Vector-Borne Pathogens With Veterinary and Public Health Significance in Melophagus Ovinus (Sheep Ked) From the Qinghai-Tibet Plateau

Qingxun Zhang
Institute of Zoology Chinese Academy of Sciences
Ye Wang
Institute of Zoology Chinese Academy of Sciences
Ying Li
Institute of Zoology Chinese Academy of Sciences
Shuyi Han
Institute of Zoology Chinese Academy of Sciences
Bo Wang
Institute of Zoology Chinese Academy of Sciences
Guohui Yuan
Institute of Zoology Chinese Academy of Sciences
Peiyang Zhang
Institute of Zoology Chinese Academy of Sciences
Ziwen Yang
Institute of Zoology Chinese Academy of Sciences
Shuangling Wang
Institute of Zoology Chinese Academy of Sciences
Jiyong Chen
Animal Diseases Prevention and Control Center of Yushu
Haishun Zhong
Animal Husbandry and Veterinary Station of Xunhua
Xueqing Han
Chinese Academy of Inspection and Quarantine
Hong-Xuan He (hehx@ioz.ac.cn)
Chinese Academy of Sciences

Research

Keywords: Melophagus ovinus, vector-borne pathogens, prevalence, reservoir, China

Posted Date: November 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-101829/v1

Version of Record: A version of this preprint was published at Pathogens on February 22nd, 2021. See the published version at https://doi.org/10.3390/pathogens10020249.

Abstract

Background

Melophagus ovinus (sheep ked) is a hematophagous ectoparasite that mainly parasitizes sheep. In addition to causing inflammation, wool loss and skin damage to the animal hosts, *M. ovinus* also serves as a vector for a variety of pathogens and is highly likely to participate in the life and transmission cycle of pathogenic organisms.

Methods

Herein, we investigated the presence and molecular characterization of vector-borne pathogens in *M. ovinus* from Qinghai-Tibet Plateau, China.

Results

A total of 92 *M. ovinus* pools (n=276) collected from Qinghai province of China were screened for the presence of selected vector-borne pathogens. The overall prevalence of *A. ovis*, *A. bovis*, *A. phagocytophilum*, and *T. ovis* in *M. ovinus* was 39.1%, 17.4%, 9.8%, and 89.1%, respectively. All of the samples were negative for BDV, other *Anaplasma* species, *Babesia* spp., *Rickettsia* spp., and *Borrelia* spp. Co-infection of different *Anaplasma* species and *T. ovis* occurred in 51.2% of all samples with *T. ovis*. The positive rates of *A. ovis*, *A. bovis*, and *A. phagocytophilum* in different region and altitude of the sampling sites were significantly different. Sequence and phylogenetic analysis of target genes confirmed their identity with corresponding pathogens.

Conclusion

Our results elucidate the occurrence and genetic diversity of *Anaplasma* spp. and *Theileria* spp. in *M. ovinus*, which could act as potential zoonotic reservoirs. To the best of our knowledge, this is the first report of the detection of *A. bovis* and *A. phagocytophilum* DNA in *M. ovinus*. This study gives the first extensive molecular survey of vector-borne pathogens with veterinary and public health significance in *M. ovinus* from the Qinghai-Tibet Plateau, China.

Introduction

Melophagus ovinus (sheep ked) belongs to the family Hippoboscidae (Diptera: Hippoboscoidea) and is a blood-feeding ectoparasite of livestock and wild animals, including sheep, goats, rabbits, dogs, Tibetan antelope, European bison, and red foxes, and has also been found in humans (Marcos and Domenico, 2020; Rudolf et al., 2016; Small, 2005). *M. ovinus* is reported to cause reproductive performance, inflammation, wool loss and skin damage of sheep and has significant economic effects in the sheep industry (Sertse and Wossene, 2007; Small, 2005). *M. ovinus* is broadly distributed in Africa, Europe, Oceania, North America, and Asia (Liu et al., 2016). In China, *M. ovinus* has recently been reported to parasitize sheep and Tibetan antelopes in Tibet, Xinjiang, Qinghai, and Gansu, and also detected in imported sheep and sheep wool in certain areas of China (Chu et al., 2011; Duan et al., 2017; Liu et al., 2016; Liu et al., 2018;).

