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Prevalence of *Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp. in *Ornithodoros lahorensis* from southern Xinjiang, China*

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Summary

Ticks are important vectors for pathogens and transmit a variety of diseases in humans and animals. Numerous studies have reported the presence of Ixodidae ticks and tick-borne pathogens in southern Xinjiang. However, data pertaining to argasid ticks and argasid tick-associated pathogens are sparse. This study collected a total of 407 ticks from southern Xinjiang. The tick species were identified on the basis of their morphological and mitochondrial 16S ribosomal DNA (16S rDNA) characteristics. The citrate synthase gene (*gltA*), 17-kilodalton antigen gene (*17-kDa*), and outer membrane protein A and B genes (*ompA*, *ompB*) were amplified using four sets of primers. The species of rickettsiae were further identified on the basis of concatenated sequences. To detect *Anaplasma* and *Ehrlichia*, nested PCR was used to amplify the *Ehrlichia* and *Anaplasma* 16S rRNA genes, respectively. All 407 ticks were *Ornithodoros lahorensis.* A total of 109 *Rickettsia*-positive samples and 19 *Anaplasma*-positive samples were detected in DNA samples from the 407 ticks. *R. sibirica*, *R. massiliae*, *Candidatus* R. barbariae, *A. ovis*, *A. marginale,* and *A. phagocytophilum* were found. All DNA samples from *Or. lahorensis* were tested for the presence of the *Ehrlichia* spp. nucleotide, but none of the samples were positive for such pathogens. This is the first time that *R. sibirica*, *R. massiliae,* and *A. marginale* were detected in *Or. lahorensis* from southern Xinjiang.

Keywords: ***Ornithodoros lahorensis*, southern Xinjiang, *Anaplasma*, *Rickettsia***

Ticks are ectoparasites. They belong to the phylum Arthropoda and are grouped under the order Acarina in the class Arachnida. They can parasitize humans, domestic animals, such as cattle, sheep, and camels, as well as wild animals. Tick larvae hatch from eggs and gradually develop into nymphs and adults. Each stage of the tick's life depends on feeding on its host's blood (20). Ticks also carry and transmit a variety of pathogenic bacteria, which cause sickness in humans and animals (2). There have been an increasing number of published studies on ticks because of growing public health concerns worldwide. Ticks are classified as Ixodidae (hard ticks), Argasidae (soft ticks), or Nuttalliellidae. Approximately 219 species of soft ticks have been identified worldwide (24). In 2019, Sun et al. discovered a new soft tick, *Ornithodoros huajianensis*, in Gansu (28). By the end of 2021, at least 15 species of soft ticks, including nine that bite humans, had been found in China (4). Few studies, however, have investigated soft ticks in China.

Xinjiang is the frontier of China, with a vast territory that borders on eight countries, including Russia, Pakistan, and Mongolia. It is one of the five major

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pastoral areas in China, where mostly cattle and sheep are raised. Xinjiang has a complex geography and diverse ecological environment, making it an ideal habitat for ticks. Forty-five tick species have been found in Xinjiang, accounting for about one-third of all tick species in China. The identified ticks include two genera and seven species of soft ticks, namely *Or. lahorensis*, *Or. papillipes*, *Or. tartakovskyi*, *Argas japonicus*, *Ar. vespertilionis*, *Ar. persicus*, and *Ar. reflexus* (39).

Ticks are the second-largest vector of pathogen transmission (22), and there have been frequent case reports of tick-borne diseases (TBDs) around the world in recent years. TBDs not only cause severe damage to human and animal health, but also affect the development of animal husbandry. Therefore, the dangers of TBDs cannot be ignored. Lyme borreliosis, tick-borne encephalitis, Q-fever, and North-Asia tick-borne spotted fever are relatively common TBDs in China (33). Over the past 40 years, many emerging TBDs have been reported worldwide (7), and the incidence of TBDs is increasing (5). Governments and relevant departments need to strengthen the monitoring of TBDs and continuously improve their knowledge to formulate effective control measures.

Microorganisms belonging to the genus *Rickettsia* are obligate intracellular gram-negative bacteria that can inhabit a variety of hosts (12), including arthropods (such as ticks, mites, and fleas) and vertebrates (such as humans, livestock, and wild animals). Currently, the four biotypes under the genus *Rickettsia* are the ancestral group (AG), the transitional group (TRG), the spotted fever group (SFG), and the typhus group (TG) (21). The SFG is the dominant group in China, comprising more than 10 *Rickettsia* species (9, 31), five of which are confirmed to be pathogenic to humans (16, 27).

The genus *Anaplasma* (Rickettsiales: Anaplasmataceae) includes *A. bovis*, *A. ovis*, and *A. centrale*, among others. *A. ovis* is an intraerythrocytic rickettsial pathogen that causes fever, progressive anemia, icterus, weight loss, and decreased milk production in the host. In severe cases, it can even lead to the death of the host (3, 15, 34). *A. phagocytophilum* is the causative agent in human granulocytic anaplasmosis (HGA) (38). Until 2001, the disease was named human granulocytic ehrlichiosis (HGE), as it was caused by the *Ehrlichia* species (18, 36).

Bacteria belonging to the genus *Ehrlichia* are grouped under the family *Anaplasmataceae* of the order Rickettsiales. They may cause symptoms such as fever, loss of appetite, anemia, and muscle pain (6). In 1935, the first *Ehrlichia* species was discovered in a dog from Algeria, and was later named *Ehrlichia canis* (29). In 1986, the world's first case of human monocytic ehrlichiosis (HME) was identified in the United States; the pathogen was isolated in 1991 and named *E. chaffeensis* (1).

