsia asembonensis* Maina et al. 2016. In the *ompA* phylogenetic trees, Haplotypes O1-O15, O18, and O20-O22 were clustered with *Rickettsia raoultii*, while O16 and O19 were clustered with *Rickettsia sibirica*. *Rickettsia* in Inner Mongolia is closely related to *Candidatus Rickettsia uralica* with 98% identity and distantly related to *Rickettsia montana* (ex Lackman et al. 1965) Weiss and Moulder 1984. The phylogenetic trees for both genes showed that the haplotypes of *Rickettsia* clustered into one branch with the ingroup, which had two genotypes, *Rickettsia raoultii* and *Rickettsia sibirica*, in this study (Fig.1, 2).

Rickettsia genetic diversity by *gltA* gene

Of 10 haplotypes recovered, 4 were shared haplotypes (G1, G2, G3, G10). Numerically, the most common haplotype was G1, with 167 sequences (80.68% of all sequences) (Table4). G1 was located in the center of the haplotype network diagram and was found in four geographic populations, indicating that it was the dominant haplotype among groups (Fig.3a). The average haplotype diversity was 0.335 and the average nucleotide diversity was 0.04922. EEDS was the region with the highest haplotype diversity ($h=0.909$). Neutrality results revealed insignificant values of Tajima's D and Fu's F_s results, confirming that the population had not experienced recent expansion (Table 5). The Wright's F index was calculated

to measure the levels of genetic differentiation among the four geographical populations. Comparing pairwise F_{ST} indices exhibited that the F_{ST} value between HLBE and SZWQ was greater than 0.25, indicating that there was high genetic differentiation among populations. The F_{ST} values among the other regions were less than 0.05, indicating that the genetic differentiation amongst these populations was very small (Table 6). The AMOVA results showed that the population variation of *Rickettsia* mainly arose from within the population, and the genetic differentiation between populations was very small (Table 7). The mismatch analysis chart exhibited double peaks, indicating that the four geographic populations did not experience rapid population expansion (Fig. 4a).

***Rickettsia* genetic diversity by ompA gene**

Of the 22 haplotypes that were found, 4 were shared haplotypes (O1, O2, O3, O16). Numerically, the most common haplotypes were O1, O2, and O3, with 170 sequences (82.13% of all sequences) (Table 8). O2 was located in the center of the network map and was found in four geographic populations, indicating that it was the dominant haplotype among groups (Fig.3b). The total average haplotype diversity was 0.735 and the total nucleotide diversity was 0.0738. SZWQ was the locality with the highest haplotype diversity ($h=1$) (Table 10). Neutrality results were the same as those for *gltA* (Table 9). Comparing pairwise F_{ST} indices exhibited that the F_{ST} value between CF and HLBE was greater than 0.25, indicating that there was a high genetic differentiation among populations. The F_{ST} values between HLBE–SZWQ and EEDS were all greater than 0.05, indicating that there was a moderate genetic differentiation among populations. The F_{ST} values among the other regions were less than 0.05, indicating that the genetic differentiation amongst these populations was very small (Table 10). The results of the AMOVA and mismatch analyses were consistent with the *gltA* results (Table 11, Fig.4b).

Discussion

In addition to evaluating the emergence of new genotypes and studying the population structure, investigations of the genetic diversity of *Rickettsia* are critical for planning effective control measures. This study analyzed the genetic diversity of the *gltA* and *ompA* genes of *Rickettsia* from four localities of Inner Mongolia. Two genotypes of *Rickettsia* have been identified: *Rickettsia raoultii* and *Rickettsia sibirica*, both belong to SFGR. However, in recent years, human infection with *Rickettsia raoultii* in many countries led to tick-borne lymphadenitis [28–31]. Public health workers and physicians should pay attention to the high risk of humans being infected by *Rickettsia raoultii*, to strengthen appropriate detection and treatment measures in endemic regions.

