frontiers in Veterinary Science

BRIEF RESEARCH REPORT
published: 30 September 2021
doi: 10.3389/fvets.2021.727166

Ehrlichia chaffeensis and Four *Anaplasma* Species With Veterinary and Public Health Significance Identified in Tibetan Sheep (*Ovis aries*) and Yaks (*Bos grunniens*) in Qinghai, China

Ye Wang[1,2†], Qingxun Zhang[1†], Shuyi Han[1†], Ying Li[3†], Bo Wang[1,4], Guohui Yuan[1], Peiyang Zhang[1,4], Ziwen Yang[1,4], Heng Zhang[5], Yali Sun[3], Jiyong Chen[6], Xueqing Han[7] and Hongxuan He[1]*

[1] National Research Center for Wildlife Borne Diseases, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, [2] College of Agriculture, Ningxia University, Yinchuan, China, [3] State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, China, [4] College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China, [5] College of Animal Science, Anhui Science and Technology University, Chuzhou, China, [6] Animal Disease Prevention and Control Center of Yushu, Yushu, China, [7] Chinese Academy of Inspection and Quarantine, Beijing, China

Tick-borne diseases (TBDs) can cause serious economic losses and are very important to animal and public health. To date, research on TBDs has been limited in Qinghai-Tibet Plateau, China. This epidemiological investigation was conducted to evaluate the distribution and risk factors of *Anaplasma* spp. and *Ehrlichia chaffeensis* in livestock in Qinghai. A total of 566 blood samples, including 330 yaks (*Bos grunniens*) and 236 Tibetan sheep (*Ovis aries*) were screened. Results showed that *A. bovis* (33.3%, 110/330) and *A. phagocytophilum* (29.4%, 97/330) were most prevalent in yaks, followed by *A. ovis* (1.2%, 4/330), *A. capra* (0.6%, 2/330), and *E. chaffeensis* (0.6%, 2/330). While *A. ovis* (80.9%, 191/236) and *A. bovis* (5.1%, 12/236) infection was identified in Tibetan sheep. To our knowledge, it is the first time that *A. capra* and *E. chaffeensis* have been detected in yaks in China. Apart from that, we also found that co-infection of *A. bovis* and *A. phagocytophilum* is common in yaks (28.2%, 93/330). For triple co-infection, two yaks were infected with *A. bovis*, *A. phagocytophilum*, and *A. capra*, and two yaks were infected with *A. bovis*, *A. phagocytophilum*, and *E. chaffeensis*. Risk analysis shows that infection with *A. bovis*, *A. phagocytophilum*, and *A. ovis* was related to region and altitude. This study provides new data on the prevalence of *Anaplasma* spp. and *E. chaffeensis* in Qinghai, China, which may help to develop new strategies for active responding to these pathogens.

Keywords: tick-borne disease, *Anaplasma capra*, *Ehrlichia chaffeensis*, Tibetan sheep, yak, Qinghai

OPEN ACCESS

Edited by:
Cornelia Silaghi,
Friedrich-Loeffler-Institute, Germany

Reviewed by:
Giulia Morganti,
University of Perugia, Italy
Snorre Stuen,
Norwegian Veterinary Institute (NVI), Norway

***Correspondence:**
Hongxuan He
hehx@ioz.ac.cn

† These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Parasitology, a section of the journal Frontiers in Veterinary Science

Received: *18 June 2021*
Accepted: *06 September 2021*
Published: *30 September 2021*

Citation:
Wang Y, Zhang Q, Han S, Li Y, Wang B, Yuan G, Zhang P, Yang Z, Zhang H, Sun Y, Chen J, Han X and He H (2021) Ehrlichia chaffeensis and Four Anaplasma Species With Veterinary and Public Health Significance Identified in Tibetan Sheep (Ovis aries) and Yaks (Bos grunniens) in Qinghai, China. Front. Vet. Sci. 8:727166. doi: 10.3389/fvets.2021.727166

INTRODUCTION

Anaplasmosis and ehrlichiosis are important diseases caused by tick-borne pathogens, which result in additional economic losses to livestock (1, 2). To date, seven *Anaplasma* species have been identified, including *A. bovis*, *A. phagocytophilum*, *A. centrale*, *A. platys*, *A. marginale*, *A. ovis*, and *A. capra* (3, 4). *A. bovis* parasitizes monocytes and macrophages of ruminants and small mammals (5). *A. phagocytophilum* infects neutrophils of humans and animals, and causing human granulocytic anaplasmosis (HGA), tick-borne fever in ruminants, and canine and equine granulocytic anaplasmosis (5). *A. centrale* and *A. marginale* mainly infect erythrocytes of cattle, while *A. ovis* primarily infect small ruminant animals such as sheep and goats. (6). *A. platys* mainly infect canine platelets and cause cyclic thrombocytopenia in dogs (6). *A. capra* is an emerging pathogen, which can infect ruminants and humans (7). In addition, as a member of the *Ehrlichia* family, *Ehrlichia chaffeensis* can cause human monocytic ehrlichiosis (HME) (8), and ehrlichiosis in animals (9).

Over the past several decades, the *Anaplasma* and *Ehrlichia* infections are very common in many countries (3, 10–12). *A. bovis* is mainly distributed in Africa, Asia, and South America, and cattle are considered the primary hosts (6). Similarly, *A. ovis* is the leading cause of anaplasmosis in small ruminants, which is widely distributed around the world (13). Recently, *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* have received much attention for their potential threats to public health (7, 14). *A. phagocytophilum* has been detected in sheep, cattle, *Capreolus pygargus*, goats, and humans in different areas of China (15–18). *E. chaffeensis* infections are very common in the United States, with an annual rate of 4.46 cases/1,000,000 population (19). For *A. capra*, it was initially isolated from goats and humans in China (7). Subsequently, it was found in many countries (20, 21).