The complex relationships between ticks, livestock, and humans may promote the spread of TBDs. In this study, species of soft ticks dwelling on the body surfaces of livestock and the corresponding tick-borne pathogens were investigated in several areas of southern Xinjiang. The data may facilitate the surveillance, prevention, and control of TBDs in southern Xinjiang.

Material and methods

Tick sampling and identification. Argasid ticks parasitizing sheep were collected in southern Xinjiang, including Kashi, Akesu, Yuli, Hetian, and Atushi, from 2019 to 2021. A single sample was collected in Yuli and Atushi, each, while two or more samples were obtained from the other areas (Tab. 1, Fig. 1). Samples were collected at approximately one-month intervals from March to May over three years. Ultimately, a total of 407 *Or. lahorensis* samples were col-

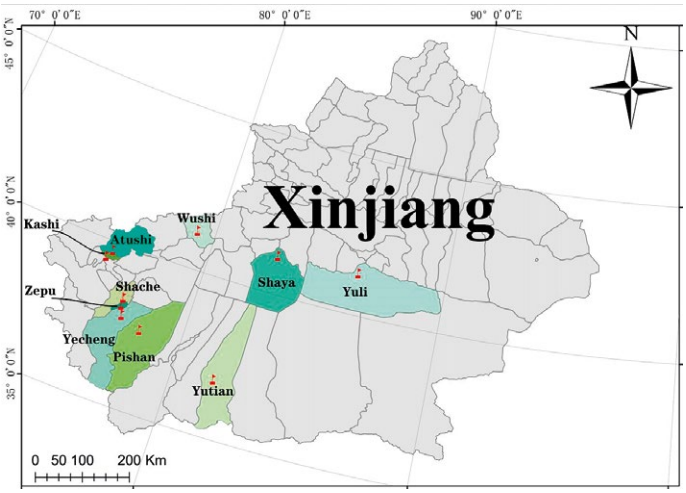

Fig. 1. Map of sample collection sites. A. The ten sampling sites are marked in different colors on the map of Xinjiang. B. Red flags are used to indicate where the samples were collected

Tab. 1. Infection rates for soft tick pathogens in specific areas of southern Xinjiang between 2019 and 2021

Cities	Points	N (2019 + 2020 + 2021)	Sex (♂/♀)	Positive for *Rickettsia* spp. N (%)	Positive for *Anaplasma* spp. N (%)
Atushi	Atushi	48 (16 + 21 + 11)	25/23	0	5 (10.4)
Kashi	Kashi	91 (47 + 25 + 19)	50/41	71 (78.0)	0
	Zepu	35 (14 + 9 + 12)	16/19	0	6 (17.1)
	Shache	24 (11 + 5 + 8)	11/13	0	2 (8.3)
	Yecheng	52 (19 + 23 + 10)	32/20	12 (23.1)	0
Hetian	Wushi	54 (23 + 18 + 13)	29/25	26 (48.1)	0
	Yutian	22 (13 + 6 + 3)	7/15	0	0
	Pishan	35 (16 + 11 + 8)	17/18	0	0
Yuli	Yuli	21 (11 + 6 + 4)	11/10	0	0
Akesu	Shaya	25 (15 + 7 + 3)	14/11	0	6 (24.0)
Total		407	212/195	109 (26.8)	19 (4.7)

lected. The host and the living habitat were not exposed to acaricides before the samples were collected. Adult argasid ticks were collected from the body surfaces of adult hosts. Detailed data on the samples were collected, including the area, time, location, and the number of samples collected. Each sample was numbered. All samples were preserved in 75% ethanol. All ticks were identified based on their morphological characteristics, including the dorsal integument, anus, stigmatic shield, genital plate, claws, capitulum, and legs, as previously reported (8). A total of 50 ticks were collected for molecular taxonomic analysis by selecting 10 ticks from each sampling area. The genomic DNA of all samples was extracted using a TIANamp Genomic DNA Kit (TIANGEN Corporation, Beijing, China) in accordance with the manufacturer's instructions. The resulting DNA samples were stored at –80°C for subsequent analysis. Partial mitochondrial (16S rDNA) gene sequences were used to analyze the DNA extracts of the 50 ticks selected. The primer sequences and reaction conditions are presented in Table 2-1 and Table 2-2.

PCR amplification of tick-associated pathogens. In order to identify the species of pathogens found in the 407 ticks and evaluate their genetic diversity, the *17-kDa*, *gltA*, *ompA*, *ompB*, and 16S rRNA genes were amplified by Polymerase Chain Reaction (PCR) using the corresponding primers. The reaction system included 2 × Taq PCR Master Mix 12.5 μL, 1 μL forward and reverse primers, and 2 μL DNA template. The volume of the reaction mixture was then made up to 25 μL with nuclease-free sterile distilled water. Nested PCR was performed using the templates of the second round as the primary PCR products and components similar to those used in the initial amplification, except that the primers were different. The primer sequences and reaction conditions are presented in Table 2-1 and Table 2-2. To avoid false-positive results, the PCR was conducted at least twice with nuclease-free sterile distilled water as the negative control.