Our results illustrated that the *gltA* and *ompA* genes have shared haplotypes in four regions, which are dominant haplotypes characterized as primitive and stable. Shared haplotypes indicate that the degree of gene communication is high in *Rickettsia* populations. We found $Hd>0.5$, $Pi>0.005$ as the highest genetic diversity [32]. The lowest genetic diversity was found in HLBE in the *gltA* gene, while the diversity of the *ompA* was markedly higher in HLBE. The genetic diversity of species is affected by many factors, including geographical distribution, population size, and other natural factors. Different gene markers are

under different selective pressures during the evolution of species, leading to inconsistent genetic diversities [33, 34]. Overall, the high genetic diversity of *Rickettsia* is in accordance with different environments. Among four geographic populations, the degree of genetic differentiation was high only between HLBE and SZWQ in the *gltA* gene. The degree of genetic differentiation was large between HLBE and the other three regions. We concluded that the genetic differentiation in some regions is not within the same range due to the different evolutionary rates of the two genes. The neutrality test and the mismatch analysis maps for the two genes exhibited genetic differentiation with no population expansion. The haplotypes of *Rickettsia* did not branch clearly according to the clustering of geographic regions, documenting that the *Rickettsia* populations did not form a pedigree geographical structure. Since *Rickettsia* can be transmitted either vertically or horizontally, the high retention and infestation rates of *Rickettsia* in *Dermacentor nuttalli*, together with the animal husbandry exchange in China gradually lead to the harmonization of genetic characteristics of *Rickettsia* across various regions. Overall, the significant genetic diversity and geographic structure exhibited among *Rickettsia* in *Dermacentor nuttalli* has critical implications for SFGR control.

Conclusion

The *gltA* and *ompA* genes were used to study the genetic diversity of *Rickettsia* from four geographic localities in Inner Mongolia. This study provides a reference for avoiding the risk of introducing new genotypes and complex genetic structures of *Rickettsia* populations, at the same time, it fills a gap in the knowledge of the genetic diversity of *Rickettsia* in Inner Mongolia.

Additional files

Abbreviations

PCR

Polymerase chain reaction

D. nuttalli

Dermacentor nuttalli

R. raoultii

Rickettsia raoultii

R. sibirica

Rickettsia sibirica

AMOVA

Analysis of molecular variance

BLAST

Basic Local Alignment Sequence Tool.

Declarations

Declarations

Ethics approval and consent to participate

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Declarations

Consent for publication

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interests.

Declarations

Abbreviation

n, number of samples; Ht, number of haplotypes; S, number of polymorphic sites; Hd, haplotype diversity; Pi, nucleotide diversity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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Authors' contributions

ZG, HC and DDQ performed laboratory analysis, analyzed data and wrote the first draft. RM, TC, and JFY revised the manuscript. YYS and DDQ participated in samples collection. SYF directed the experiment and helped to revise the manuscript. All authors read and approved the final manuscript.

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References

1. Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin Infect Dis*. 2001;32(6):897–928.
2. Černý J, Buyannemekh B, Needham T, Gankhuyag G, Oyuntsetseg D. Hard ticks and tick-borne pathogens in Mongolia-A review. *Ticks Tick Borne Dis*. 2019;10(6):101268.
3. Guo WP, Wang YH, Lu Q, Xu G, Luo Y, Ni X, et al. Molecular detection of spotted fever group rickettsiae in hard ticks, northern China. *Transbound Emerg Dis*. 2019;66(4):1587–1596.
4. Satjanadumrong J, Robinson MT, Hughes T, Blacksell SD. Distribution and Ecological Drivers of Spotted Fever Group Rickettsia in Asia. *Ecohealth*. 2019;16(4):611–626.
5. Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev*. 1997;10(4):694–719.
6. Fischer T, Myalkhaa M, Krücken J, Battsetseg G, Batsukh Z, Baumann MPO. Molecular detection of tick-borne pathogens in bovine blood and ticks from Khentii, Mongolia. *Transbound Emerg Dis*. 2020;67(2):111–118.
7. Song S, Chen C, Yang M, Zhao S, Wang B, Hornok S, et al. Diversity of Rickettsia species in border regions of northwestern China. *Parasite Vectors*. 2018;11(1): 634.
8. Yin X, Guo S, Ding C, Cao M, Kawabata H, Sato K, et al. Spotted Fever Group Rickettsiae in Inner Mongolia, China, 2015–2016. *Emerging Infect Dis*. 2018;24(11):2105–2107
9. Wang Q, Guo WB, Pan YS, Jiang BG, Du CH, Que TC, et al. Detection of Novel Spotted Fever Group Rickettsiae (Rickettsiales: Rickettsiaceae) in Ticks (Acari: Ixodidae) in Southwestern China. *J Med Entomol*. 2021;58(3):1363–1369.
10. Guo WP, Wang YH, Lu Q, Xu G, Luo Y, Ni X, et al. Molecular detection of spotted fever group rickettsiae in hard ticks, northern China. *Transbound Emerg Dis*. 2019;66(4):1587–1596.
11. Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. *Lancet Infect Dis*. 2015;15(12):1467–1479.

