Coxiella burnetii is widespread in ticks (Ixodidae) in the Xinjiang border areas of China

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Abstract

Background The gram-negative Coxiella burnetii bacterium is the pathogen that causes Q fever. The bacterium is transmitted to animals via ticks and can cause infection in domestic animals, wild animals, and humans. As the provincial-level administrative region with the largest land area in China, Xinjiang has many endemic tick species; however, the distribution of C. burnetii in ticks in Xinjiang border areas has not been studied in detail.

Results For the current study, 1507 ticks were collected from livestock at 22 sampling sites in ten border regions of the Xinjiang Uygur Autonomous region from 2018 to 2019. C. burnetii was detected in 205/348 (58.91%) Dermacentor nuttalli ; in 110/146 (75.34%) Dermacentor pavlovskyi ; in 66/80 (82.50%) Dermacentor silvarum ; in 15/32 (46.90%) Dermacentor niveus ; in 28/132 (21.21%) Hyalomma rufipes ; in 24/25 (96.00%) Hyalomma anatolicum ; in 219/312 (70.19%) Hyalomma asiaticum ; in 252/338 (74.56%) Rhipicephalus sanguineus ; and in 54/92 (58.70%) Haemaphysalis punctata . Among these samples, C. burnetii was detected in D. pavlovskyi for the first time. The infection rate of R. sanguineus was 74.56% (252/338), which was the highest among the four tick genera sampled, whereas the infection rate of H. anatolicum was 96% (24/25), which was the highest among the nine tick species sampled. A sequence analysis indicated that 63 16S rRNA sequences could be found in four newly established genotypes: CXJ-1 (n = 18), CXJ-2 (n = 33), CXJ-3 (n = 6), and CXJ-4 (n = 6).

Conclusions This study indicates that CXJ-2 might represent the main C. burnetii genotype in the ticks in Xinjiang because it was detected in eight of the tick species studied. The high infection rate of C. burnetii detected in the ticks found in domestic animals may indicate a high likelihood of Q fever infection in both domestic animals and humans.

Introduction

Coxiella burnetii, an obligate gram-negative intracellular bacterium, can cause Q fever disease in humans, survive in the environment for long periods of time, and is often found in the phagolysosome of infected mammalian cells[1, 2]. Given its impact on global public health, it has attracted significant attention for research pur poses[3]. In humans, infection with C. burnetii causes acute symptoms that include vomiting, headache, pneumonia, fever, and hepatitis, as well as chronic symptoms related to hepatitis, osteomyelitis, endocarditis, and intravascular infection[4, 5]. In animals, infection with C. burnetii causes various reproductive problems, including delivery of weak offspring, infertility, postpartum metritis, stillbirth, and abortion[6].

Q fever was first detected in workers at a slaughterhouse in Brisbane, Australia, in 1935 by E.H. Derrick, who named the illness “question fever”[7]. It has also been reported in humans in other countries, including Great Britain, the Netherlands, Spain, Germany, and Switzerland[8–12]. It was first reported to be in China during the 1950s, with C. burnetii antibodies being reported in humans from 32 prefectures in 15 provinces of China[13]. Slaughterhouse workers, veterinarians, and farmers are currently at high risk of contracting this relatively rare zoonotic disease[14].

Ticks are widely distributed around the world and are among the most important vectors of human disease, second only to mosquitoes; they are also the main carrier of pathogens in wild animals and livestock[15]. C. burnetii maintains a symbiotic relationship with ticks and can infect ticks at all tick life stages[16, 17]. It has been isolated from more than 40 species of hard ticks and at least 14 species of soft ticks, indicating the importance of ticks in its transmission[18]. Animals become infected with C. burnetii via tick bites, whereas humans become infected mainly via contact with tick excreta[19]. Although the direct transmission of C. burnetii to humans through tick bites has not been reported in detail[20], C. burnetii has been reported in the milk, birth products, faeces, and urine of the infected animals, to which humans can be exposed and thus become infected with C. burnetii[1].

In the current study, molecular biological methods were used to detect Q fever in tick species collected from the border area of Xinjiang, China, to reveal the species and pathogen-carrying status of the ticks in this region, to analyse the cross-border spread of Q fever, to assess risk for human and livestock infections and to provide basic information on the development of effective prevention and control measures for this important tick-borne disease.