The PCR amplification products were assessed via electrophoresis on 1.0% agarose gel. After decontamination with a TIANgel Midi Decontamination Unit (TIAN-GEN Corporation, Beijing, China), the PCR products were cloned into the pGEM-T Easy vector, and then submitted for sequencing.

Sequencing and phylogenetic analysis. GENEWIZ Inc. (Suzhou, China) conducted the sequencing of positive PCR amplicons. Bidirectional sequencing was used to authenticate and align nucleotide sequences with reference sequences sourced from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). The MEGA 7.0 software (http://www.megasoftware.net/) was used to identify new variant strains of *Rickettsia* spp., *Ehrlichia* spp., and *Anaplasma* spp. Phylogenetic trees were created

Tab. 2-1. PCR primers, target genes, nucleotide sequences, and product sizes

Species	Target gene	Primer	Nucleotide sequences (5'-3')	Product size (bp)	Reference
Tick	*16S rDNA*	16S rDNA-F	CCGGTCTGAACTCAGATCAAGT	460	(34)
		16S rDNA-R	CTGCTCAATGATTTTTTAAATTGCTGTGG		
Rickettsia	*17-kDa*	17KD-W-F	GCTTTACAAAATTCTAAAAACCATATA	434	(35)
		17KD-W-R	TGTCTATCAATTCACAACTTGCCGTT		
		17KD-N-F	GCTCTTGCAACTTCTATGTT		
		17KD-N-R	CATTGTTCGTCAGGTTGGCG		
	gltA	gltA-F	ATGACCAATGAAAATAATAAT	1060	(36)
		gltA-R	ATTGCAAAAAGTACAGTGAACA		
	ompA	ompA-F	ATGGCGAATATTTCTCCAAAA	533	(37)
		ompA-R	AGTGCAGCATTCGCTCCCCCT		
	ompB	ompB-F	TACTTCCGGTTACAGCAAAGT	812	(38)
		ompB-R	AAACAATAATCAAGGTACTGT		
Anaplasma	*16S rRNA*	Eh-out-F	TTGAGAGTTTGATCCTGGCTCAGAACG	389	(39)
		Eh-out-R	CACCTCTACACTAGGAATTCCGCTATC		
		HGA-F	GTCGAACGGATTATTCTTTATAGCTTG		
		HGA-R	TATAGGTACCGTCATTATCTTCCCTAC		
Ehrlichia	*16S rRNA*	Eh-out-F	TTGAGAGTTTGATCCTGGCTCAGAACG	282	(40)
		Eh-out-R	CACCTCTACACTAGGAATTCCGCTATC		
		Eh-gs-F	GTAATACTGTATAATCCCTG		
		Eh-gs-R	GTACCGTCATTATCTTCCCTA		

Tab. 2-2. Amplification conditions (temperature and time) corresponding to each set of primers

Primer	Initial denaturation	Denaturation	Renaturation	Extension	Cycles	Final extension
16S rDNA	94°C/5 min	92°C/30 s	54°C/30 s	72°C/30 s	37	72°C/8 min
17kD-W	95°C/5 min	95°C/1 min	58°C/30 s	72°C/30 s	35	72°C/5 min
17kD-N	95°C/5 min	95°C/30 s	61°C/30 s	72°C/30 s	35	72°C/5 min
gltA	95°C/5 min	95°C/1 min	50°C/1 min	72°C/1 min	35	75°C/8 min
ompA	95°C/5 min	95°C/1 min	50°C/30 s	72°C/30 s	35	72°C/8 min
ompB	95°C/5 min	95°C/30 s	50°C/30 s	68°C/90 s	35	68°C/7 min
HGA	94°C/5 min	92°C/30 s	57°C/30 s	72°C/30 s	30	72°C/8 min
Eh-out	94°C/5 min	92°C/30 s	56°C/30 s	72°C/30 s	40	72°C/8 min
Eh-gs	94°C/5 min	92°C/30 s	57°C/30 s	72°C/30 s	30	72°C/8 min

Tab. 3. All accession numbers provided by the GenBank database

Pathogen	Gene	Accession number
Rickettsia massiliae	*17-kDa*	OM475671
	gltA	OM475668
	ompA	OM475665
	ompB	OM475662
Rickettsia sibirica	*17-kDa*	OM475659
	gltA	OM475656
	ompA	OM475653
	ompB	OM475650
Candidatus Rickettsia barbariae	*17-kDa*	OM475683
	gltA	OM475680
	ompA	OM475677
	ompB	OM475674
Anaplasma ovis	*16S rRNA*	OL826839
Anaplasma marginale	*16S rRNA*	OM065781
Anaplasma phagocytophilum	*16S rRNA*	OL824941, MN795629

using MEGA 7.0. They were built by the maximum-likelihood (ML) method on the basis of a matrix of evolutionary distances estimated using the Kimura 2-parameter model. The confidence interval of the ML was assessed by bootstrap analysis with 1000 replicates. Sequences reported in this article were submitted to NCBI's GenBank database; the sequence names and accession numbers are listed in Table 3.

Results and discussion

Tick collection and morphological identification. A total of 407 adult argasid ticks were collected at 10 sites in five counties and cities in southern Xinjiang between 2019 and 2021. The key points for the morphological identification of the specimens included a wrinkled, densely star-shaped fossa on the body surface, the absence of preanal and postanal transverse grooves, the presence of postanal grooves immediately behind the anus, and the presence of several pairs of irregular depressions on either side of the postanal grooves (Fig. 2). The morphological identification of the specimens revealed 212 male and 195 female argasid ticks of a single species. The 16S rDNA PCR sequencing analysis of 50 tissue samples showed 99% sequence identity with the 16S rDNA sequences of *Or. lahorensis* (KX530874 and KX530876). The results of morphological identification and PCR sequencing were consistent and confirmed that all 407 ticks were *Or. lahorensis*.