M. ovinus serves as potential vectors of a variety of pathogens and has been reported to be responsible for the transmission of pathogenic organisms such as helminths, protozoa, bacteria and viruses due to their blood-feeding behavior towards hosts (Marcos and Domenico, 2020; Zhao et al., 2019). *M. ovinus* was reported to mechanically transmit Bluetongue virus in sheep (Rudolf et al., 2016). Additionally, *M. ovinus* may be a carrier for *Bartonella schoenbuchensis* and *B. chomeli* in USA (Halos et al., 2004), *Anaplasma ovis* in Hungary (Hornok et al., 2011), *Acinetobacter* spp. in Ethiopia (Kumsa et al., 2012), and *Bartonella* in Central Europe (Rudolf et al., 2016). Chen et al (2011) reported *Borrelia burgdorferi* sensu lato in sheep keds in Tibet, China. Recently, in China, *Anaplasma ovis* (Zhao et al., 2018), *Rickettsia raoultii* and *R. slovaca* (Liu et al., 2016), *Theileria ovis* (Zhao et al., 2019), and Border disease virus (BDV) (Liu et al., 2019) have also been detected in *M. ovinus* in Xinjiang Uygur Autonomous Region, northwestern China.

Qinghai province is the traditional animal husbandry base and a small number of reports have recorded the presence of *M. ovinus* in this region (Liu et al., 2016). However, very little is known about the prevalence of arthropod-borne pathogens in *M. ovinus* from the Qinghai. Given the veterinary and public health significance of *M. ovinus*, the objective of the present study was to investigate the presence of vector-borne pathogens in *M. ovinus* from Qinghai-Tibet Plateau of China.

Materials And Methods

Study sites and sample collection

Adult sheep keds (n=276) were collected at four sites: Xunhua, Haidong (n=24, altitude 3000 m, 35°39'N 102°41'E), Maqin, Golog (n=24, altitude 3800 m, 35°2'N 99°12'E), Dari, Golog (n=24, altitude 4100 m, 33°43'N 99°38'E), and Zhiduo, Yushu (n=24, altitude 4100 m, 33°37'N 95°58'E) during June 2020 in Qinghai province, China (Figure 1). After collection, sheep keds were shipped into the laboratory in cooled flasks and pooled (n=92, three adults per tube according to gender and the sampling sites) before being frozen at −80 °C until testing. Morphological studies (Figure 1) and 18S rRNA gene sequence analysis (data not shown) confirmed that the collected samples belong to sheep keds.

Nucleic acid extraction and PCR amplification

All samples were sterilized with 70 % ethanol and distilled water and were mechanically disrupted in 200 μL of PBS. Genomic DNA and RNA were extracted from 100 μL of the homogenate with the commercially TIANamp Genomic DNA Kit (TIANGEN BIOTECH (BEIJING) CO., LTD) and Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. cDNA was synthesized using GoScript Reverse Transcription System and 5'-UTR of BDV was amplified according to Access RT-PCR System (Promega, USA) (Liu et al., 2019). The DNA samples were detected for the presence of the genus *Anaplasma* by PCR-based amplification of the 16S rRNA gene for *A. bovis*, *A. phagocytophilum*, *A. centrale* and *A. platys*, the msp4 gene for *A. ovis* and *A. marginale*, and the citrate synthase (gltA) gene and 16S rRNA gene for *A. capra*, respectively, as previously described (Li et al., 2015; Torina et al., 2012; Wang et al., 2019; Yang et al., 2016). For piroplasm (*Theileria* spp. and *Babesia* spp.) detection, all samples were screened using nested PCR assays targeting the 18S rRNA gene (Wang et al., 2019). Other vector-borne bacteria including *Rickettsia* spp. and *Borrelia* spp. were also detected (Anstead and Chilton, 2013; Rijpkema et al., 1995; Roux et al., 1996) and the PCR primers and cycling conditions were shown in S1 Table. The DNAs extracted from the domestic animals and ticks in Qinghai infected with *A. ovis*, *A. bovis, A. phagocytophilum*, *Theileria* spp., and *Rickettsia* spp. were used as positive controls. The PCR products were detected by 1% agarose gel electrophoresis with M5 Hipure Next III Gelred (Mei5 Biotechnology Co., Ltd) stained.

Sequencing and phylogenetic analysis

The PCR products from positive samples were bi-directionally sequenced at BGI Sequencing (Beijing, China) and subjected to BLAST searches and MEGA software for nucleotide sequence analysis and alignments. Phylogenetic trees were constructed using the neighbor-joining method executed in with Kimura 2-parameter model MEGA X as previously described. Bootstrap values were assessed with 1000 bootstrap replicates. The representative nucleotide sequences of this study have been deposited in the GenBank database under accession number MW147462 for *A. ovis*, MW142384 for *A. bovis*, MW142385 for *A. phagocytophilum* and MW142379 for *T. ovis*.