12. Liu H, Liang X, Wang H, Sun X, Bai X, Hu B, et al. Molecular evidence of the spotted fever group Rickettsiae in ticks from Yunnan Province, Southwest China. *Exp Appl Acarol.* 2020;80(3):339–348.
13. Miranda J, S Mattar. Molecular detection of Rickettsia bellii and Rickettsia sp. strain Colombianensi in ticks from Cordoba, Colombia. *Ticks Tick Borne Dis.* 2014;5(2):208–212.
14. Qin XR, Han HJ, Han FJ, Zhao FM, Zhang ZT, Xue ZF, et al. Rickettsia japonica and Novel Rickettsia Species in Ticks, China. *Emerg Infect Dis.* 2019;25(5):992–995.
15. Pillay AD, Mukaratirwa S. Genetic diversity of Rickettsia africae isolates from Amblyomma hebraeum and blood from cattle in the Eastern Cape province of South Africa. *Exp Appl Acarol.* 2020;82(4):529–541.
16. Song S, Chen C, Yang M, Zhao S, Wang B, Hornok S, et al. Diversity of Rickettsia species in border regions of northwestern China. *Parasit Vectors.* 2018;11(1):634.
17. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate Synthase Gene Comparison, a New Tool for Phylogenetic Analysis, and Its Application for the Rickettsiae. *INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY.* 1997;47(2):252–261.
18. Gui Z, Wu L, Cai H, Mu L, Yu JF, Fu SY, et al. Genetic diversity analysis of Dermacentor nuttalli within Inner Mongolia, China. *Parasit Vectors.* 2021;14(1):131.
19. Halos L, Jamal T, Vial L, Maillard R, Suau A, Le Menach A, et al. Determination of an efficient and reliable method for DNA extraction from ticks. *Vet Res.* 2004;35:709–713.
20. Bermúdez S, Martínez-Mandiche J, Domínguez L, Gonzalez C, Chavarria O, Moreno A, et al. Diversity of Rickettsia in ticks collected from wild animals in Panama. *Ticks Tick Borne Dis.* 2021;12(4):101723.
21. Mendell NL, Reynolds ES, Blanton LS, Hermance ME, Londoño AF, Hart CE, et al. Detection of Rickettsiae, Borreliae, and Ehrlichiae in Ticks Collected from Walker County, Texas, 2017-2018. *Insects.* 2019;10:315.
22. Li K, Stanojević M, Stamenković G, Ilić B, Paunović M, Lu M, et al. Insight into diversity of bacteria belonging to the order Rickettsiales in 9 arthropods species collected in Serbia. *Sci Rep.* 2019;9:18680.
23. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol.* 2016;33:1870–1874.
24. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 2009;25:1451–1452.
25. Rozas J. DNA sequence polymorphism analysis using DnaSP. *Methods Mol Biol.* 2009;537:337–350.
26. Meirmans PG, Hedrick PW. Assessing population structure: F(ST) and related measures. *Mol Ecol Resour.* 2011;11:5–18.
27. Santibáñez S, Portillo A, Santibáñez P, Palomar AM, Oteo JA. Usefulness of rickettsial PCR assays for the molecular diagnosis of human rickettsioses. *Enferm Infecc Microbiol Clin.* 2013;31(5):283–288.