Detection of *Rickettsia* spp. Among the 109 positive samples, we detected three spotted-fever group *Rickettsia* species, *R. massiliae*, *R. sibirica*, and *Candidatus* R. barbariae, with positive rates of 23.9% (26/109), 11.0% (12/109), and 65.1% (71/109), respectively. Each of the sequences of *R. massiliae* generated from 26 *Or. lahorensis* obtained from WuShi during

Fig. 2. External morphology of *Ornithodoros lahorensis* (dorsal view on the left, ventral view on the right)

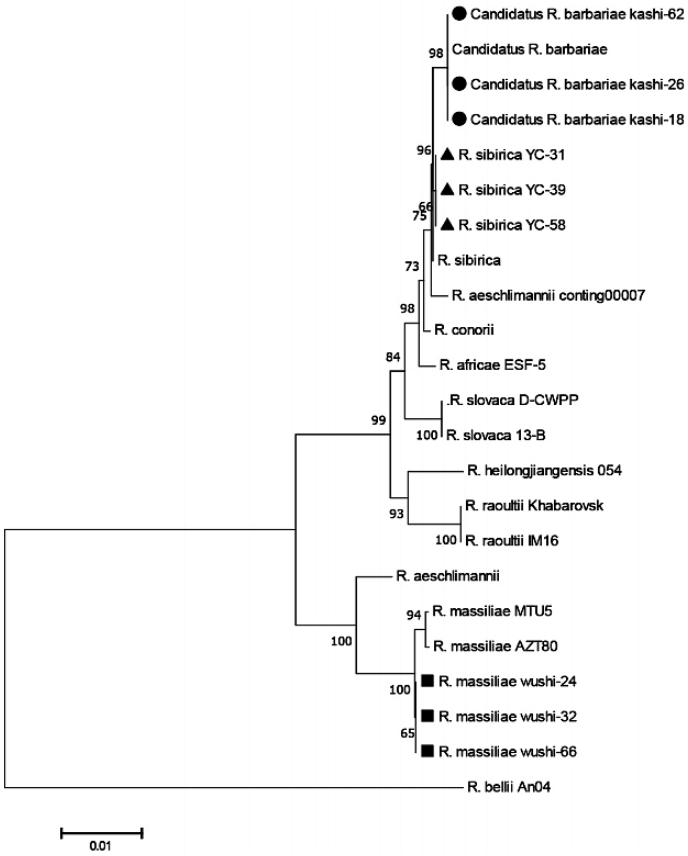

Fig. 3. Phylogenetic analysis of *Rickettsia* species found in ticks collected in the border regions of northwestern China
Explanations: The maximum likelihood (ML) tree was constructed using MEGA7.0 based on concatenated sequence data obtained from the *17KDa-ompA-ompB-gltA* genes, with 1000 bootstrap replicates. The species sequences from the ticks analyzed in this study are denoted by shapes, specifically squares (■), circles (●), and triangles (▲).

these three years were identical to each other. The *17-kDa* sequences showed 99.72% and 99.45% similarity to the *R. massiliae* reference sequences derived from *Rhipicephalus turanicus* in China (KY069262) and the *R. massiliae* MTU5 complete genome in France (CP000683), respectively. The *gltA* sequence was 100% (1178/1178 bp) identical to the corresponding sequence of *R. massiliae* from China (MT309037) and 99.92% (1177/1178) identical to the *R. massiliae*

MTU5 complete genome from France (CP000683). The *ompA* sequence was 100% (533/533 bp) identical to the corresponding sequence of *R. massiliae* from Spain (KR401143). The *ompB* sequence was 100% (533/533 bp) identical to the corresponding sequence of *R. massiliae* from China (MF002502). However, the *ompA* and *ompB* sequences were identical to the *R. massiliae* MTU5 complete genome sequence. Phylogenetic analyses inferred from data concatenation of the four rickettsial gene sequences described above revealed that *R. massiliae* clustered with previous *R. massiliae* MTU5 complete genome sequences and belonged to the spotted fever group (Fig. 3).