Data analysis

The data were grouped into three variables in terms of sheep keds gender and the region and the altitude of the sampling sites. Differences in infection rates of each group were statistically calculated using the Chi-square test in SPSS 25.0. A P-value of < 0.05 was considered significant.

Results

A total of 92 *M. ovinus* pools were screened for the presence of selected vector-borne pathogens. Of the 92 samples tested, 46 (50.0%) pools were positive for one or more *Anaplasma* species. The average infection rates were 39.1%, 17.4%, and 9.8% for *A. ovis*, *A. bovis*, and *A. phagocytophilum* in *M. ovinus*, respectively (Table 1). Importantly, *A. bovis*, and *A. phagocytophilum* were detected in *M. ovinus* for the first time. 82 samples (89.1%) for piroplasm infection were found, and all belonging to *Theileria ovis*. No positive results were obtained for other tested pathogens, including BDV, *A. centrale*, *A. platys*, *A. capra*, *A. marginale*, *Babesia* spp., Rickettsia spp. and *Borrelia* spp. Mixed infections of both *Theileria ovis* and *Anaplasma* species accounted for 51.2%(42/82) of all samples with *Theileria ovis*. And *A. ovis* co-infections with *A. bovis* and *A. phagocytophilum* accounted for 26.1% (12/46) and 2.2% (1/46) of *Anaplasma* species infections, respectively. The molecular characterization of *Anaplasma* spp. and *Theileria* spp. identified from *M. ovinus* was also performed. Sequence analysis of the msp4 sequences of *A. ovis* (sequence similarity 99.9-100%), 16S rRNA sequences of *A. bovis* (sequence similarity 99.9-100%) and *A. phagocytophilum* (sequence similarity 100%), 18S rRNA gene sequences of *Theileria ovis* (sequence similarity 99.9-100%) confirmed their identity with corresponding pathogens by using BLASTn search. The phylogenetic analysis showed that the representative strain MW147462 was classified as *A. ovis* Genotypes II, Sequence MW142385 of *A. phagocytophilum* was classified into cluster I and sequence of MW142384 was identical with strains isolated from sheep (MT036513), tick (KC311345), horse (MK028574), and deer (KJ659040) (Figure 2A-C). The phylogenetic analysis of 18S rRNA gene confirmed that the obtained piroplasm (MW142379) was *T. ovis* (Figure 2D).

Risk factors including *M. ovinus* gender, the region and altitude of the sampling sites were used as variables for statistical analysis of the infection patterns of *Anaplasma* spp. and *Theileria* spp. prevalence (Table 2). As a result, the region of the sampling sites was positively associated with the presence of *A. ovis*, *A. bovis*, and *A. phagocytophilum* but not *Theileria ovis*, and *M. ovinus* in Haidong had a higher risk than other *M. ovinus* in Golog and Yushu to be infected with *A. ovis* and *A. bovis*. *A. phagocytophilum* infection rates in *M. ovinus* collected in Yushu were significantly higher than Haidong and Golog. *M. ovinus* collected at 3000 m areas had a higher risk for being infected by *A. ovis* and *A. bovis* than in the *M. ovinus* collected at elevations of 3800 m and 4100 m. In addition, the results showed no significant difference in gender.

Discussion

To date, few publications have described the distribution and prevalence of vector-borne pathogens in *M. ovinus* from Qinghai-Tibet Plateau, China. As the traditional animal husbandry base, epidemiological investigations into vector-borne pathogens with veterinary and public health significance in Qinghai are of particular importance. In this study, the first extensive molecular survey of BDV, *Anaplasma* spp., piroplasm, *Rickettsia* spp. and *Borrelia* spp. in *M. ovinus* was performed. In our results, only *A. ovis*, *A. bovis*, *A. phagocytophilum*, and *Theileria ovis* were positive and other tested pathogens were negative. To the best of our knowledge, this is the first molecular evidence of *A. bovis* and *A. phagocytophilum* in *M. ovinus*. Many factors including biogeographic, season of sample collection, number of samples etc, may contribute to the differences between investigations of pathogenic organisms in *M. ovinus* in other regions in China or other conutries.