Materials And Methods

Sample collection and morphological identification of the ticks

A total of 1507 ticks were collected from cattle and sheep at 22 sampling sites in ten border regions of Xinjiang, China from 2018 to 2019 (Fig. 1). The collected samples were stored in 50-mL centrifuge tubes and taken to the laboratory. The ticks were identified based on morphological criteria following the descriptions provided by D eng GF[21].

DNA extraction from tick samples

Tick samples were placed in 50-mL sterilization centrifuge tubes and washed individually twice with 75% ethanol, followed by ddH2O rinsing until the liquid was clear. For each sample, DNA was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, and the extracted DNA was stored at −20°C.

PCR amplification and sequencing

As described in previous studies[22-25], primers were designed with conserved regions of the *C. burnetii* IS1111 and 16S rRNA gene sequences (Table 1); the expected product from the *C. burnetii* primers used for IS1111 amplification was 517 bp, and for the 16S rRNA primers, it was 592 bp. The 25 μL of PCR mixture comprised 2 μL of DNA sample, 12.5 μL of DreamTaq Green PCR Master Mix (2×) (Thermo Fisher Scientific, Lithuania, MA, USA), 8.5 μL of ddH2O, and 1 μL of 10 μM forward primer and 10 μM reverse primer (TSINGKE Biotech, Xian, China). A negative control was prepared with double-distilled water. Finally, the 25 μL reaction mixture was subjected to PCR under the following conditions: degeneration at 95°C for 5 min, 95°C for 30 s, 55°C for 30 s, and 35 cycles of

72°C for 1 min, and the final step at 72°C for 5 min. Next, 5 μL of the PCR products was subjected to 1.5% agarose gel electrophoresis and visualized after being stained with Goldview; three positive samples (IS1111 gene) from each sampling site were selected for the amplification of *C. burnetii* 16S rRNA sequences.

Phylogenetic analysis

The sequenced 16S rRNA nucleotide sequence was aligned with the reference sequence from GenBank (www.ncbi.nlm.nih.gov/nucleotide/) using SeqMan (www.dnastar.com/); correct calibration was performed after manual adjustment as needed. Phylogeny was inferred via a neighbour-joining analysis of the 16S rRNA gene sequences based on distances as calculated with the Kimura 2-parameter model using Mega 7.0 (www.megasoftware.net/). The confidence values for each branch of the phylogenetic tree were determined by using 1000 repeat analyses.

Results

In total, 1507 tick samples were collected from livestock in different regions of the Xinjiang border; the samples belonged to one family (Ixodidae), four genera (606 Dermacentor, 471 Hyalomma, 338 Rhipicephalus, and 92 Haemaphysalis), and ten species (348 D. nuttalli, 146 D. pavlovskyi, 80 D. silvarum, 32 D. niveus, 132 H. rufipes, 2 H. scupense, 25 H. anatolicum, 312 H. asiaticum, 338 R. sanguineus, and 92 H. punctata).

In this study, the tick samples were analysed to detect C. burnetii. The IS1111 DNA of C. burnetii was detected in 973 (973/1507) DNA samples in the following proportions: D. nuttalli, 205 (58.91%); D. pavlovskyi, 110 (75.34%); D. silvarum, 66 (82.50%); D. niveus, 15 (46.90%); H. scupense, 0 (0.00%); H. rufipes, 28 (21%); H. anatolicum, 24 (96.00%); H. asiaticum, 219 (70.19%); H. punctata, 54 (58.70%); and R. sanguineus 252 (74.56%) (Table 2). Representing each sampling site, three of the 16S rRNA amplification samples were selected for sequencing form positive IS1111 amplification samples. After multisequence alignment, 63 16S rRNA gene sequences were obtained and characterized into four genotypes: CXJ-1 (n = 18), CXJ-2 (n = 33), CXJ-3 (n = 6), and CXJ-4 (n = 6).