Qinghai is the source of the Yangtze River, the Yellow River, and the Lancang River, located in the northeast of Qinghai-Tibet Plateau and northwest of China with an average altitude of more than 3,000 meters. The complicated topographic features and changeable climate bless the region with advantageous conditions of rich natural resources. Tibetan sheep (*Ovis aries*) and yaks (*Bos grunniens*) are the main domestic animals in Qinghai and an important source of life and income for herders. Ixodid tick infestation in livestock is a common and severe problem, and more than 25 tick species in six genera have been reported in Qinghai (22, 23). However, information about tick-borne diseases (TBDs) in the region has been limited. Therefore, to better understand the situation of TBDs in Qinghai, China, a molecular epidemiologic study was conducted investigating exposure to *Anaplasma* spp. and *E. chaffeensis* in domestic animals across the area.

MATERIALS AND METHODS

Blood Sample Collection of Yaks and Tibetan Sheep

A total of 566 blood samples of yaks ($n = 330$) and Tibetan sheep ($n = 236$) were collected using random sampling from six sampling sites in Maqin (35°2′38″N, 99°12′5″E; altitude 3,877 m), Dari (33°43′4″N, 99°38′2″E; altitude 4,130 m), and Banma (32°43′24″N, 100°42′41″E; altitude 3,864 m) of Guoluo Tibetan Autonomous Prefecture (GL), and Yushu (32°51′18″N, 96°48′57″E; altitude 4,317 m), Zhiduo (33°37′5″N, 95°58′51″E; altitude 4,177 m) and Qumalai (34°10′15″N, 95°49′57″E; altitude 4,279 m) of Yushu Tibetan Autonomous Prefecture (YS) during June 2020 in Qinghai, China (**Figure 1**). GL and YS are similar in altitude and climate, and both belong to the continental climate of the plateau. Except for about 400 Tibetan sheep in Maqin, the number of yaks and Tibetan sheep in other sampling sites is between 100 and 200. All animals adopt a free grazing system. Ticks and *Melophagus ovinus* and their bites can be seen in Tibetan sheep, while ticks are rarely found on yaks.

Extraction and Quantification of DNA

According to the manufacturer's operation manual, genomic DNA was extracted from 200 uL whole blood samples by the TIANamp Genomic DNA kit (TIANGEN biotech, Beijing). The concentration of the extracted DNA was detected by NanoDrop 2,000 (Thermo Fisher Scientific, USA) and then stored at −20°C for pathogens detection.

Detection of *Anaplasma* spp. and *E. chaffeensis*

Conventional PCR or nested PCR was used to screen for *Anaplasma* spp. and *E. chaffeensis* in extracted DNA. Nested PCRs were employed to detect *A. bovis*, *A. phagocytophilum*, *A. centrale*, *A. platys*, and *E. chaffeensis* based on *16S* rRNA gene. Conventional PCR based on the *msp4* genes was employed to detect *A. marginale* and *A. ovis*, while *16S* rRNA gene for detection of *A. capra*. PCR primers and cycling conditions used in this study, as shown in **Table 1**. The DNAs extracted from the whole blood of Tibetan sheep and yaks infected with *A. bovis*, *A. phagocytophilum*, *A. ovis*, *A. capra*, and *E. chaffeensis* that had been verified by sequencing, were used as a positive control for corresponding PCR reactions; double-distilled water was used as a negative control. The PCR products were detected by 1.5% agarose gel electrophoresis with M5 Hipure Next III Gelred (Mei5 Biotechnology Co., Ltd., Beijing, China) stained.

Sequencing and Phylogenetic Analysis

PCR products of all positive samples for *Anaplasma* spp. and *E. chaffeensis* randomly selected from each sampling site were sequenced by BGI (Beijing, China). The sequence obtained by BGI sequencing was submitted to NCBI for BLASTn search (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and sequence alignment and analysis. The representative nucleotide sequences of this study have been deposited in the GenBank database. Phylogenetic trees were constructed using the neighbor-joining method executed with the p-distance model in MEGA X. Bootstrap values were assessed with 1,000 bootstrap replicates (28, 29).

Statistical Analysis

The data were grouped into four variables according to animal species, gender, sampling sites, and the altitude of sampling