Aanaplasma spp. prevalence in *M. ovinus* demonstrated a wide distribution of *A. ovis*, *A. bovis*, and *A. phagocytophilum* in Qinghai-Tibet Plateau, China. *A. ovis* has been considered as the etiological agents of anaplasmosis of domestic ruminants and it has been widely detected in sheep, goat, cattle, wild deer and many tick species around the world (Battilani et al., 2017; Han et al., 2019). In previous reports, all sheep keds (100%, 81/81) were found to harbor *A. ovis* in Hungary (Hornok et al., 2011) and 28 specimens (including five pupal specimens) (31.8%,28/88) collected in 2016 and 2017 in Xinjiang, China tested positive for *A. ovis* (Zhao et al., 2018). The prevalence (39.1%) and genetic characteristic (Genotype I) of *A. ovis* in *M. ovinus* in this study was concurred with other reports published in Xinjiang, but lower than Hungary. *Anaplasma* bovis mainly affecting cattle with fever, progressive anemia, and even death, and the subclinical infections of this agent has also been found in small mammals and ruminants, indicating the reservoir competence of those animals for *A. bovis* (Yang et al., 2016). In addition, *A. bovis* can be found in many tick species (*Haemaphysalis longicorn*is, *H. lagrangei*, *H.concinna*, and *Rhipicephalus evertsi* etc) in Asia, Europe, and Africa (Han et al., 2019; Qin et al., 2018). We detected *A. bovis* with the prevalence of 17.4% in *M. ovinus* for the first time, which indicated that *M. ovinus* may be the potential reservoirs or maintenance hosts of this agent. Among the *Anaplasma* species detected, *A. phagocytophilum* is an emerging zoonotic pathogen of human and animal granulocytic

anaplasmosis and can be transmitted to a wide range of mammals including humans, ruminants, horses, cats, dogs, rodents, birds and reptiles through the bite of ticks (Stuen et al., 2013). In the present study, this is the first time that *A. phagocytophilum* DNA has been detected in *M. ovinus* using molecular identification. Statistics analysis indicated that *Aanaplasma* spp. infections showed significant correlations with the region and altitude of the sampling sites and the co-infection. Our results expand the potential vector spectrum of *A. bovis* and *A. phagocytophilum* and emphasize the veterinary and public health significance of *M. ovinus*.

Theileria spp. is the causative agent of Theileriosis and has a wide geographical and host-species distribution. Among the *Theileria* species, *Theileria ovis* mainly causes benign theileriosis in sheep, goats, and cattle, which is easily overlooked (Qi et al., 2018). In China, *T. ovis* has mainly been reported in animal, tick, and sheep keds from Xinjiang (Li et al., 2011; Zhao et al., 2019), Inner Mongolia (Yang et al., 2014), Qinghai (Li et al., 2020), Sichuan (Hao et al., 2020). Recently, two reports have shown the absence of *Theileria* spp. in *M. ovinus* in Xinjiang (*T. ovis* with the prevalence of 16%) and Sichuan (*T. luwenshuni* with the prevalence of 30.8%) (Hao et al., 2020; Zhao et al., 2019). Herein, a high prevalence (89.1%, 82/92) of *T. ovis* DNA was demonstrated in *M. ovinus*, which need more attention.

Vector-borne diseases including *Anaplasma* species, BDV, *Babesia* spp., *Rickettsia* spp., and *Borrelia* spp. cause economic losses in the livestock industry and pose a risk to humans. Although these infectious agents were negative in this study, some of these pathogens were found in tick, yak, and Tibetan sheep samples (unpublished data), implying that this region tend to have a higher risk of vector-borne diseases. Future study should systematically screen *M. ovinus* for the presence of potential animal as well as human pathogens.

Conclusions

We demonstrated the prevalence of *A. ovis*, *A. bovis*, and *A. phagocytophilum*, and *T. ovis* with veterinary and medical significance in *M. ovinus* in Qinghai of China. *A. bovis* and *A. phagocytophilum* was found for the first time and the present study extended the spectrum of pathogens potentially present in *M. ovinus*. The prevalence of these pathogens in *M. ovinus* may be a threat to animal and public health in Qinghai-Tibet Plateau, China. Future investigations are warranted to elucidate the genetic diversity of vector-borne pathogens in *M. ovinus* and the role of *M. ovinus* as the specific biological vectors of some pathogens.

Abbreviations

PCR: Polymerase chain reaction; BLAST: Basic Local Align-ment Search Tool;

Declarations

Acknowledgments

We thank the support of morphological identification of *M. ovinus* provided by the Dr. Qingsong Zhou.

Authors' contributions

HHX and ZQX conceived and designed the study and critically revised the manuscript. ZQX, WY, and LY collected samples, conducted the laboratory experiments and analyzed the data. CJY and ZHS performed the sheep ked collection. WB, YGH, ZPY, YZW, and WSL performed DNA and RNA extraction and PCR analyses. All the authors read and approved the final manuscript.