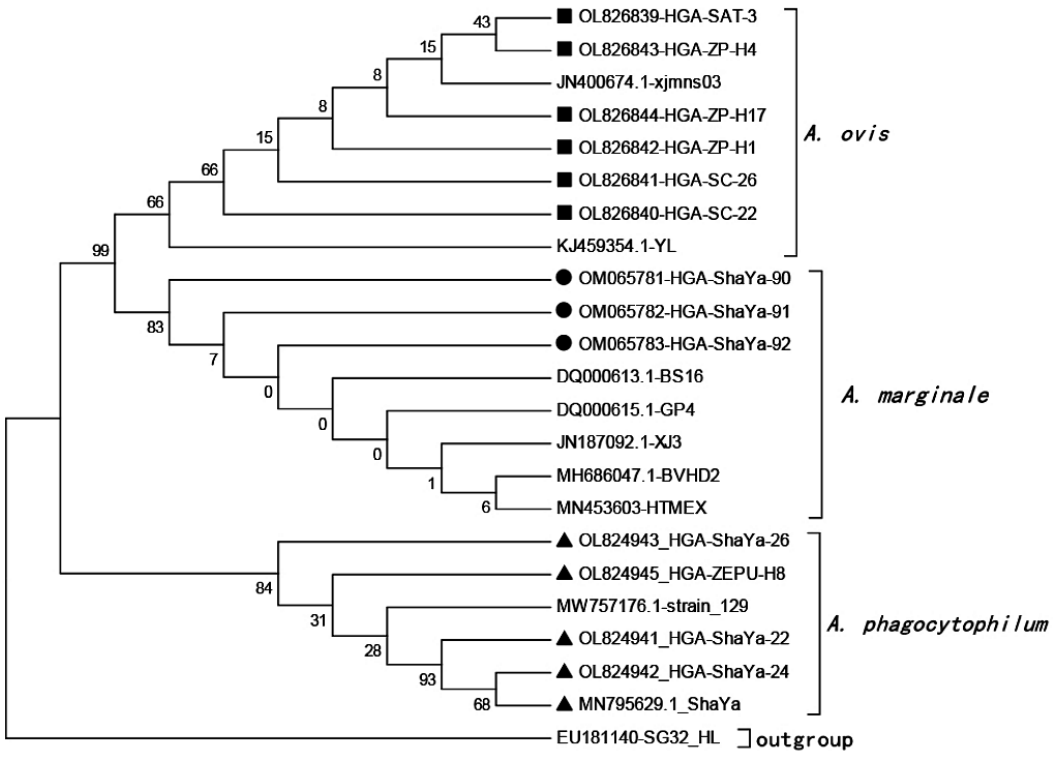

Fig. 4. Phylogenetic analysis of *Anaplasma* species found in ticks collected in southern Xinjiang
Explanations: The maximum likelihood (ML) tree, with 1000 bootstrap replicates, was constructed using concatenated sequence data for the 16S rRNA genes in MEGA7.0. Squares (■), circles (●), and triangles (▲) represent *Anaplasma* species sequences from the ticks investigated in this study.

Twelve of the 52 samples collected in Yecheng were positive, and the positive samples were obtained from two sheep flocks. The sequences of four genes of *R. sibirica* (*17-kDa*, *gltA*, *ompA*, and *ompB*) showed 97.2% to 99.92% similarity to sequences of the reference strains in the GenBank database. Samples positive for *Candidatus* Rickettsia barbariae were collected from *Or. lahorensis* in KaShi. The *ompA* and *ompB* loci of *Candidatus* R. barbariae were identical in each sample, and BLAST analysis showed 100% nucleotide sequence identity to the corresponding sequences of *Candidatus* R. barbariae in the GenBank database. The genes *17-kDa* and *gltA* did not show the same sequences in the BLAST search. Therefore, we downloaded the two sequences of known *17-kDa* from the GenBank database. They showed 98.3% to 100% similarity to the *Candidatus* R. barbariae isolate SHZ (KY069264) and *Candidatus* R. barbariae isolate Alaer-8 (MT309044) reference strains at the nucleotide level. We selected known *gltA* fragments from China, *Candidatus* R. barbariae type II citrate synthase (KT284716) and *Candidatus* R. barbariae isolate SHZ (KY069260) as reference strains for comparison and found that they showed 100% nucleotide sequence identity. A phylogenetic tree comparing the four rickettsial genes with those derived from GenBank is displayed in Figure 3.

Detection of *Anaplasma* spp. Of the 407 samples tested, 4.7% (19/407) were positive for *Anaplasma*. The infection rates for *A. ovis*, *A. marginale*, and *A. phagocytophilum* were 63.1% (12/19), 21.1% (4/19), and 15.8% (3/19), respectively. More importantly, *A. marginale* was found in *Or. lahorensis* in southern Xinjiang for the first time. Sequence comparison revealed that the selected *A. ovis* strains were 97.0-99.5% similar to *A. ovis* isolate Alaer-8 (MT309044). The DNA sequencing results for *A. marginale* isolates identified in *Or. lahorensis* were 99.4% to 100% identical to isolates from different host species in Italy, Vietnam, Mexico, and China. Sequence analysis of the selected *A. phagocytophilum* 16S rRNA amplicons revealed that these isolates exhibited 96.6% to 100% identity with the *Anaplasma* sp. strain 129 (MW757176) in the GenBank database. The phylogenetic tree revealed that the *A. ovis*, *A. marginale*, and *A. phagocytophilum* sequences obtained in this study clustered with *A. ovis*, *A. marginale*, and *A. phagocytophilum* isolates from Genbank (Fig. 4).

Detection of *Ehrlichia* spp. A total of 407 *Or. lahorensis* samples were tested to identify *Ehrlichia* spp. DNA. The results revealed no traces of these pathogens in any of the samples.

Ticks hold significant importance in human and veterinary medicine. All soft ticks identified in this study were *Or. Lahorensis*, and it is hypothesized that this may be the dominant soft tick species in southern Xinjiang. In addition to *Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp. mentioned in this study, these ticks can also carry pathogens implicated in the

Crimean-Congo hemorrhagic fever virus, *Coxiella burnetii*, *Brucella abortus*, and *Francisella tularensis* (8). According to the above study, *Or. lahorensis* is a pathogen vector for many zoonotic pathogens and may pose a high risk of transmission. It is possible for a tick to be co-infected with two or more pathogens. In this study, three *Rickettsia* species and three *Anaplasma* species were molecularly detected among the 407 *Or. lahorensis* ticks sampled from southern Xinjiang, northwestern China. Changes in tick tissues carrying primary infection may prevent secondary infection (19). This is consistent with our experimental results, which showed only a single species of *Rickettsia* in most samples.