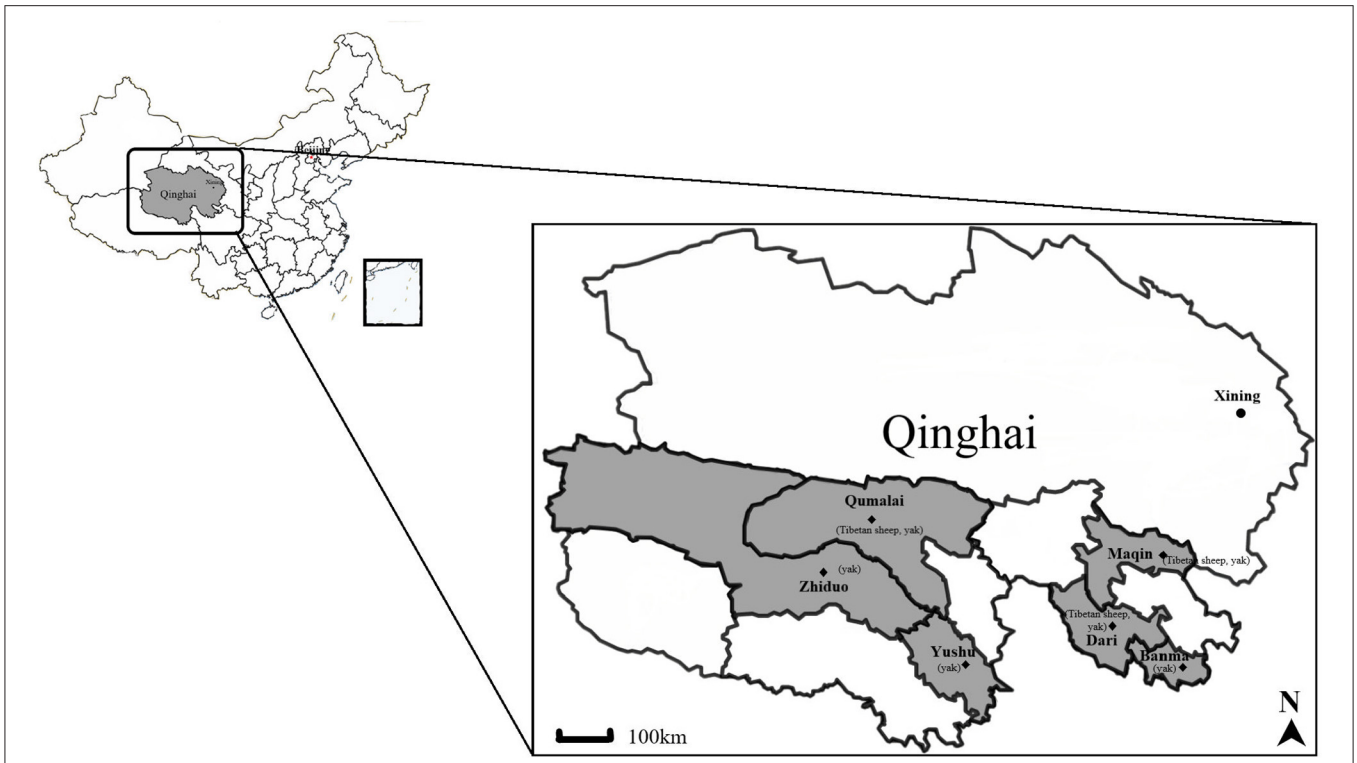

FIGURE 1 | Map of the Qinghai Province, showing the sampling sites and animals included. The yak and Tibetan sheep samples were collected at six sites indicated by ♦. The figure was generated and modified using DITUHUI (https://g.dituhui.com).

sites. The chi-square test was used to calculate the difference of infection rate in SPSS 25.0 software in each group. When $p < 0.05$, the difference was significant.

RESULTS

Prevalence of *Anaplasma* spp. and *E. chaffeensis* in Tibetan Sheep and Yaks

This study identified four *Anaplasma* species and *E. chaffeensis* from Tibetan sheep and yaks (**Table 2**). Of the 566 samples tested, 50% (283/566) were positive for at least one pathogen. The infection rates of *A. bovis* and *A. ovis* were 33.3% and 1.2% in yaks, 5.1% and 80.9% in Tibetan sheep. The infection rates of *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* were 29.4%, 0.6%, and 0.6% in yaks, respectively. This is the first time that *A. capra* and *E. chaffeensis* have been detected in yaks in China. Interestingly, we noticed *A. ovis* infection in yaks and *A. bovis* in Tibetan sheep. The most common co-infection was *A. bovis* and *A. phagocytophilum*, with an infection rate of 28.2% (93/330) in yaks. For co-infection with three pathogens, the infection rate of *A. bovis*, *A. phagocytophilum*, and *A. capra* was 0.6% (2/330), and the infection rate of *A. bovis*, *A. phagocytophilum*, and *E. chaffeensis* was 0.6% (2/330) (**Table 2**). No co-infections by two or more pathogens were detected in Tibetan sheep.

Sequencing and Phylogenetic Analysis

In the current study, 15 representative sequences were obtained and submitted to GenBank (**Table 3**). We compared and analyzed the partial *16S* rRNA gene sequences of *A. bovis*, *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* obtained from blood samples of Tibetan sheep and yaks. BLASTn analysis of the *16S* rRNA gene showed that the *Anaplasma* spp. obtained in this study had 99.04–100% identities to either of *A. bovis*, *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* sequences, respectively. The *E. chaffeensis* sequences (MW048788, MW048789) from yaks were 99.44–100% identical to *E. chaffeensis* isolated from goats (KX505292) in China. Phylogenetic analysis of *16S* rRNA gene sequences confirmed *A. bovis*, *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* in this study (**Figures 2A,B**, **3A,B**). Additionally, we analyzed the *msp4* genomic region of three *A. ovis* (MZ231113-MZ231115) obtained in this study. The results showed that the three sequences were consistent with the homology of the Iranian *A. ovis* (MH790273). *A. ovis* were classified as *A. ovis msp4* Genotypes II based on $T^{366}C^{470}$ (25). Phylogenetic analysis of *msp4* gene sequences confirmed the identity of *A. ovis* in this study (**Figure 4**).