Funding

This study was financially supported by the Regular Assistance Project of International Department of the Ministry of Science and Technology of China (KY201904013), the Strategic Priority Research Program of the Chinese Academy of Sciences

(XDA19050204 and XDA19090115), Beijing Innovation Consortium of Agriculture Research System (BAIC04-2020), National Forestry and Grassland Administration, China, and Beijing Wildlife Rescue Center, China.

Ethics approval and consent to participate

The study was conducted in compliance with the ethical policies of the journal and the rules of the ethic committee of the Institute of Zoology, Chinese Academy of Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

Author details

1. National Research Center for Wildlife-Borne Diseases, Institute of Zoology, Chinese Academy of Sciences. Beijing, China; 2. University of Chinese Academy of Sciences, Beijing, China; 3. Ningxia University; 4. Qinghai University. Xining, Qinghai, China; 5. Animal Disease Prevention and Control Center of Yushu, Qinghai, China; 6. Animal Husbandry and Veterinary Station of Xunhua, Qinghai, China; 7. Chinese Academy of Inspection and Quarantine, Beijing, China.

†These authors contributed equally to this work and should be considered co-first authors.

References

Anstead CA, Chilton NB. A novel *Rickettsia* species detected in vole ticks (Ixodes angustus) from Western Canada. Appl. Environ. Microbiol. 2013; 79:7583–7589.

Battilani M, Arcangeli SD, Balboni A, Dondi F. Genetic diversity and molecular epidemiology of *Anaplasma*. Infect Genet Evol. 2017; 49:195–211.

Chu CY, Jiang BG, Qiu EC, Zhang F, Zuo SQ, Yang H, et al. *Borrelia burgdorferi sensu lato* in sheep keds (*Melophagus ovinus*), Tibet China. Vet Microbiol. 2011; 149:526–529.

Duan DY, Liu H, Cheng TY, Wang YQ. Microbial population analysis of the midgut of *Melophagus ovinus* via high-throughput sequencing. Parasit Vectors. 2017; 10:382.

Halos L, Jamal T, Maillard R, Girard B, Guillot J, Chomel B, et al. Role of Hippoboscidae flies as potential vectors of *Bartonella* spp. infecting wild and domestic ruminants. Appl Environ Microbiol. 2004; 70(10):6302–6305.

Han R, Yang J, Mukhtar MU, Chen Z, Niu Q, Lin Y, et al. Molecular detection of *Anaplasma* infections in ixodid ticks from the Qinghai-Tibet Plateau. Infect Dis Poverty. 2019; 8: 12.

Hao L, Yuan D, Li S, Jia T, Guo L, Hou W, et al. Detection of *Theileria* spp. in ticks, sheep keds (*Melophagus ovinus*), and livestock in the eastern Tibetan Plateau, China. Parasitol Res. 2020; 119: 2641–2648.

Hornok S, de la Fuente J, Biró N, de Fernández Mera IG, Meli ML, Elek V, et al. First molecular evidence of Anaplasma ovis and Rickettsia spp. in keds (Diptera: Hippoboscidae) of sheep and wild ruminants. Vector Borne Zoonotic Dis. 2011; 11(10):1319–21.

Kumsa B, Socolovschi C, Parola P, Rolain JM, Raoult D. Molecular detection of Acinetobacter species in lice and keds of domestic animals in Oromia Regional State, Ethiopia. PLoS One. 2012; 7(12): e52377.

Li J, Jian Y, Jia L, Galon EM, Benedicto B, Wang G, et al. Molecular characterization of tick-borne bacteria and protozoans in yaks (Bos grunniens), Tibetan sheep (Ovis aries) and Bactrian camels (Camelus bactrianus) in the Qinghai-Tibetan Plateau Area, China. Ticks Tick Borne Dis. 2020; 11(5): 101466.

Li H, Zheng YC, Ma L, Jia N, Jiang BG, Jiang RR, et al. Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. Lancet Infect Dis. 2015; 15:663–670.

Li YQ, Guan GQ, Ma ML, Liu JL, Ren QY, Luo JX, et al. 2011. *Theileria ovis* discovered in China. Exp. Parasitol. 2011; 127: 304–307.

Liu D, Wang YZ, Zhang H, Liu ZQ, Wureli HZ, Wang SW, et al. First report of *Rickettsia raoultii* and *R. slovaca* in *Melophagus ovinus*, the sheep ked. Parasit Vectors. 2016; 9:600.

Liu YH, He B, Li F, Li KR, Zhang LY, Li XQ, et al. Molecular Identification of *Bartonella melophagi* and *Wolbachia* Supergroup F from Sheep Keds in Xinjiang, China. Korean J Parasitol. 2018; 56:365–370.

