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Anaplasma ovis and *Anaplasma phagocytophilum* Infection in Sheep and Wild Rodents from Northern Xinjiang, Northwest China

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Abstract

The zoonotic rickettsial pathogen *Anaplasma* species have a broad geographical distribution and are important intracellular agents. Domestic animals and wild rodents may play an important role in the epidemiology of this disease. The aim of this study was to estimate the prevalence of infection with *Anaplasma* species among domestic animals and wild rodents in northern Xinjiang Uygur Autonomous Region (XUAR), Northwest China, during 2015-2016. In this study, DNA from *Anaplasma ovis* was detected by nested PCR in blood samples from 21/137 sheep (15.3%) in Ili Kazakh Autonomous Prefecture (Ili), 18/79 sheep (22.8%) in Bole Mongol Autonomous Prefecture (Bole) and 13/71 sheep (18.3%) in Shihezi City. In addition, detection of *Anaplasma phagocytophilum* DNA in spleen samples from great gerbils *(Rhombomys opimus)* in Urumqi (37/356, 10.4%) and Bole (21/134, 15.7%). Interestingly, co-infection *A. ovis* and *A. phagocytophilum* in blood of sheep (9/137, 6.7%) from Ili, northern XUAR. Based on 16S rRNA sequence, phylogenetic analyses showed that *A. ovis* were separated into one clade, while the *A. phagocytophilum* was separated into another clade. This analysis demonstrated that there are at least two different *Anaplasma* species widespread. The present investigation revealed high infection rates of *A. phagocytophilum* and *A. ovis,* which shed light on making effective measures to prevent and control *Anaplasma* species infection in animals in XUAR, Northwest China.

Keywords: *Anaplasma ovis, Anaplasma phagocytophilum, Prevalence, 16S rRNA, Northwest China, Phylogenetic analyzes*

Kuzeybatı Çin ve Kuzey Sincan'da Koyun ve Yabani Kemirgenlerde *Anaplasma ovis* ve *Anaplasma phagocytophilum* Enfeksiyonu

Öz

Zoonotik rickettsial patojen olan *Anaplasma* türleri geniş bir coğrafi dağılıma sahiptir ve önemli hücre içi ajanlardır. Evcil hayvanlar ve vahşi kemirgenler bu hastalığın epidemiyolojisinde önemli rol oynarlar. Bu çalışmanın amacı, 2015-2016 yıllarında Kuzey Sincan Uygur Özerk Bölgesinde (XUAR), evcil hayvanlar ve vahşi kemirgenler arasında *Anaplasma* türleriyle enfeksiyon prevalansının tespitidir. Bu çalışmada, Ili Kazak Özerk Bölgesi'nde (Ili) 21/137 koyun (%15.3), Bole Mongol Özerk Bölgesi'nde (Bole) 18/79 koyun (%22.8) ve Shihezi Şehrinde 13/71 koyun (%18.3) kan örneğinde yuvalanmış PCR yöntemi ile *Anaplazma ovis* DNA'sı tespit edildi. Ek olarak, Urumçi'deki (37/356, %10.4) ve Bole'deki (21/134, %15.7) büyük rodentlerin *(Rhombomys opimus)* dalak örneklerinde *Anaplasma phagocytophilum* DNA'sı belirlendi. İlginç biçimde, Ili, kuzey XUAR'dan alınan koyunların kanında (9/137, %6.7) *A. ovis* ve *A. phagocytophilum* ile eş zamanlı enfeksiyon mevcuttu. 16S rRNA sekansına dayanarak yapılan filogenetik analizler, *A. ovis*'in bir kola ayrıldığını, *A. phagocytophilum*'un ise başka bir kola ayrıldığını gösterdi. Bu analiz bu bölgelerde en az iki farklı *Anaplasma* türünün yaygın olduğunu göstermiştir. Mevcut araştırma, XUAR, Kuzeybatı Çin'deki hayvanlarda *A. phagocytophilum* ve *A. ovis* enfeksiyon oranlarının yüksek olduğunu ve *Anaplasma* türlerinin enfeksiyonunu önlemek ve kontrol altına almak için etkili önlemler alınmasının gerekliliğini ortaya koydu.