The experimental data indicated that *Rickettsia* was the most prevalent pathogen in the sampled areas. *Candidatus* R. barbariae was the predominant species. The prevalence rate of *Anaplasma* was low, and most of the positive DNA samples were identified as *A. ovis*.

The total positive rates of rickettsial pathogens detected by Wang et al. in northeast China in 2019 and Zhao et al. in Xinjiang in 2017 (30, 40) were 41.2% and 41.8%, respectively. These rates are significantly higher than those reported in our study, indicating that rickettsial pathogens are widespread in northern China. The results also suggest that the prevention and disinfestation efforts in Xinjiang have yielded positive outcomes in recent years. However, these studies used relatively few sampling sites. In 2017, Ghafar et al. (11) investigated pathogens carried by Bovine ticks in six districts of Pakistan and reported that the positivity rate for *R. massiliae* was 1.7%. Fernández de Mera et al. (10) investigated the infection of ticks with spotted-fever group *Rickettsia* in Lebanese farms and found that *Candidatus* R. barbariae (10.2%) had the highest positive rate, which is consistent with our results.

The results of the current study show that the total positive rate for *Anaplasma* was 4.7%, including 2.9% for *A. ovis*, 1.0% for *A. marginale*, and 0.7% for *A. phagocytophilum*. In a study by Ghafar et al (11), the positive rates were 1.7% for *A. ovis*, 7.7% for *A. marginale*, and 0.4% for *A. phagocytophilum*. Overall, no significant differences were found between these data and our findings. A study by Yan et al. (34), which examined ticks carrying *Anaplasma* on the body surfaces of goats from four Chinese provinces (Shaanxi, Shanxi, Henan, and Guizhou), found an overall positive rate of 26.68%, and the prevalence rates of 13.21% for *A. ovis* and 1.35% for *A. marginale*. Li et al. (17) studied pathogens carried by domestic ticks in northern Xinjiang and found that 22.4% of the samples were infected with *A. ovis*. In contrast, the overall *Anaplasma* spp. positive rate and the positive rate for *A. ovis* in our study were lower, while that for *A. marginale* was the same. The present study expands our understanding of the distribution of *Anaplasma* areas and the pathogens carried by *Or. lahorensis*. The findings indicate that the role of soft ticks in disease transmission should not be overlooked.

The ticks analyzed in this study had sucked blood before collection, and it is possible that the pathogens may have been acquired from the host blood. Our study found pathogens in soft ticks collected at 10 sites in Xinjiang, but not all ticks from each host tested positive for a specific pathogen. If the pathogen had originated from the host blood, then all ticks from that host should have been positive for the pathogen. However, in examining blood-sucking ticks only, it cannot be concluded with certainty that the pathogen was derived from the tick sample. To confirm whether ticks are vectors of *Rickettsia* spp. and *Anaplasma* spp., it is necessary to collect blood for detection from the corresponding host as well as from fasting ticks. This will be the focus of our future research.

Due to the large area of southern Xinjiang, we faced difficulties in sampling during the study. We had few sampling points, and they were unevenly distributed, which resulted in limited geographical scope. Thus, the final experimental data do not fully reflect the overall situation in southern Xinjiang.

The Tarim River Basin is the largest river basin in China, covering an area of over 1,020,000 km^2 (35), accounting for most of southern Xinjiang. This vast area has a complex topography with many unknown ecological conditions. The Tarim River Basin provides a suitable habitat for argasid ticks.

Xinjiang is the largest province in China, and transportation in the province is extremely challenging. Almost every household in the countryside raises cattle and sheep, but many farmers lack knowledge about epidemics and how to prevent them relevant technologies. Few grassroots service personnel are equipped with basic technologies. Therefore, it is not easy to implement pest control for all cattle and sheep. Xinjiang borders many countries, and its unique geographical location provides an important gateway for China to the world. China conducts massive livestock trade with many countries through Xinjiang's trade ports, which is undoubtedly an important route for the transmission of ticks and tick-borne pathogens. Numerous livestock are frequently transported between Xinjiang and other provinces, which contributes to tick migration and tick-borne pathogen transmission among domestic provinces. The prevention of TBD transmission from abroad into the country is a challenge.

This study provides fundamental evidence of the prevalence of several pathogens, including *Rickettsia* and *Anaplasma* in *Or. lahorensis*. *Or. lahorensis* plays a potential role in transmitting these diseases. Three species of *Rickettsia* and *Anaplasma* each were molecularly detected in 407 *Or. lahorensis*. As far as we know, this is the first time that *R. sibirica*, *R. massiliae*, and *A. marginale* were detected in *Or. lahorensis* in southern Xinjiang. This discovery enriches the basic research data on soft ticks in China.