28. Li H, Zhang PH, Huang Y, Du J, Cui N, Yang ZD, et al. Isolation and Identification of *Rickettsia raoultii* in Human Cases: A Surveillance Study in 3 Medical Centers in China. *Clin Infect Dis*. 2018;66(7):1109–1115.
29. Wen J, Jiao D, Wang JH, Yao DH, Liu ZX, Zhao G, et al. *Rickettsia raoultii*, the predominant *Rickettsia* found in *Dermacentor silvarum* ticks in China-Russia border areas. *Exp Appl Acarol*. 2014;63(4):579–585.
30. Dong Z, Yang Y, Wang Q, Xie S, Zhao S, Tan W, et al. A case with neurological abnormalities caused by *Rickettsia raoultii* in northwestern China. *BMC Infect Dis*. 2019;19(1):796.
31. Seo MG, Kwon OD, Kwak D. High Prevalence of *Rickettsia raoultii* and Associated Pathogens in Canine Ticks, South Korea. *Emerg Infect Dis*. 2020;26(10):2530–2532.
32. Was G, Bowen B W. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*. 1998(5):415–426.
33. Feng X, Liu J, Chiang YC, Gong X. Investigating the Genetic Diversity, Population Differentiation and Population Dynamics of *Cycas segmentifida* (Cycadaceae) Endemic to Southwest China by Multiple Molecular Markers. *Front Plant Sci*. 2017;8:839.
34. [1] Hamrick J L, Godt M J W, Sherman-Broyles S L. Factors influencing levels of genetic diversity in woody plant species. *New Forests*. 1992;6(1):95–124.

Tables

Table 1 Sample information of *Dermacentor nuttalli* populations

Location	Collection time	Area code	Longitude	Latitude	Samples
ChiFeng	2019.4	CF	121°64'	43°46'	219
Siziwang Banner	2019.5	SZWQ	111°63'	40°81'	30
Hulun Buir	2019.8	HLBE	116°82'	48°67'	74
Ordos	2019.7	EEDS	108°32'	37°70'	85

Table 2 Primers of PCR and amplification conditions

Gene target	Primer sequence 5'~3'	Amplification size bp	Annealing temperature °C
gltAF gltAR	CGATTGCTTTACTTACGACCC GAGCATTTCTTTCCATTGTGCC	1090	53
ompAF	ACTAGGTGCGAATATAGACCC	712	56
ompAR	CGTACCTTTTGC GTTAACACT		

Table 3 *Rickettsia* infection in *Dermacentor nuttalli*

Statistic	CF	EEDS	SZWQ	HLBE	Total
Tested	219	85	30	74	408
Infected	106	23	15	63	207
Infection rate	48.40	27.06	50	85.14	50.74

Table 4 Haplotype distribution of *Rickettsia* based on gltA gene

Hap	Num	CF	EEDS	HLBE	SZWQ
G1	167	77	20	60	10
G2	25	20	1	2	2
G3	7	6	0	0	1
G4	1	1	0	0	0
G5	1	1	0	0	0
G6	1	1	0	0	0
G7	1	0	0	0	1
G8	1	0	0	0	1
G9	1	0	1	0	0
G10	2	0	1	1	0

Table 5 Summary statistics for polymorphisms and neutrality tests of *gltA* gene from *Rickettsia* in Inner Mongolia

	n	Ht	S	Hd	Pi	Tajima's	Fu's Fs
CF	106	14	400	0.484	0.06725	-1.47773	47.607
EEDS	23	12	445	0.909	0.04918	-2.72424**	8.007
HLBE	63	6	10	0.313	0.00064	-2.13550*	-2.666
SZWQ	15	9	308	0.800	0.13230	-1.12826	9.686
TOTAL	207	10	282	0.335	0.04922	-1.65779	46.426