Based on the 16S rRNA gene sequence analysis, the CXJ-1 (n = 18) genotype shared 99.5% and 97.1% homology with the C. burnetii in R. sanguineus from India (MG050151.1) and the C. burnetii in A ra ararauna from the USA (KX611832.1), respectively; the CXJ-2 (n = 33) genotype shared 99.3% and 99.5% homology with the C. bur netii in D. steini from Malaysia (LT009435.1) and the C. burnetii in a human (a patient with Q fever endocarditis) from Denmark (FJ787329.1), respectively; the CXJ-3 (n = 6) genotype shared 98.6% and 97.6% homology with the C. burnetii in H. punctata from England (KP994816.1) and the C. burnetii in H. tibetensis from China (KU758902.1), respectively; and the CXJ-4 (n = 6) genotype shared 99.2% and 99.0% homology with the C. burnetii in D. silvarum from China (KP994814.1) and the C. burnetii in D. silvarum from Russia (KM079618.1), respectively. The CXJ-1, CXJ-2, CXJ-3 and CXJ-4 shared 96.6%, 99.5%, 96.3% and 96.6% homology with the C. burnetii in a human (a patient with Q fever endocarditis) from Denmark (FJ787329.1) (Table 3).

As shown by the phylogenetic analysis, three of the newly characterized genotypes identified (CXJ-1, CXJ-3, and CXJ-4) clearly belonged in group A (represented by the genus Rhipicephalus of the Ixodidae family), whereas the CXJ-2 genotype clearly belonged in group B (including the Ornithodoros genus of the Argasidae family and the Dermacentor genus of the Ixodidae family) (Fig. 2).

The CXJ-1 genotype is closely related to that of the C. burnetii in R. sanguineus from India (MG050151.1), Brazil (KP994844.1), Marshall Islands (HQ116458.1), and Japan (D84559.1); that of the C. burnetii in Rhipicephalus sp. from Cote d'Ivoire (KP994850.1); and that of the C. burnetii in R. turanicus from Israel (JQ480822.1) (100% bootstrap support) (Fig. 2). The CXJ-2 genotype was closely related to that of the C. burnetii in D. steini from Malaysia (LT009435.1); that of the C. burnetii in a human from Denmark (FJ787329.1); that of the C. burnetii in O. capensis from Chile (KJ459063.1), Tunisia (KJ459070.1), and Spain (KJ459072 and KJ459073.1); that of the C. burnetii in O. maritimus from Tunisia (KP994782.1) and Spain (KP994784.1); and that of the C. burnetii in O. spheniscus from Chile (KP994800.1) (94% bootstrap support) (Fig. 2).

Discussion

As the second largest group of vectors in the world, ticks are hosts to pathogens of a variety of important zoonoses[26–28], such as Forest encephalitis, Q fever, Lyme disease, Spot fever, tularemia, and babesiosis [29, 30]. In recent years, new tick-borne diseases, such as human granulocytic anaplasmosis (HGA), severe fever with thrombocytopenia syndrome (SFTS), and Guertu virus (GTV), have been reported[31, 32]. Ticks transmit pathogens primarily by biting the host[33, 34] but also by aerosol transmission (C. burnetii)[5]. Previous reports indicated that ticks are widely distributed in China, with 42 species of ticks from nine genera reported in Xinjiang alone, accounting for more than one-third of the total tick species in China[35–37]. I. persulcatus, D. nuttalli, H. asiaticum, D. marginatus, and D. niveus are the dominant tick species in Xinjiang[38], and their wide distribution has a significant impact on the development of animal husbandry and public health.

Of the 1507 tick samples collected for the current study, 64.57% (973/1507) contained C. burnetii IS1111 DNA. C. burnetii was detected in D. nuttalli (58.91%), D. pavlovskyi (75.34%), D. silvarum (82.50%), D. niveus (46.90%), H. rufipes (21.21%), H. anatolicum (96.00%), H. asiaticum (70.19%), R. sanguineus (74.56%), and H. punctata (58.70%). We did not detect C. burnetii in H. scupense (0/2; 0.00%), an outcome that may be related to the small sample size (2 samples of H. scupense). In addition, the presence of C. burnetii in D. pavlovskyi was first reported here. The high infection rate of C. burnetii in D. silvarum, H. asiaticum, and R. sanguineus seems to be related to the symbiosis and vertical transmission between them[39, 40]. Although there is no related article reporting the existence of symbiotic and vertical propagation of C. burnetii in D. nuttalli, D. pavlovskyi, D. niveus, H. rufipes, H. anatolicum, or H. punctata, the findings lead us to suspect that C. burnetii has these relationships in these ticks, particularly because some reports have shown that C. burnetii has a high infection rate and vertical transmission relationship among some ticks[41–44]. Confirmation of our hypothesis requires more literature support and research to prove; here we are merely stating the supposition. Overall, our results indicate that C. burnetii is widespread in the border areas of Xinjiang.