Risk Factors of Tibetan Sheep and Yaks Infected With *Anaplasma* spp. and *E. chaffeensis*

These factors include animal species, gender, sampling sites, and altitude of sampling sites, which were used as variables for statistical analysis of the infection patterns of *Anaplasma* spp. and *E. chaffeensis*. The results indicate that the prevalence of *Anaplasma* spp. and *E. chaffeensis* in female animals was similar

TABLE 1 | Primers used in this study to detect *Anaplasma* spp. and *E. chaffeensis* in Tibetan sheep and yaks in Qinghai, China.

Pathogens	Target gene		Primers (5′ → 3′)	Product (bp)	Annealing temperature (°C)	Reference
A. bovis	*16S* rRNA	EE1	TCCTGGCTCAGAACGAACGCTGGCG	1,430	55	(24)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG			
		AB1f	CTCGTAGCTTGCTATGAGAAC	551	55	(12)
		AB1r	TCTCCCGGACTCCAGTCTG			
A. phagocytophilum	*16S* rRNA	EE1	TCCTGGCTCAGAACGAACGCTGGCG	1,430	55	(24)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG			
		SP2f	GCTGAATGTGGGGATAATTTAT	641	55	(12)
		SP2r	ATGGCTGCTTCCTTTCGGTTA			
A. centrale	*16S* rRNA	EE1	TCCTGGCTCAGAACGAACGCTGGCG	1,430	55	(24)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG			
		AC1f	CTGCTTTTAATACTGCAGGACTA	426	60	(17)
		AC1r	ATGCAGCACCTGTGTGAGGT			
A. platys	*16S* rRNA	EE1	TCCTGGCTCAGAACGAACGCTGGCG	1,430	55	(24)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG			
		Apf	TCCTGGCTCAGAACGAACGCTGGCGGC	506	60	(17)
		APr	AGTCACTGACCCAACCTTAAATGGCTG			
A. marginale/ A. ovis	*msp4*	MSP45	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	870	60	(25)
		MSP43	CCGGATCCTTAGCTGAACAGGAATCTTGC			
A. capra	*16S* rRNA	Capra-F	GCAAGTCGAACGGACCAAATCTGT	1,261	58	(26)
		Capra-R	CCACGATTACTAGCGATTCCGACTTC			
E. chaffeensis	*16S* rRNA	ECB	CGTATTACCGCGGCTGCTGGCA	450	60	(27)
		ECC	AGAACGAACGCTGGCGGCAAGCC			
		HE1	CAATTGCTTATAACCTTTTGGTTATAAAT	3,90	55	(27)
		HE3	TATAGGTACCGTCATTATCTTCCCTAT			

TABLE 2 | The prevalence of *Anaplasma* spp. and *E. chaffeensis* in Tibetan sheep and yaks in Qinghai, China.

Species	Pathogens	GL*				YS*			
		No. infected/(%)				No. infected/(%)			
		Maqin	Dari	Banma	Total	Yushu	Qumalai	Zhiduo	Total
Yak	No. tested	95	35	84	**214**	56	30	30	**116**
	A. bovis	1 (1.1)	0	84 (100)	**85 (39.7)**	21 (37.5)	0	4 (13.3)	**25 (21.6)**
	A. phago[*]	0	0	74 (88.1)	**74 (34.6)**	19 (33.9)	1 (3.3)	3 (10)	**23 (19.8)**
	A. ovis	0	0	0	**0**	0	4 (13.3)	0	**4 (3.5)**
	A. capra	0	0	2 (2.4)	**2 (0.9)**	0	0	0	**0**
	E. chaffeensis	0	0	1 (1.2)	**1 (0.5)**	1 (1.8)	0	0	**1 (0.9)**
	A. bovis + *A. phago*	0	0	74 (88.1)	**74 (34.6)**	16 (28.6)	0	3 (10)	**19 (16.4)**
	A. bovis + *A. phago* + *A. capra*	0	0	2 (2.4)	**2 (0.9)**	0	0	0	**0**
	A. bovis + *A. phago* + *E. chaffeensis*	0	0	1 (1.2)	**1 (0.5)**	1 (1.8)	0	0	**1 (0.9)**
Tibetan sheep	No. tested	143	51	0	**194**	0	42	0	**42**
	A. bovis	12 (8.4)	0	0	**12 (61.9)**	0	0	0	**0**
	A. ovis	109 (76.2)	48 (94.1)	0	**157 (80.9)**	0	34 (81)	0	**34 (81)**

**A. phago = A. phagocytophilum, GL: Guoluo Tibetan Autonomous Prefecture, YS: Yushu Tibetan Autonomous Prefecture.*

to that of male animals ($P > 0.05$). The infection rates of *A. bovis*, *A. phagocytophilum*, and *A. ovis* in yaks in GL and YS were 39.7 and 21.6% ($P = 0.001$), 34.6 and 19.8% ($P = 0.006$), 0 and 3.5% ($P = 0.005$), respectively. In addition, the infection rate of *A. bovis* and *A. phagocytophilum* below 4,000 m was significantly higher than those above 4,000 m ($P = 0.000$). In Tibetan sheep,

TABLE 3 | The DNA sequences submitted to the gene bank in this study.