References

1. *Anderson B., Dawson J., Jones D., Wilson K.*: Ehrlichia chaffeensis, a new species associated with human ehrlichiosis. Journal of clinical microbiology 1992, 29, 2838-2842.
2. *Bartikova P., Holikova V., Kazimirova M., Stibraniova I.*: Tick-borne viruses. Acta Virol. 2017, 61 (4), 413-427.
3. *Berthelsson J., Ramabu S. S., Lysholm S., Aspán A., Wensman J. J.*: Anaplasma ovis infection in goat flocks around Gaborone, Botswana. Comparative Clinical Pathology 2020, 29 (1), 167-172.
4. *Chen Z., Liu J.*: A review of argasid ticks and associated pathogens of China. Front Vet. Sci. 2022, 9, 865664.
5. *Dantas-Torres F., Chomel B. B., Otranto D.*: Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 2012, 28 (10), 437-446.
6. *Diarra A. Z., Kelly P., Davoust B., Parola P.*: Tick-borne diseases of humans and animals in West Africa. Pathogens 2023, 12 (11), 1276.
7. *Estrada-Pena A., Ayllon N., de la Fuente J.*: Impact of climate trends on tick-borne pathogen transmission. Front Physiol. 2012, 3, 64.
8. *Estrada-Peña A., Mihalca A. D., Petney T. N.* (ed.): Ticks of Europe and North Africa: a guide to species identification, Springer, Switzerland 2017.
9. *Fang L. Q., Liu K., Li X. L., Liang S., Yang Y., Yao H. W., Sun R. X., Sun Y., Chen W. J., Zuo S. Q., Ma M. J., Li H., Jiang J. F., Liu W., Yang X. F., Gray G. C., Krause P. J., Cao W. C.*: Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect. Dis. 2015, 15 (12), 1467-1479.
10. *Fernandez de Mera I. G., Blanda V., Torina A., Dabaja M. F., El Romeh A., Cabezas-Cruz A., de la Fuente J.*: Identification and molecular characterization of spotted fever group rickettsiae in ticks collected from farm ruminants in Lebanon. Ticks Tick Borne Dis. 2018, 9 (1), 104-108.
11. *Ghafar A., Cabezas-Cruz A., Galon C., Obregon D., Gasser R. B., Moutailler S., Jabbar A.*: Bovine ticks harbour a diverse array of microorganisms in Pakistan. Parasit. Vectors 2020, 13 (1), 1.
12. *Gillespie J. J., Beier M. S., Rahman M. S., Ammerman N. C., Shallom J. M., Purkayastha A., Sobral B. S., Azad A. F.*: Plasmids and rickettsial evolution: insight from Rickettsia felis. Plos One 2007, 2 (3), e266.
13. *Glatz M., Müllegger R. R., Maurer F., Fingerle V., Achermann Y., Wilske B., Bloemberg G. V.*: Detection of Candidatus Neoehrlichia mikurensis, Borrelia burgdorferi sensu lato genospecies and Anaplasma phagocytophilum in a tick population from Austria. Ticks Tick Borne Dis. 2014, 5 (2), 139-144.
14. *Heise S. R., Elshahed M. S., Little S. E.*: Bacterial diversity in Amblyomma americanum (Acari: Ixodidae) with a focus on members of the genus Rickettsia. J. Med. Entomol. 2010, 47 (2), 258-268.
15. *Jiménez C., Benito A., Arnal J. L., Ortín A., Gómez M., López A., Villanueva-Saz S., Lacasta D.*: Anaplasma ovis in sheep: Experimental infection, vertical transmission and colostral immunity. Small Ruminant Research. 2019, 178, 7-14.
16. *Li H., Cui X. M., Cui N., Yang Z. D., Hu J. G., Fan Y. D., Fan X. J., Zhang L., Zhang P. H., Liu W., Cao W. C.*: Human infection with novel spotted fever group Rickettsia Genotype, China, 2015. Emerg. Infect. Dis. 2016, 22 (12), 2153-2156.
17. *Li Y., Wen X., Li M., Moumouni P. F. A., Galon E. M., Guo Q., Rizk M. A., Liu M., Li J., Ji S., Tumwebaze M. A., Byamukama B., Chahan B., Xuan X.*: Molecular detection of tick-borne pathogens harbored by ticks collected from livestock in the Xinjiang Uygur Autonomous Region, China. Ticks Tick Borne Dis. 2020, 11 (5), 101478.
18. *Liz J. S., Sumner J. W., Pfister K., Brossard M.*: PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (Capreolus capreolus) and chamois (Rupicapra rupicapra). J. Clin. Microbiol. 2002, 40 (3), 892-897.
19. *Macaluso K., Sonenshine D., Ceraul S., Azad A.*: Macaluso KR, Sonenshine DE, Ceraul SM, Azad AF.. Rickettsial infection in Dermacentor variabilis (Acari : Ixodidae) inhibits transovarial transmission of a second Rickettsia. J. Med. Entomol. 2002, 39, 809-813.
20. *Mediannikov O., Fenollar F.*: Looking in ticks for human bacterial pathogens. Microb. Pathog. 2014, 77, 142-148.
21. *Miranda J., Mattar S.*: Molecular detection of Rickettsia bellii and Rickettsia sp. strain Colombianensi in ticks from Cordoba, Colombia. Ticks Tick Borne Dis. 2014, 5 (2), 208-212.
22. *Papa A., Tsioka K., Kontana A., Papadopoulos C., Giadinis N.*: Bacterial pathogens and endosymbionts in ticks. Ticks Tick Borne Dis. 2017, 8 (1), 31-35.