Phylogenetic analyses have indicated the presence of C. burnetii worldwide, including in the environment and in both vertebrate and invertebrate hosts (Fig. 2). C. burnetii from different genera (Rhipicephalus and Ornithodoros) form different branches: A and B. CXJ-1 (from R. sanguineus and H. asiaticum), CXJ-3 (from H. punctata), and CXJ-4 (from D. nuttalli) clearly belong to group A, whereas CXJ-2 (from D. nuttalli, D. pavlovskyi, D. silvarum, D. niveus, H. rufipes, H. anatolicum, H. asiaticum, and R. sanguineus) clearly belong to group B, suggesting that there is a correlation between C. burnetii from different tick species. Notably, C. burnetii isolated from ticks in Iceland (KP994860.1), Morocco (KP994851.1), and France (KP994857.1) were highly correlated. Such diversity of C. burnetii among tick species suggests that this microbiome had a role in both maternal inheritance and horizontal transmission during evolution.

In this study, 16S rRNA assays were used to detect previous unknown genotypes (CXJ-1, CXJ-2, CXJ-3, and CXJ-4) of C. burnetii from nine species of tick: D. nuttalli, D. pavlovskyi, D. silvarum, D. niveus, H. rufipes, H. anatolicum, H. asiaticum, R. sanguineus, and H. punctata; however, CXJ-1 was detected only in R. sanguineus and H. asiaticum; CXJ-3 was detected only in H. punctata; and CXJ-4 was detected only in D. nuttalli, whereas CXJ-2 was detected in D. nuttalli, D. pavlovskyi, D. silvarum, D. niveus, H. rufipes, H. anatolicum, H. asiaticum, and R. sanguineus. Thus, these results suggest that CXJ-2 is the main genotype in ticks in Xinjiang. More importantly, CXJ-2 showed 99.5% identity with the C. burnetii in the human from Denmark (FJ787329.1), suggesting that C. burnetii infection may be related to ticks. There have been few reports of the direct transmission of C. burnetii to humans through ticks[20]. However, C. burnetii has been reported in the milk, birth products, faeces, and urine of infected animals, to which humans can be exposed and thus be infected[1]. At particularly high risk are veterinary personnel, stockyard workers, farmers, hide tannery workers and others who work closely with animals. Sporadic cases of C. burnetii in humans are reported each year, although occasionally there are large outbreaks in humans[45, 46]. For example, between 2007 and 2011, a Q fever pandemic occurred in The Netherlands, affecting 4107 people and causing the death of > 50 000 dairy goats. It was thought that most of these infected people developed Q fever by inhaling air in which C. burnetii had been released during the birthing season of both goats and sheep (February–May)[9, 47]. Similarly, C. burnetii has been detected in cattle, goats, dogs, pigs, mice, and ticks from China through serological and molecular biology methods[48].

In summary, this study reports, for the first time, the Q fever infection in ticks in the border areas of Xinjiang, China, indicating that the abundant tick species and high infection rates of C. burnetii in the border areas of Xinjiang pose potential threats to domestic animals and humans. Xinjiang, located in northwest China, is bordered by Pakistan, Tajikistan, Mongolia, Kyrgyzstan, India, Afghanistan, Russia and Kazakhstan. Ticks are widely distributed in the wild animals and domestic animals across the region, providing increased opportunities for cross-border transmission of C. burnetii as global trade intensifies. Thus, there is a need for farmers to adhere to livestock testing and to implement tick control strategies.

Conclusions

This study confirmed, for the first time, that C. burnetii is widely distributed in ticks in Xinjiang, China, indicating that domestic animals and humans in this region are at risk of being infected with C. burnetii. Therefore, there is a need for further research on C. burnetii cross-border transmission via ticks. More importantly, there is a need to monitor domestic animals and humans in the region and to eradicate ticks in the region to the greatest extent possible to ensure animal and public safety.