	Obtained sequences				Reference sequences from GenBank	
Pathogen	**Host**	**Target gene**	**Accession number**	**Length (bp)**	**Identity (%)**	**Accession number (host, country)**
A. bovis	yak	*16S* rRNA	MW048790	516	99.61	MT036513 (sheep, Russia)
	yak	*16S* rRNA	MW048791	525	99.04	MT036513 (sheep, Russia)
	Tibetan sheep	*16S rRNA*	MW048792	524	99.42	MT036513 (sheep, Russia)
	yak	*16S rRNA*	MZ231111	524	99.61	MT036513 (sheep, Russia)
	yak	*16S rRNA*	MZ231112	525	99.81	MN213735 (giraffe, Pakistan)
A. phago	yak	*16S rRNA*	MW048793	620	99.34	MW142385 (*M. ovinus*, China)
	yak	*16S rRNA*	MW048794	617	99.67	MW142385 (*M. ovinus*, China)
	yak	*16S rRNA*	MZ231109	618	99.83	MW142385 (*M. ovinus*, China)
	yak	*16S rRNA*	MZ231110	617	99.67	MW142385 (*M. ovinus*, China)
A. capra	yak	*16S rRNA*	MW577114	1106	100	MF066918 (sheep, Gansu)
A. ovis	Tibetan sheep	*msp4*	MZ231113	826	100	MH790273 (sheep,Iran)
	Tibetan sheep	*msp4*	MZ231114	824	100	MH790273 (sheep,Iran)
	Tibetan sheep	*msp4*	MZ231115	824	100	MH790273 (sheep,Iran)
E. chaffeensis	yak	*16S* rRNA	MW048788	360	100	KX505292 (goat, China)
	yak	*16S* rRNA	MW048789	362	99.44	KX505292 (goat, China)

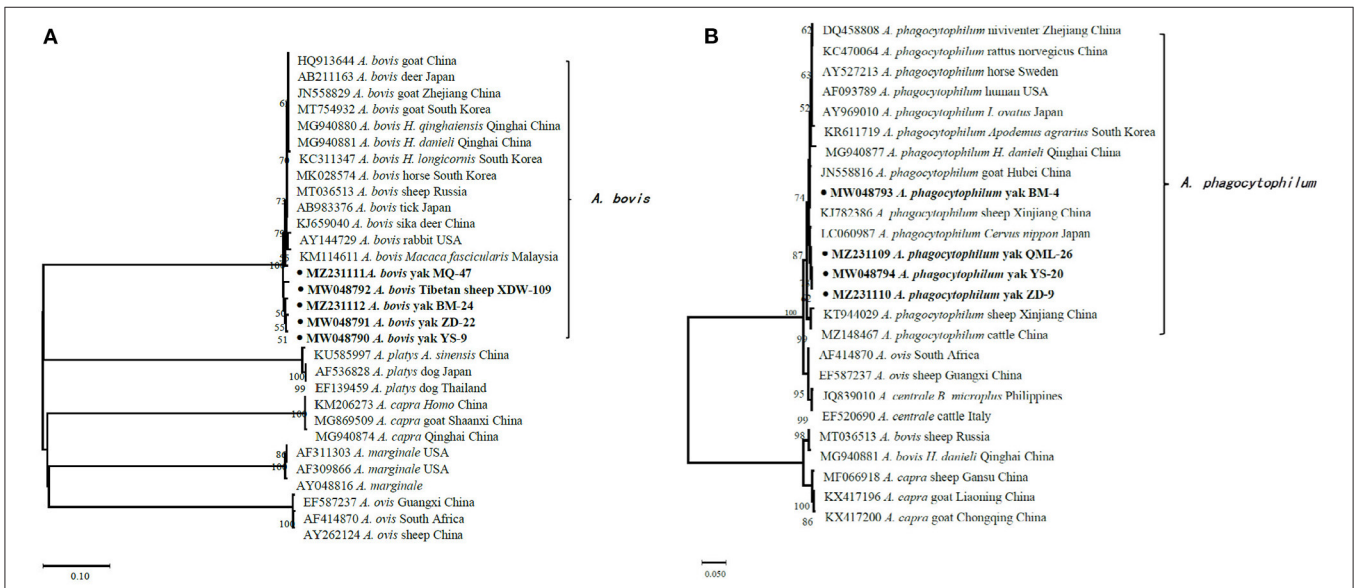

FIGURE 2 | Phylogenetic trees were constructed based on partial sequences of the *16S* rRNA genes of *A. bovis* **(A)** and *A. phagocytophilum* **(B)**. Phylogenetic trees were con-structed by the neighbor-joining method with the p-distance model using the MEGA X software, and the bootstrap test was assessed with 1,000 replicates. The species identified in this study are indicated by • and highlighted in bold.

the infection rate of *A. ovis* above 4,000 m was higher than that below 4,000 m ($P = 0.022$) (**Table 4**).

DISCUSSION

In the present study, *Anaplasma* spp. and *E. chaffeensis* were investigated in domestic animals in Qinghai, China. Four *Anaplasma* species (*A. bovis*, *A. phagocytophilum*, *A. ovis*, and *A. capra*) and *E. chaffeensis* were identified in Tibetan sheep and yaks. Among them, *E. chaffeensis* and *A. capra* were detected in yaks for the first time in China.