Liu YH, He B, Li KR, Li F, Zhang LY, Li XQ, et al. First report of border disease virus in *Melophagus ovinus* (sheep ked) collected in Xinjiang, China. PLoS ONE. 2019; 14(8): e0221435.

Marcos ABS, Domenico O. Keds, the enigmatic flies and their role as vectors of pathogens. Acta Trop. 2020; 209:105521.

Qi M, Cui YY, Song XM, Zhao AY, Bo J, Zheng ML, et al. Common occurrence of *Theileria annulata* and the first report of *T. ovis* in dairy cattle from Southern Xinjiang, China. Ticks Tick. Borne. Dis. 2018; 9: 1446–1450.

Qin XR, Han FJ, Luo LM, Zhao FM, Han HJ, Zhang ZT, et al. *Anaplasma* species detected in *Haemaphysalis longicornis* tick from China. Ticks Tick Borne Dis. 2018; 9:840–843.

Rijpkema SG, Molkenboer MJ, Schouls LM, Jongejan F, Schellekens JF. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi sensu lato* in Dutch Ixodes ricinus ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J Clin Microbiol. 1995; 33:3091–5.

Roux V, Fournier PE, Raoult D. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J Clin Microbiol. 1996; 34:2058–65.

Rudolf I, Betášová L, Bischof V, Venclíková K, Blažejová H, Mendel J, et al. Molecular survey of arthropod-borne pathogens in sheep keds (*Melophagus ovinus*), Central Europe. Parasitol Res. 2016; 115(10): 3679–3682.

Sertse T, Wossene A. Effect of ectoparasites on quality of pickled skins and their impact on the tanning industries in Amhara regional state, Ethiopia. Small. Rum. Res. 2007; 69: 55–61.

Small RW. A review of *Melophagus ovinus*, (L.), the sheep ked. Vet Parasitol. 2005; 130(1–2):141–55.

Stuen S, Granquist EG, Silaghi C. Anaplasma phagocytophilum-a widespread multi-host pathogen with highly adaptive strategies. Front. Cell. Infect. Microbiol. 2013; 3:31.

Torina A, Agnone A, Blanda V, Alongi A, D'Agostino R, Caracappa S, et al. Development and validation of two PCR tests for the detection of and differentiation between *Anaplasma ovis* and *Anaplasma marginale*. Ticks and Tick-Borne Diseases, 2012; 3(5-6), 283–287.

Wang HN, Yang JF, Mukhtar MU, Liu ZJ, Zhang MH, Wang XL. Molecular detection and identification of tick-borne bacteria and protozoans in goats and wild Siberian roe deer (Capreolus pygargus) from Heilongjiang Province, northeastern China. Parasit Vectors. 2019; 12(1): 296.

Yang Y, Mao Y, Kelly P, Yang Z, Luan L, Zhang J, et al. A pan-Theileria FRET-qPCR survey for *Theileria* spp. in ruminants from nine provinces of China. Parasit. Vectors. 2014; 7: 413.

Yang J, Liu Z, Niu Q, Liu J, Han R, Liu G, et al. Molecular survey and characterization of a novel *Anaplasma* species closely related to *Anaplasma capra* in ticks, northwestern China. Parasit Vectors. 2016; 9:603.

Zhao L, He B, Li KR, Li F, Zhang LY, Li XQ, et al. First report of *Anaplasma ovis* in pupal and adult *Melophagus ovinus* (sheep ked) collected in South Xinjiang, China. Parasit Vectors. 2018; 11:258.

Zhao L, Wang J, Ding Y, Li K, He B, Li F, et al. *Theileria ovis* (Piroplasmida: Theileriidae) detected in *Melophagus ovinus* (Diptera: Hippoboscoidea) and *Ornithodoros lahorensis* (Ixodida: Argasidae) removed from sheep in Xinjiang, China. J Med Entomol. 2020; 57(2): 631-635.

Tables

Table 1. Detection of *Anaplasma* spp. and *Theileria* spp. in the *M. ovinus* collected from four counties belonging to three cities in Qinghai Province.

County/Average altitude	Number of pooled	Number of infected (n)/Infection rate (%)			
		A. ovis	*A. bovis*	*A. phagocytophilum*	*T. ovis*
Xunhua/3000m	8	6/75.0	3/37.5	1/12.5	8/100
Maqin/3800	37	10/27.0	1/2.7	1/2.7	32/86.5
Dari/4100	15	4/26.7	2/13.3	0/0	14/93.3
Zhiduo/4100	32	16/50.0	10/31.3	7/21.9	28/87.5
Total	92	36/39.1	16/17.4	9/9.8	82/89.1

Table 2. Patterns of *Anaplasma* spp. and *Theileria* spp. prevalence in the *M. ovinus*, grouped by *M. ovinus* gender, the region and altitude of the sampling sites.