Anahtar sözcükler: *Anaplasma ovis, Anaplasma phagocytophilum, Prevalans, 16S rRNA, Kuzeybatı Çin, Filogenetik analiz*

INTRODUCTION

Xinjiang Uygur Autonomous Region (XUAR) covers 1.66 million square kilometers, which is located in the hinterland of the Eurasian continent is geographically divided into two parts by Tianshan Mountain, namely northern XUAR and southern XUAR [1]. In northern XUAR, which is composed of the desert, Gobi desert, saline beach and

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patched oases. There is a great diversity of tick species in XUAR, owing to the variability of geographical landscape and the availability of multiple vertebrate host species for these parasites. Ticks were reported to transmit bacterial, viral and parasitic diseases to animals and humans [2]. Anaplasmosis is a tick-borne disease and considered emerging or reemerging pathogens with serious public health implications, which are obligate intracellular pathogens that infect humans and animals [3]. Although these agents could infect humans through various routes, animal hosts play an important role in transmission.

Since the first case was reported in the USA in 1990, *Anaplasma* species have been described in both Europe and Asia [4-7]. The major *Anaplasma* species that impact animal and human health include *Anaplasma marginale*, *Anaplasma ovis*, *Anaplasma centrale*, *Anaplasma bovis*, *Anaplasma phagocytophilum*, and *Anaplasma platys* [8]. Among them, *A. phagocytophilum* is distributed worldwide and infects a variety of hosts [9,10]. *A. ovis* is the main inter-erythrocytic pathogens of ovine, which are responsible for ovine anaplasmosis in tropical and subtropical areas [11]. Although studies on *Anaplasma* species have been carried out in part of China [9,12] information is scarce on the animal reservoirs of *Anaplasma* spp. in northern XUAR. Therefore, the aim of this study was to identify *A. ovis* and *A. phagocytophilum* infection in wild and domestic hosts in different districts of northern XUAR, Northwest China

MATERIAL and METHODS

During 2015-2016, blood samples were collected from 137 sheep in Ili Kazakh Autonomous Prefecture (Ili), 79 sheep in Bole Mongol Autonomous Prefecture (Bole) and 71 sheep in Shihezi City. Spleen samples were collected from 356 great gerbils *(Rhombomys opimus)* in Urumqi City and 134 *R. opimus* in Bole in northern XUAR, Northwest China. The study area ranged between latitude 43°49′31.42″N - 44°54′18.04″N and longitude 81°31′27.78″ - 87°36′50.35″E. In accordance with the different types of landscapes, 1-4 sampling sites were selected in each county or city. The sheep bloods were sampled at different intervals under the owner agreements. As to wild rodents (i.e. the great gerbils *(R. opimus)*), their carcasses were submitted for postmortem examination to the Xinjiang Uygur Autonomous Region Wildlife Management Office, and then sent to our laboratory for scientific research. This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2015-01).

Genomic DNA was extracted from blood or dissected tissues/organs of the animals (including sheep and the great gerbil) (i.e. spleen in this study) by using the 96 flux automatic nucleic acid extraction instrument with a matching commercial kit (Cell & Tissue Kit, Bioteke, Beijing, China) according to the manufacturer's instructions, cloned into the pBS-T vector, and used for the transformation of One-shot® Top10 *Escherichia coli* cells. To prevent contamination problems, as negative control, used purified sterile water, were tested after every sample in our PCR. To determine genetic variability and regional differences of *A. phagocytophilum* and *A. ovis*, all samples were examined targeting *16S rRNA* genes by polymerase chain reaction (PCR) according to previous descriptions [13,14]. An approximately 400bp fragment was amplified using primers *16S rRNA*-Outer for the first round, then *16S rRNA*-Inner for the second round PCR. Each amplified product was repeatedly sequenced three times. Sequences were compared with GenBank data using BLAST *(http://www.ncbi.nlm.nih.gov/BLAST/)*. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [15]. Evolutionary analyses were conducted in MEGA7 [16].

RESUTS

In this study, *A. ovis* was found in the blood samples from sheep in Ili (21/137, 15.3%), Bole (18/79, 22.8%) and Shihezi City (13/71, 18.3%) in northern XUAR, respectively. In addition to the above results, DNA from *A. phagocytophilum* was detected in spleen samples from great gerbils *(R. opimus)* in Urumqi City (37/356, 10.4%) and Bole (21/134, 15.7%), northern XUAR. Interestingly, co-infection *A. ovis* and *A. phagocytophilum* in blood of 9/137 sheep (6.7%) from Ili, northwest China *(Table 1)* was detected.