23. *Regnery R. L., Spruill C. L., Plikaytis B. D.*: Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. 1991, 173 (5), 1576-1589.
24. *Rodriguez M. M., Oviedo A., Flores F. S., Castro L. R.*: First report of Ornithodoros puertoricensis (Argasidae) and Amblyomma dissimile (Ixodidae) on Tamandua mexicana (Myrmecophagidae). Systematic and Applied Acarology 2022, 27 (8), 1495-1499.
25. *Roux V., Raoult D.*: Phylogenetic analysis of members of the genus Rickettsia using the gene encoding the outer-membrane protein rOmpB (ompB). Int. J. Syst. Evol. Microbiol. 2000, 50 Pt 4, 1449-1455.
26. *Roux V., Rydkina E., Eremeeva M., Raoult D.*: Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int. J. Syst. Bacteriol. 1997, 47 (2), 252-261.
27. *Shao J. W., Zhang X. L., Li W. J., Huang H. L., Yan J.*: Distribution and molecular characterization of rickettsiae in ticks in Harbin area of Northeastern China. PLoS Negl Trop Dis. 2020, 14 (6), e0008342.
28. *Sun Y., Xu R., Liu Z., Wu M., Qin T.*: Ornithodoros (Ornithodoros) huajianensis sp. nov. (Acari, argasidae), a new tick species from the Mongolian marmot (Marmota bobak sibirica), Gansu province in China. Int. J. Parasitol. Parasites Wildl. 2019, 9, 209-217.
29. *Unver A., Perez M., Orellana N., Huang H., Rikihisa Y.*: Molecular and antigenic comparison of Ehrlichia canis isolates from dogs, ticks, and a human in Venezuela. J. Clin. Microbiol. 2001, 39 (8), 2788-2793.
30. *Wang Q., Pan Y. S., Jiang B. G., Ye R. Z., Chang Q. C., Shao H. Z., Cui X. M., Xu D. L., Li L. F., Wei W., Xia L. Y., Li J., Zhao L., Guo W. B., Zhou Y. H., Jiang J. F., Jia N., Cao W. C.*: Prevalence of multiple tick-borne pathogens in various tick vectors in Northeastern China. Vector Borne Zoonotic Dis. 2021, 21 (3), 162-171.
31. *Wei Q. Q., Guo L. P., Wang A. D., Mu L. M., Zhang K., Chen C. F., Zhang W. J., Wang Y. Z.*: The first detection of Rickettsia aeschlimannii and Rickettsia massiliae in Rhipicephalus turanicus ticks, in northwest China. Parasit. Vectors 2015, 8, 631.
32. *Wen B., Jian R., Zhang Y., Chen R.*: Simultaneous detection of Anaplasma marginale and a new Ehrlichia species closely related to Ehrlichia chaffeensis by sequence analyses of 16S ribosomal DNA in Boophilus microplus ticks from Tibet. J. Clin. Microbiol. 2002, 40 (9), 3286-3290.
33. *Wu X. B., Na R. H., Wei S. S., Zhu J. S., Peng H. J.*: Distribution of tick-borne diseases in China. Parasit Vectors 2013, 6, 119.
34. *Yan Y., Wang K., Cui Y., Zhou Y., Zhao S., Zhang Y., Jian F., Wang R., Zhang L., Ning C.*: Molecular detection and phylogenetic analyses of Anaplasma spp. in Haemaphysalis longicornis from goats in four provinces of China. Sci. Rep. 2021, 11 (1), 14155.
35. *Yaning C., Takeuchi K., Xu C., Chen Y., Xu Z.*: Regional climate change and its effects on river runoff in the Tarim Basin, China. Hydrological Processes 2006, 20, 2207-2216.
36. *Zhang L., Liu Y., Ni D., Li Q., Yu Y., Yu X. J., Wan K., Li D., Liang G., Jiang X., Jing H., Run J., Luan M., Fu X., Zhang J., Yang W., Wang Y., Dumler J. S., Feng Z., Ren J., Xu J.*: Nosocomial transmission of human granulocytic anaplasmosis in China. JAMA 2008, 300 (19), 2263-2270.
37. *Zhang L., Wang G., Liu Q., Chen C., Li J., Long B., Yu H., Zhang Z., He J., Qu Z., Yu J., Liu Y., Dong T., Yao N., Wang Y., Cheng X., Xu J.*: Molecular analysis of Anaplasma phagocytophilum isolated from patients with febrile diseases of unknown etiology in China. Plos One 2013, 8 (2),e57155.
38. *Zhang Y., Chen T., Polimera H., Evans M., Bayerl M. G., George M. R.*: Hemophagocytic Lymphohistiocytosis induced by human granulocytic anaplasmosis: A case report and literature review into the immunopathogenesis. Human Pathology Reports 2022, 27, 300598.
39. *Zhao L., Lin X. M., Li F., Li K. R., He B., Zhang L. Y., Pan J. J., Wang Q. R., Gao J. M., Johnson N., Yuan X. F., Lv J. Z., Wu S. Q., Liu Y. H.*: A survey of argasid ticks and tick-associated pathogens in the Peripheral Oases around Tarim Basin and the first record of Argas japonicus in Xinjiang, China. Plos One 2018, 13 (12), e0208615.
40. *Zhao S., Yang M., Jiang M., Yan B., Zhao S., Yuan W., Wang B., Hornok S., Wang Y.*: Rickettsia raoultii and Rickettsia sibirica in ticks from the long-tailed ground squirrel near the China-Kazakhstan border. Exp. Appl. Acarol. 2019, 77 (3), 425-433.

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