The genus *Anaplasma* are widely distributed in domestic animals, wild animals, ticks, and other vectors (23, 30–32). This study found relatively high *A. ovis* infection rates of 76.2, 94.1, and 81.3% in Tibetan sheep in three sampling sites, Maqin, Dari, and Qumalai, respectively, which is higher than in sheep

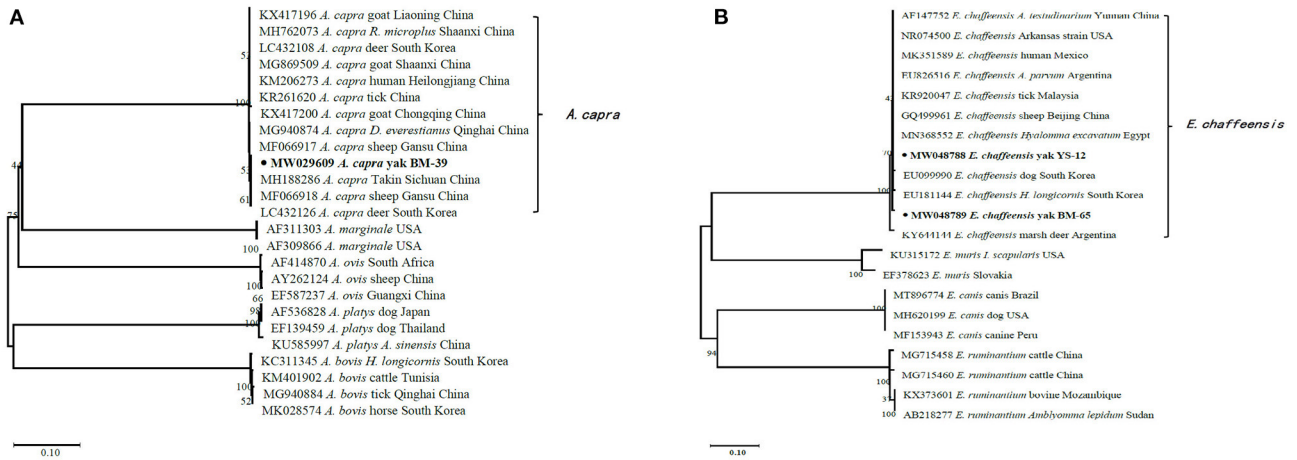

FIGURE 3 | Phylogenetic trees were constructed based on partial sequences of the 16S rRNA genes of *A. capra* **(A)** and *E. chaffeensis* **(B)**. Phylogenetic trees were constructed by the neighbor-joining method with the p-distance model using the MEGA X software, and the bootstrap test was assessed with 1,000 replicates. The species identified in this study are indicated by • and highlighted in bold.

in Xinjiang (40.5%) (16) and Gansu (5.7%) (33), and Tibetan sheep in northeast Qinghai (58%) (34). An explanation for higher infection rates of *A. ovis* in this area could be the bites of ticks and other arthropods. Ticks and *M. ovinus* were found in Tibetan sheep in Maqin and Dari, and data on that *M. ovinus* carried *A. ovis* has been reported in our previous study (31). In addition, we carried out the comparative analysis and phylogenetic analysis of the *msp4* gene sequence of *A. ovis* (25). The results showed that the *A. ovis* strains isolated from Tibetan sheep were identical to those isolated in *M. ovinus* in our previous study (31). Whereas, the *A. ovis* isolated from *Dermacentor nuttalli* in Qinghai by Han et al. (23) belongs to genotypes III, which is in the same clade as those obtained from sheep in Italy (**Figure 4**) (25). Genotypes II and III were also isolated from *M. ovinus* in Xinjiang by Zhao et al. (35). Remarkably, an *A. ovis* variant was reported in humans (36), indicating that this agent has zoonotic potential. Taken together, there are two *A. ovis* genotypes prevalent in domestic animals in northwest China, and arthropods (including *M. ovinus* and ticks) may be the main vectors of *A. ovis*.

A. phagocytophilum and *A. bovis* are frequently detected in ruminants around the world. This study confirms that both *A. phagocytophilum* and *A. bovis* can infect yaks. The infection rate of *A. phagocytophilum* in yaks (29.4%) in this study was higher than that reported in sheep (9.9%), dairy cattle (12%), and white yaks (5.3%) in other areas of China (1, 13, 37), and lower than that in *C. pygargus* (33.3%) from Heilongjiang China (17). Since the first case of HGA, caused by *A. phagocytophilum*, was reported in Anhui, China (38), HGA has been reported in the USA, Europe, Africa, and Asia (11, 39, 40). For *A. bovis*, the infection rate in yaks (33.3%) was higher than that in cattle (4.8%) and white yaks (6.2%) from China (16, 37), cattle (1.0%) from South Korea (20). Recent studies have shown that climate, altitude, longitude, latitude, season, tick bites, contact with wild animals, and feeding methods are important factors affecting *Anaplasma* infection (41). Previous reports have shown that *Haemaphysalis qinghaiensis*, *Dermacentor abaensis*, *D. nuttalli*, and *Dermacentor silvarum* are common ectoparasites among grazing livestock in high altitude areas (2,800 to 4,300 m), and the risk of tick bites with *Anaplasma* spp. was related to altitude and tick species (23). Our results also showed that the risk of infection with *Anaplasma* spp. in Tibetan sheep and yaks is mainly related to altitude and sampling sites. Furthermore, all animals in this study adopted a free grazing system, which increased the risk of domestic animals being exposed to ticks.

A. capra is a novel *Anaplasma* species that emerged in recent years. The novel species was first found in goats and then in sheep (30), *C. pygargus* (17), dogs (42), and ticks (23) in China. In addition, *A. capra* has also been detected in goats, cattle, and *Hydropotes inermis argyropus* in South Korea (32, 43), cattle in Malaysia (10), and *Cervus elaphus* and *Rucervus duvaucelii* in France (21). In 2015, it was isolated from the blood samples of patients with a history of tick bites in northeastern China (7). Subsequently, Peng et al. (44) confirmed the ability of *A. capra* to infect human erythrocytes, HL-60 and TF-1, and further confirmed its zoonotic characteristics. In this study, we detected *A. capra* DNA in yaks in China for the first time. In Qinghai, *H. qinghaiensis* is the most dominant tick species infected with *A. capra*, followed by *D. abaensis* and *D. nuttalli* (23). The above evidence suggests that *A. capra* is widely distributed and could infect a wide range of hosts.