Based on the analysis of BLAST and phylogenic tree, six *Anaplasma* genotypes *(A. ovis* and *A. phagocytophilum)* were detected *(Fig. 1)*. The sequences of *16S rRNA* fragments amplified from the three *A. ovis* isolates were 100% identical in our study [GenBank No. MK260043, MK260044

Table 1. *Prevalence of Anaplasma ovis and Anaplasma phagocytophilum from northern Xinjiang, northwest China*

District	Host	*Anaplasma ovis* (%)	*Anaplasma phagocytophilum* (%)	Coordinate
Ili	sheep	21/137 (15.3%)	9/137 (6.6%)	43°58′33.93″N 81°31′27.78″E
Bole	sheep	18/79 (22.8%)	-	44°54′18.04″N 82°03′48.45″E
	the great gerbil *(Rhombomys opimus)*	-	21/134 (15.7%)	
Urumqi	the great gerbil *(Rhombomys opimus)*	-	37/356 (10.4%)	43°49′31.42″N 87°36′50.35″E
Shihezi	sheep	13/71 (18.3%)	-	44°18′19.04″N 85°18′19.04″E

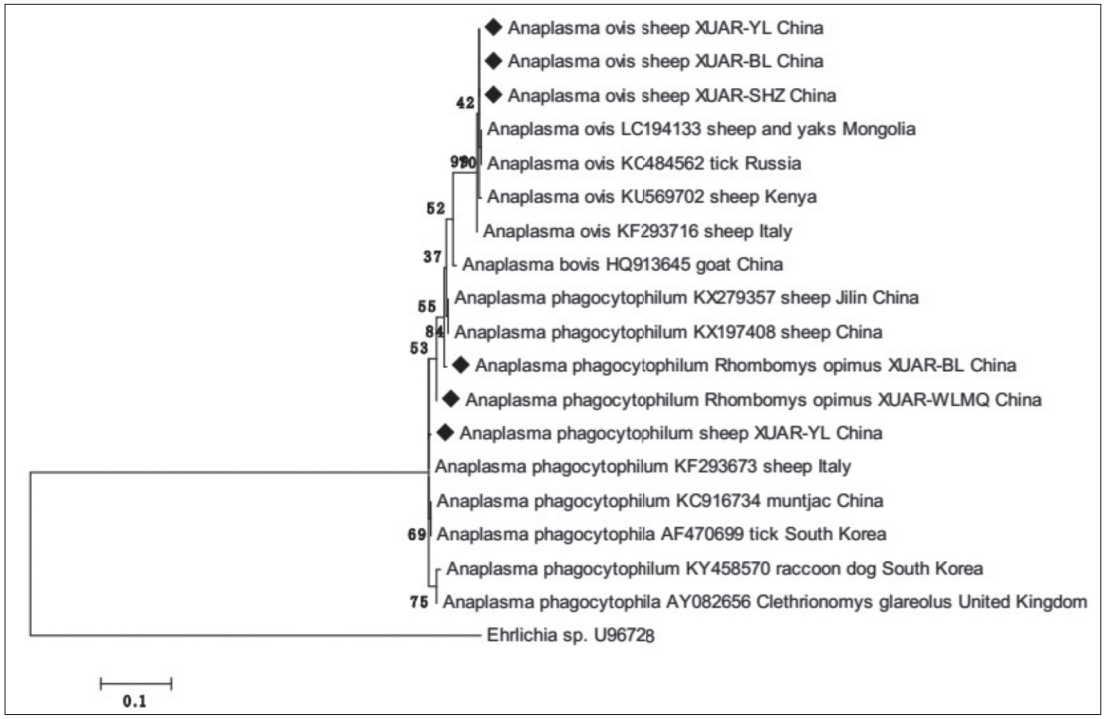

Fig 1. Phylogenetic comparison of *16S rRNA* gene sequence of *A. ovis* and *A. phagocytophilum* (◆) in this study and relevant sequences from GenBank. The results are based on the Maximum-likelihood (ML; 500 bootstrap replicates) approximation of the standard likelihood ratio test score. Branch lengths correlate to the number of substitutions inferred according to the scale shown