Group		Number of pooled	Number of infected (n)/Infection rate (%)							
			A. ovis	P-value	*A. bovis*	P-value	*A. phagocytophilum*	P-value	*T. ovis*	P-value
Region	Haidong	8	6/75.0	**0.01**	3/37.5	**0.003**	1/12.5	**0.011**	8/100	0.581
	Golog	52	14/26.9		3/5.8		1/1.9		46/88.5	
	Yushu	32	16/50.0		10/31.3		7/21.9		28/87.5	
Gender	Female	41	18/43.9	0.40	5/12.2	0.238	6/14.6	0.160	36/87.8	0.714
	Male	51	18/35.3		11/21.7		3/5.9		46/90.2	
Altitude	3000m	8	6/75.0	**0.033**	3/37.5	**0.007**	1/12.5	0.169	8/100	0.537
	3800m	37	10/27.0		1/2.7		1/2.7		32/86.5	
	4100m	47	20/42.6		12/25.5		7/14.9		42/89.4	

Figures

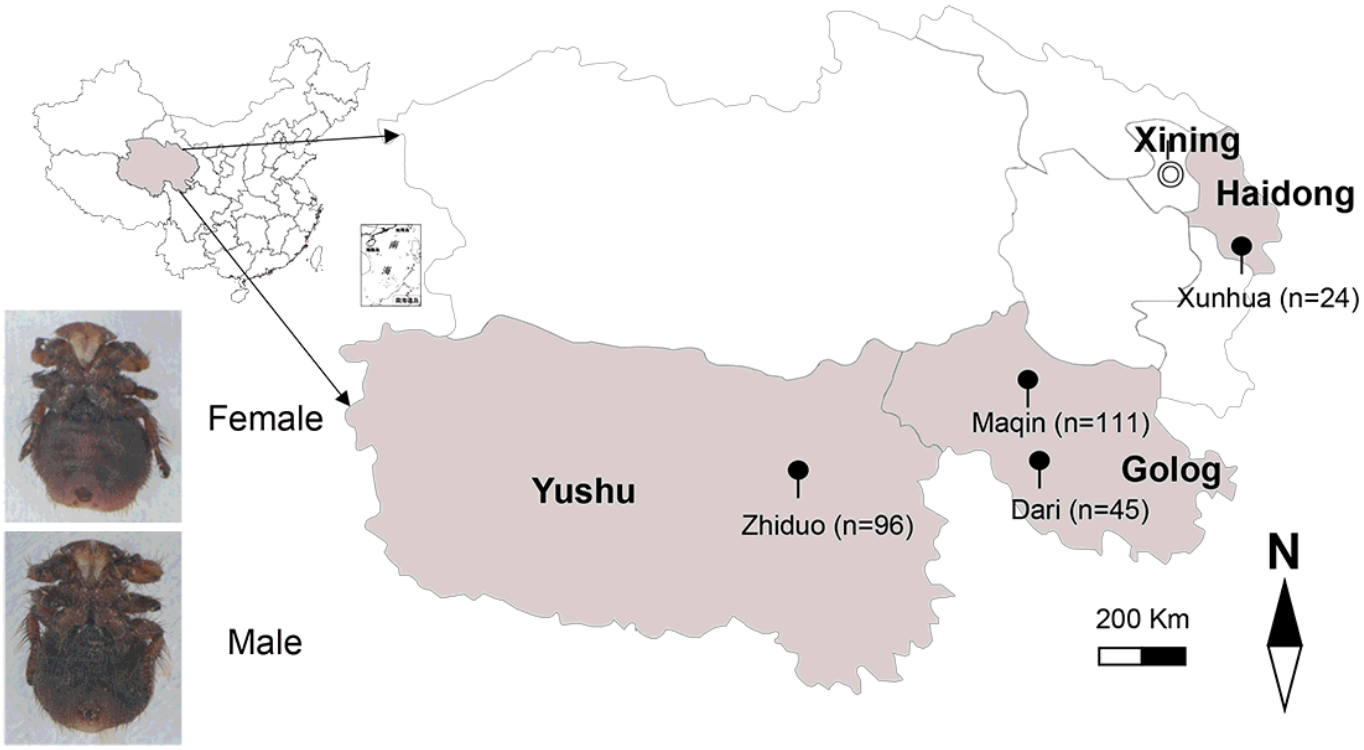

Figure 1

Sampling locations of M. ovinus (●) for the present survey in Qinghai province of China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