Ehrlichia species include *E. chaffeensis*, *E. canis*, *E. ewingii*, *E. equi*, *E. muris*, and *E. ruminantium*. These species have been detected in many ticks in China, for instance, *Amblyomma testudinarium*, *Haemaphysalis yeni*, *Haemaphysalis longicornis*, *Ixodes sinensis*, *D. silvarum*, *Rhipicephalus sanguineus*, and *Rhipicephalus microplus* (45–48). In previous studies, *E. canis*

FIGURE 4 | Phylogenetic trees were constructed based on the msp4 genes of *A. ovis*. Phylogenetic trees were constructed by the neighbor-joining method with the p-distance model using the MEGA X software, and the bootstrap test was assessed with 1,000 replicates. The species identified in this study are indicated by • and highlighted in bold.

infection was detected in *Cervus nippon* in Gansu (49), and high infection rates of *E. canis* and *E. chaffeensis* were reported in dogs, cattle, sheep, goats, donkeys, and humans in Xinjiang (9, 18, 50). *Ehrlichia* species were also detected in birds and small mammals in other parts of China (51, 52). In the current study, the prevalence rate of *E. chaffeensis* was 0.61%. We present the first report of *Ehrlichia* infection caused by *E. chaffeensis* in yaks in China. However, it is unclear which ticks are responsible for the pathogen. Therefore, further study is needed to determine the vector or reservoir host for this pathogen.

Moreover, mixed-infection is also an important issue that would need to be considered in livestock. The present study results illustrate that mixed infection of *A. phagocytophilum* and *A. bovis* are very common in yaks in Qinghai. Co-infection involving three *Anaplasma* species of *A. bovis*, *A. phagocytophilum,* and *A. capra* was also observed in two yaks in this study. In addition, we found that two yaks were co-infected with *A. bovis*, *A. phagocytophilum* and *E. chaffeensis*. Currently, *A. phagocytophilum*, *A. capra*, and *E. chaffeensis* have been recognized as causative agents of human infection. Mixed-infection of tick-borne pathogens has also been observed in animals in other countries and regions (1, 30, 34, 53). Above all, co-infection of tick-borne pathogens emphasizes the need for differential diagnosis of these pathogens in animal hosts and humans to improve the prevention and control of TBDs.

Notably, all pathogens were detected from apparently healthy animals in this study, consistent with other studies (54–56). This indicates that the appearance of clinical symptoms is mainly dependent on the pathogenicity of these pathogens strains and the breed or species of the infected animals (54). Alternatively, these animals have previously been infected with these pathogens and developed immunity against these pathogens (56). Further research is necessary to confirm these speculations.

TABLE 4 | Patterns of *Anaplssasma* spp. and *E. chaffeensis* prevalence in the yaks and Tibetan sheep, grouped by animal species, gender, sampling sites, and the altitude of sampling sites.

Parameter		Yak							Tibetan sheep				
		No. infected/(%)							No. infected/(%)				
		A. bovis	*p*-value	*A. phago*	*p*-value	*A. ovis*	*p*-value	*A. capra*	*p*-value	*E. chaffeensis*	*p*-value	*A. bovis*	*p*-value	*A. ovis*	*p*-value
Gender	Female	69 (33.8)	0.810	58 (28.4)	0.625	3 (1.5)	0.585	0	0.071	2 (1)	0.265	10 (4.8)	0.597	168 (80.8)	0.862
	Male	41 (32.5)		39 (31)		1 (0.8)		2 (1.6)		0		2 (7.1)		23 (82.1)	
Sampling sites	GL	85 (39.7)	**0.001***	74 (34.6)	**0.006**	0	**0.005**	2 (0.9)	0.296	1 (0.5)	0.659	12 (61.9)	0.098	157 (80.9)	0.997
	YS	25 (21.6)		23 (19.8)		4 (3.5)		0		1 (0.9)		0		34 (81)	
Altitude	3,500–4,000 m	85 (47.5)	**0.000**	74 (41.3)	**0.000**	0	**0.028**	2 (1.1)	0.193	1 (0.6)	0.904	12 (8.4)	**0.04**	109 (76.2)	**0.022**
	>4000 m	25 (16.6)		23 (15.2)		4 (2.7)		0		1 (0.7)		0		82 (88.2)	

*, *Bold indicates significant difference.*

In conclusion, we investigated the epidemic situation of the TBDs in yaks and Tibetan sheep in Qinghai province, China, and confirmed that Tibetan sheep and yaks could be infected with *A. bovis*, *A. phagocytophilum*, *A. ovis*, *A. capra*, and *E. chaffeensis*. This is the first report of *A. capra* and *E. chaffeensis* infection in yaks in China. These pathogens could pose a significant threat to livestock and human health. Thus, future studies should focus more on systematically assessing these pathogens' threats to veterinary and public health.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences approved the procedures of collecting blood samples from Tibetan sheep and yaks, and obtained the livestock owner's consent. Written informed consent for participation was not obtained from the owners because all the samples in this study were collected by local veterinarians during the daily epidemic surveillance.