and MK260045], and varied from all known *A. ovis* sequences in GenBank, with 99.2%-99.7% nucleotide identity from sheep, yaks and ticks in other countries [GenBank No. KF293716 (Italy), LC194134 (Mongolia), KU569702 (Kenya) and KC484562 (Russia), respectively], and separated into different clusters in the phylogenetic tree. *A. phagocytophilum* from sheep and great gerbils *(R. opimus)* in this work, which had 97.5%-98.8% similarity with the corresponding sequences of *A. phagocytophilum* originated from sheep, muntjac, tick and malebank voles *(Clethrionomys glareolus)* derived from different areas [GenBank No. KX279357 (China), KX197408 (China), KF293673 (Italy), KC916734 (China), AY082656 (United Kingdom), AF470699 and KY458570 (South Korea), respectively]. Interestingly, the sequence divergence between *A. phagocytophilum* from sheep and great gerbils *(R. opimus)* was 2.53% from different regions in our study. The phylogenetic analysis confirmed these results: the separation of genus *Anaplasma* genotypes in the study area were distinct from previously reported in other continents, geographic and host-associated cluster was strongly supported *(Fig. 1)*. All obtained sequences were deposited in GenBank *(A. ovis*: MK260043-MK260045; *A. phagocytophilum*: MK260046-MK260047), but the number has not been released.

DISCUSSION

Since it was first recognised, anaplasmosis caused by *A. ovis* and *A. phagocytophilum* is considered to have a worldwide distribution [4-7]. Studies about the extent of its occurrence in animals and humans have been detected mostly in Europe, the USA and part of Asia [4,6,7]. Here, we isolated four strains of *A. ovis* and *A. phagocytophilum* from sheep, and two strains of *A. phagocytophilum* originated from rodents and used molecular methods to investigate the occurrence of *A. ovis* and *A. phagocytophilum* in northern XUAR, Northwest China.

Ovine anaplasmosis is caused by *A. ovis* in sheep, goats and ticks, which is widely distributed in different regions of the world [1]. The sequences, named as SHZ, YL and BL in XUAR were clustered together, and close to *A. ovis* genotypes from sheep in Kenya and Italy, sheep and yaks in Mongolia and ticks in Russia *(Fig. 1)*. The phylogenetic analysis indicated that *A. ovis* genotype diversity exist for sheep in Italy, Kenya and Mongolia, for yaks in Mongolia and for ticks in Russia, but the geographical differences for each species was not distinguished yet. Although these results add new information on the reservoirs of this disease agent we still need to have more sequence information to identify the differences of every *A. ovis* species.

Anaplasma phagocytophilum is reportedly maintained in various animal reservoirs, such as white-footed mice, goats, sheep, ticks and horses [4,17,18]. The sequence variation in the *16S rRNA* gene among different *A. phagocytophilum* strains confirmed that three genotypes of *A. phagocytophilum* were detected in this study *(Fig. 1)*. *A. phagocytophilum* infection in sheep from Ili compared with another two

A. phagocytophilum genotypes derived from different geographical regions in XUAR showed high diversity to *A. phagocytophilum* of the sheep, raccoon dogs, ticks, *Clethrionomys glareolus* and muntjacs from Italy, South Korea, United Kingdom and other China areas, respectively. These results showed *A. phagocytophilum* genotypes and displayed a high degree of genetic diversity, geographical and host tropisms, and the results coincides with Barakova et al.[19] report. To determine the level of infectivity in rodents as well as domestic animals, further studies are needed.

In northern XUAR, tick species distributed widespread, it is important to mention that animals located in this area suffer from heavy infestations by ticks. Here, we concluded that the emerging tick-borne *A. ovis* and *A. phagocytophilum* infection is already prevalent in different areas of ChinaThese results thus add new information on the reservoirs of those disease agents. Despite the existence of the biggest livestock industry in northwest China, there is still a considerable gap in our knowledge regarding the distribution of these pathogens and its economical relevance. In the future, it is important that tick-borne *Anaplasma* species involving domestic animals, wildlife, and humans should be paid more attention to the cooperation of Central Asia countries.

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Conflict of Interest

These authors have no conflict of interest related with this study.

REFERENCES

1. Song R, Wang Q, Guo F, Liu X, Song S, Chen C, Tu C, Wureli H, Wang Y: Detection of *Babesia* spp. *Theileria* spp. and *Anaplasma ovis* in border regions, northwestern China. *Transbound Emerg Dis*, 65 (6): 1537-1544, 2018. DOI: 10.1111/tbed.12894

2. Sheng J, Jiang M, Yang M, Bo X, Zhao S, Zhang Y, Hazihan W, Wang B, Tu C, Wang Y: Tick distribution in border regions of Northwestern China. *Ticks Tick Borne Dis*, 10 (3): 665-669, 2019. DOI: 10.1016/j.ttbdis.2019.02.011

3. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR: Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order Rickettsiales: Unification of some species of *Ehrlichia* with *Anaplasma*, Cowdria with *Ehrlichia* and *Ehrlichia* with Neorickettsia, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*, 51, 2145-2165, 2001. DOI: 10.1099/00207713-51-6-2145

4. Wormser GP: Accuracy of diagnosis of human granulocytic Anaplasmosis in China. *Emerg Infect Dis,* 22 (10): 1728-1731, 2016. DOI: 10.3201/eid2210.160161

5. Chen SM, Dumler JS, Bakken JS, Walker DH: Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol*, 32 (3): 589-595, 1994.

6. Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, Grab DJ, Bakken JS: Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. *Emerg Infect Dis,* 11, 1828-1834, 2005. DOI: 10.3201/eid1112.050898

7. Fourie JJ, Evans A, Labuschagne M, Crafford D, Madder M, Pollmeier M, Schunack B: Transmission of *Anaplasma phagocytophilum* (Foggie, 1949) by *Ixodes ricinus* (Linnaeus, 1758) ticks feeding on dogs and artificial membranes. *Parasit Vectors*, 12:136, 2019. DOI: 10.1186/s13071-019-3396-9

8. Liu Z, Ma M, Wang Z, Wang J, Peng Y, Li Y, Guan G, Luo J, Yin H: Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Appl Environ Microbiol*, 78 (2): 464-470, 2012. DOI: 10.1128/AEM.06848-11

9. Yang J, Liu Z, Guan G, Liu Q, Li Y, Chen Z, Ma M, Liu A, Ren Q, Luo J, Yin H: Prevalence of *Anaplasma phagocytophilum* in ruminants, rodents and ticks in Gansu, north-western China. *J Med Microbiol*, 62, 254-258, 2013. DOI: 10.1099/jmm.0.046771-0

10. Perez G, Bastian S, Chastagner A, Agoulon A, Plantard O, Vourc'h G, Butet A: Ecological factors influencing small mammal infection by *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in agricultural and forest landscapes. *Environ Microbiol*, 19 (10): 4205-4219, 2017.

11. de la Fuente J, Atkinson MW, Naranjo V, Fernández de Mera IG, Mangold AJ, Keating KA, Kocan KM: Sequence analysis of the msp4 gene of *Anaplasma ovis* strains. *Vet Microbiol*, 119 (2-4): 375-381, 2007.

12. Zhan L, Cao WC, Chu CY, Jiang BG, Zhang F, Liu W, Dumler JS, Wu XM, Zuo SQ, Zhang PH, Huang HN, Zhao QM, Jia N, Yang H, Richardus JH, Habbema JDF: Tick-borne agents in rodents, China, 2004-2006. *Emerg Infect Dis*, 15 (12): 1904-1908, 2009. DOI: 10.3201/eid1512.081141

13. Liu Z, Luo J, Bai Q, Ma M, Guan G, Yin H: Amplification of 16S rRNA genes of *Anaplasma* species in China for phylogenetic analysis. *Vet Microbiol*, 107 (1-2): 145-148, 2005. DOI: 10.1016/j.vetmic.2005.01.007

14. 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*, 8 (2): e57155, 2013. DOI: 10.1371/journal.pone.0057155

15. Tamura K, Nei M: Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*, 10 (3): 512-526, 1993. DOI: 10.1093/oxfordjournals.molbev.a040023

16. Kumar S, Stecher G, Tamura K: MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*, 33 (7): 1870-1874, 2016. DOI: 10.1093/molbev/msw054

17. Ravyn MD, Kodner CB, Carter SE, Jarnefeld JL, Johnson RC. Isolation of the etiologic agent of human granulocytic *Ehrlichiosis* from the white-footed mouse *(Peromyscus leucopus). J Clin Microbiol*, 39 (1): 335-338, 2001.

18. Scorpio DG, Leutenegger C, Berger J, Barat N, Madigan JE, Dumler JS: Sequential analysis of *Anaplasma phagocytophilum msp2* transcription in murine and equine models of human granulocytic anaplasmosis. *Clin Vaccine Immunol*, 15 (3): 418-424, 2008. DOI: 10.1128/CVI.00417-07

19. Barakova I, Derdakova M, Carpi G, Rosso F, Collini M, Tagliapietra V, Ramponi C, Hauffe HC, Rizzoli A: Genetic and ecologic variability among *Anaplasma phagocytophilum* strains, northern Italy. *Emerg Infect Dis*, 20 (6): 1082-1084, 2014. DOI: 10.3201/eid2006.131023