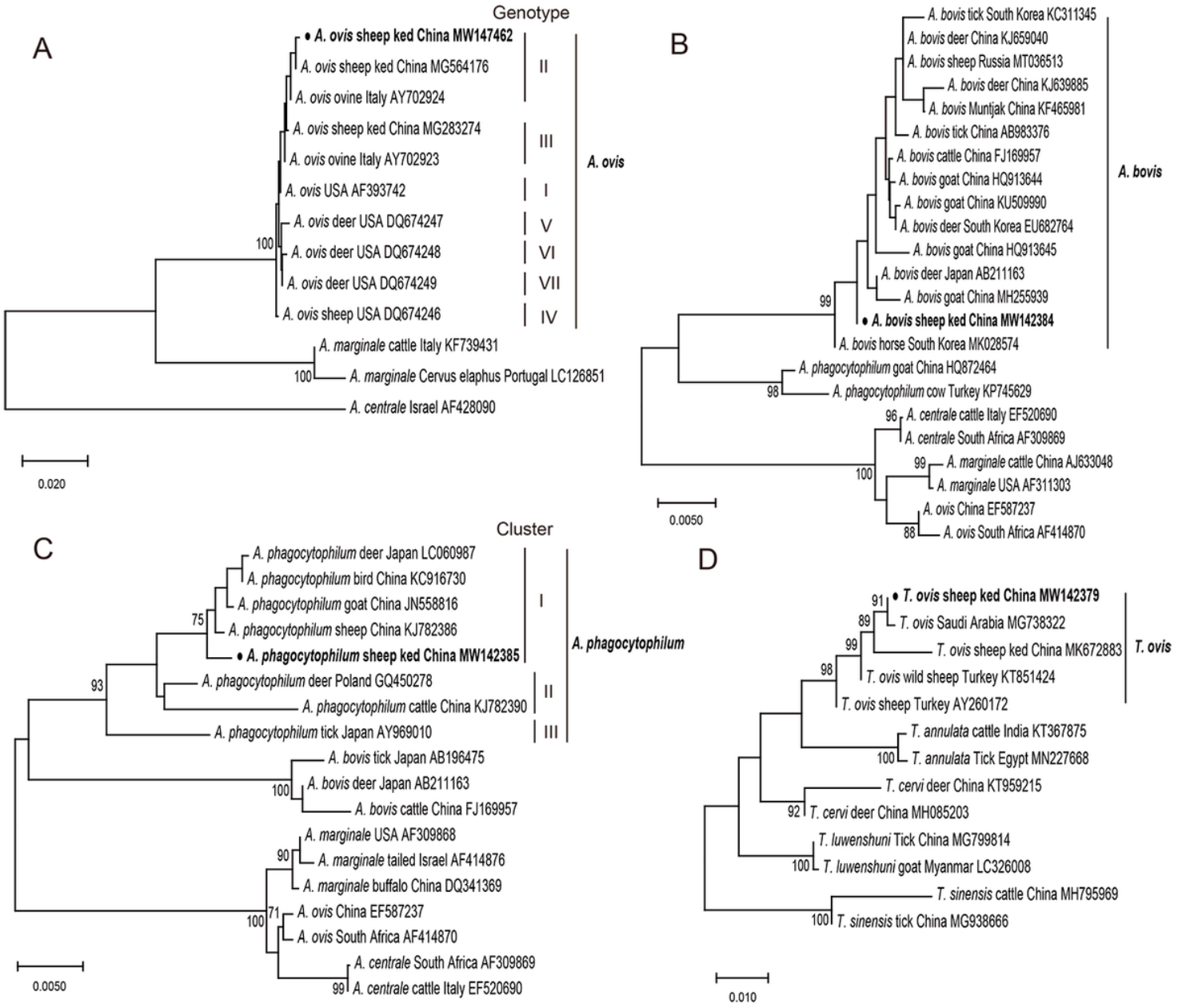

Figure 2

Phylogenetic relationship of partial segment msp4 of A. ovis (A), 16S rRNA gene for A. bovis (B), A. phagocytophilum (C), and 18S rRNA gene of T. ovis (D) identified in the present study and reference strains. All molecular phylogenetic trees were constructed by the neighborjoining method with Kimura 2-parameter model using the MEGAX software. The species identified in this study are indicated by ● and highlighted in bold.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1Table.pdf