Abbreviation: n, number of samples; Ht, number of haplotypes; S, number of polymorphic sites; Hd, haplotype diversity; Pi, nucleotide diversity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 6 F_{ST} values among different groups of *Rickettsia* based on *gltA* gene

Locality	CF	EEDS	HLBE	SZWQ
CF				
EEDS	-0.02141			
HLBE	0.04136	0.04878		
SZWQ	-0.00537	-0.00118	0.25240	

Table 7 AMOVA of *gltA* gene of *Rickettsia* population

Source of variation	d.f	Sum of squares	Variance Components	Percentage of variation	Fixation Index F_{ST}
Among population	3	75.094	0.30572Va	2.52684	0.02527
Within population	203	294.027	11.79324Vb	97.47316	
Total	206	2469.121	12.09896		

Table 8 Haplotype distribution of *Rickettsia* based on ompA gene

Hap	Num	CF	EEDS	HLBE	SZWQ
O1	59	33	8	10	8
O2	85	35	13	34	3
O3	26	21	1	2	2
O4	3	3	0	0	0
O5	2	2	0	0	0
O6	2	2	0	0	0
O7	1	1	0	0	0
O8	1	1	0	0	0
O9	1	1	0	0	0
O10	3	3	0	0	0
O11	1	1	0	0	0
O12	1	1	0	0	0
O13	1	1	0	0	0
O14	1	1	0	0	0
O15	1	0	0	0	1
O16	1	0	1	0	1
O17	8	0	0	8	0
O18	5	0	0	5	0
O19	1	0	0	1	0
O20	1	0	0	1	0
O21	1	0	0	1	0
O22	1	0	0	1	0

Table 9 Summary statistics for polymorphisms and neutrality tests of ompA gene from *Rickettsia* in Inner Mongolia

	n	Ht	S	Hd	Pi	Tajima's	Fu's Fs
CF	106	15	11	0.765	0.00394	-0.26745	-3.216
EEDS	23	12	43	0.889	0.00947	-2.21147**	-1.104
HLBE	63	15	365	0.739	0.19544	1.53899	58.425
SZWQ	15	15	57	1	0.02468	-1.48940	-5.059
TOTAL	207	22	349	0.735	0.07308	-1.08797	48.645

Abbreviation: n, number of samples; Ht, number of haplotypes; S, number of polymorphic sites; Hd, haplotype diversity; Pi, nucleotide diversity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 10 F_{ST} values among different groups of *Rickettsia* based on ompA gene

Locality	CF	EEDS	HLBE	SZWQ
CF				
EEDS	0.04464			
HLBE	0.25852	0.13410		
SZWQ	0.01902	0.01996	0.11367	

Table 11 AMOVA of ompA gene of *Rickettsia* population

Source of variation	d.f	Sum of squares	Variance Components	Percentage of variation	Fixation Index F_{ST}
Among population	3	691.719	4.90433Va	21.21763	0.21218
Within population	203	3696.644	18.21007Vb	78.78237	
Total	206	4388.362	23.11439		

Figures

Figure 1

Phylogenetic tree based on gltA gene of *Rickettsia raoultii*

Figure 2

Phylogenetic tree based on ompA gene of *Rickettsia raoultii*

a **b** **Figure 3**

a TCS haplotypes network of *Rickettsia raoultii* based on *gltA* gene from four different populations in Inner Mongolia b TCS haplotypes network of *Rickettsia raoultii* based on *ompA* gene from four different populations in Inner Mongolia

a **b** **Figure 4**

a Mismatch distribution analysis for the *Rickettsia raoultii* groups based on *gltA* b Mismatch distribution analysis for the *Rickettsia raoultii* groups based on *ompA*

Supplementary Files

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