Declarations

Additional file

Additional file: **Table 1.** PCR primers used to detect DNA extracted from the ticks taken from Xinjiang. **Table 2.** Tick species and the PCR results of *C. burnetii* from the Xinjiang samples. **Table 3.** Comparison of 16S rRNA sequences based on the four genotypes (CXJ-1, CXJ-2, CXJ-3, and CXJ-4) and *C. burnetii* FJ787329.1. **Fig. 1.** Locations of the sample sites for tick collection in the border areas of Xinjiang (different locations are coded by colour; A–V indicate the sampling points). **Fig. 2.** Phylogenetic relationships of the CXJ-1, CXJ-2, CXJ-3, and CXJ-4 genotypes identified in the current study with other *C. burnetii* samples. The phylogeny was inferred via a neighbour-joining analysis of the 16S rRNA gene sequences based on distances calculated with the Kimura 2-parameter model. *Mycoplasma gallinarum* (L24105.1) and *Mycoplasma agalactiae* (M24290.2) were used as the outgroups. Bootstrap values from 1000 replicates are shown on the nodes. The genotypes detected in this study are shown as triangles.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Authors' contributions

JN, XX, QR and GL performed experiments and revised the manuscript. JN, HL, and JL participated in sample collection. JN, XX, ZC and YT performed data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experiments were carried out in accordance with the guidelines approved by the Science and Technology Department of Gansu province, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. PCR primers used to detect DNA extracted from the ticks taken from Xinjiang.

Primers	Target gene	Primer sequence (5′→ 3′)	Annealing temp(°C)	Target fragment(bp)	Reference sequence
F	IS1111	GTGATCTACACGAGACGGGTT	55	517	M80806.1, KT391016.1, KT391020.1, KT391019.1, KT391018.1, KT391017.1, KT954146.1, KT391015.1, KT391014.1, KT391013.1, EU430257.1
R		CGTAATCACCAATCGCTTCGT			
16S-Fw	16S rRNA	TCGGTGGHGAAGAAATTCTC	55	592	KP994776.1, GU797243.1, KP994812.1, KP994826.1, KP994854.1, D89792.1, NR_104916.1, FJ787329.1, HM208383.1, AY342037.1, MH769217.1, MK182891.1
16S-Rv		AGGCACCAARTCATYTCTGACAAG			

Table 2. Tick species and the PCR results of *C. burnetii* from the Xinjiang samples.

Family	Genus	Species	No. positive/No. examined	Infection rate (%)	95% CI	No. positive/No. examined	Infection rate (%)	95% CI
Ixodidae	*Dermacentor*	*D. nuttalli*	205/348	58.91	53.74–64.08	396/606	65.35	61.56–69.14
		D. pavlovskyi	110/146	75.34	68.35–82.33			
		D. silvarum	66/80	82.50	74.17–90.83			
		D. niveus	15/32	46.90	29.58–64.17			
	Hyalomma	*H. scupense*	0/2	-	-	271/471	57.54	53.07–62.00
		H. rufipes	28/132	21.21	14.24–28.19			
		H.anatolicum	24/25	96.00	88.32–103.68			
		H. asiaticum	219/312	70.19	65.12–75.27			
	Haemaphysalis	*H.punctata*	54/92	58.70	48.63–68.76	54/92	58.70	48.63–68.76
	Rhipicephalus	*R. sanguineus*	252/338	74.56	69.91–79.20	252/338	74.56	69.91–79.20
	Total		973/1507	64.57	62.15–66.98	973/1507	64.57	62.15–66.98

Table 3. Comparison of 16S rRNA sequences based on the four genotypes (CXJ-1, CXJ-2, CXJ-3, and CXJ-4) and *C. burnetii* FJ787329.1.

410	433	434	440	445	446	448	462	535	549	564	588	592	599	604	606	609	618	620	622	626	634	673	686	692	707	717	718
T	G	G	T	-	-	G	C	C	C	A	G	T	G	T	G	C	G	G	A	T	G	G	A	C	A	C	C
*	*	A	C	T	T	A	*	*	*	*	A	*	*	*	A	T	A	*	*	C	A	*	*	T	G	G	*
*	*	*	*	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	A	T	C	A	A	-	T	T	*	*	*	C	A	*	*	T	*	*	*	C	*	*	G	T	G	*	T
*	*	C	C	-	-	*	*	*	T	G	*	*	A	C	*	T	*	A	G	C	*	A	*	*	G	*	T

FJ787329.1:Human (a patient with Q fever endocarditis) , Denmark * indicates the same as the reference sequence, - i

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