AUTHOR CONTRIBUTIONS

YW: investigation, conceptualization, methodology, data curation, visualization, writing—original draft, and writing—review & editing. QZ: investigation, methodology, visualization, writing—original draft, and writing—review & editing. YL: investigation, methodology, data curation, and funding acquisition. SH: investigation, methodology, and writing—original draft. BW, GY, PZ, ZY, and HZ: investigation and methodology. YS, XH, and JC: investigation. HH: investigation, visualization, supervision, validation, writing—review & editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Regular Assistance Project of the International Department of the Ministry of Science and Technology of China (KY201904013), the Chinese Academy of Sciences (CZBZX-1), National Forestry, and Grassland Administration, China.

ACKNOWLEDGMENTS

We want to express our heartfelt thanks to all the herdsmen and all the Guoluo and Yushu Animal Disease Prevention and Control Center staff for their strong support for this study, enabling us to smoothly carry out the research.

REFERENCES

1. Li Y, Galon EM, Guo Q, Rizk MA, Moumouni PFA, Liu M. et al. Molecular detection and identification of *Babesia* spp, *Theileria* spp, and *Anaplasma* spp in sheep from border regions, northwestern China. *Front Vet Sci.* (2020) 7:630. doi: 10.3389/fvets.2020.00630
2. Von Fricken ME, Voorhees MA, Koehler JW, Asbun C, Lam B, Qurollo B, et al. Molecular characteristics of *Rickettsia* in ticks collected along the southern border of mongolia. *Pathogens.* (2020) 9:943. doi: 10.3390/pathogens9110943
3. Liu Z, Ma M, Wang Z, Wang J, Peng Y, Li Y, et al. Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Appl Environ Microbiol.* (2012) 78:464–70. doi: 10.1128/AEM.06848-11
4. Yang J, Liu Z, Niu Q, Mukhtar MU, Guan G, Liu G, et al. A novel genotype of "*Anaplasma capra*" in wildlife and its phylogenetic relationship with the human genotypes. *Emerg Microbes Infect.* (2018) 7:210. doi: 10.1038/s41426-018-0212-0
5. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, et al. 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.* (2001) 51:2145–65. doi: 10.1099/00207713-51-6-2145
6. Battilani M, de Arcangeli S, Balboni A, Dondi F. Genetic diversity and molecular epidemiology of *Anaplasma*. *Infect Genet Evol.* (2017) 49:195–211. doi: 10.1016/j.meegid.2017.01.021
7. Li H, Zheng Y, Ma L, Jia N, Jiang B, Jiang R, et al. Human infection with a novel tick-borne *Anaplasma* species in China: a surveillance study. *Lancet Infect Dis.* (2015) 15:663–70. doi: 10.1016/S1473-3099(15)70051-4
8. Peng SH, Yang SL, Ho YN, Chen HF, Shu PY. Human case of *Ehrlichia chaffeensis* infection, Taiwan. *Emerg Infect Dis.* (2019) 25:2141–3. doi: 10.3201/eid2511.190665
9. Chahan B, Jian Z, Xuan X, Sato Y, Kabeya H, Tuchiya K, et al. Serological evidence of infection of *Anaplasma* and *Ehrlichia* in domestic animals in xinjiang uygur autonomous region area, China. *Vet Parasitol.* (2005) 134:273–8. doi: 10.1016/j.vetpar.2005.07.024
10. Koh FX, Panchadcharam C, Sitam FT, Tay ST. Molecular investigation of *Anaplasma* spp. in domestic and wildlife animals in Peninsular Malaysia. *Vet Parasitol Reg Stud Reports.* (2018) 13:141–7. doi: 10.1016/j.vprsr.2018.05.006
11. Rodino KG, Theel ES, Pritt BS. Tick-borne diseases in the United States. *Clin Chem.* (2020) 66:537–48. doi: 10.1093/clinchem/hvaa040
12. Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki A, et al. Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. *Appl Environ Microbiol.* (2006) 72:1102–9. doi: 10.1128/AEM.72.2.1102-1109.2006
13. Yan Y, Jiang Y, Tao D, Zhao A, Qi M, Ning C. Molecular detection of *Anaplasma* spp. in dairy cattle in southern Xinjiang, China. *Vet Parasitol Reg Stud Reports.* (2020) 20:100406. doi: 10.1016/j.vprsr.2020.100406
14. Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuiston JH. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000-2007. *Am J Trop Med Hyg.* (2011) 85:124–31. doi: 10.4269/ajtmh.2011.10-0613
15. Yang J, Liu Z, Guan G, Liu Q, Li Y, Chen Z, et al. Prevalence of *Anaplasma phagocytophilum* in ruminants, rodents and ticks in Gansu, north-western China. *J Med Microbiol.* (2013) 62:254–8. doi: 10.1099/jmm.0.046771-0
16. Yang J, Li Y, Liu Z, Liu J, Niu Q, Ren Q, et al. Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. *Parasit Vectors.* (2015) 8:108. doi: 10.1186/s13071-015-0727-3
17. Wang H, Yang J, Mukhtar MU, Liu Z, Zhang M, Wang X. 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) 12:296. doi: 10.1186/s13071-019-3553-1
18. Zhang L, Liu H, Xu B, Zhang Z, Jin Y, Li W, et al. Rural residents in China are at increased risk of exposure to tick-borne pathogens *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. *Biomed Res Int.* (2014) 2014:313867. doi: 10.1155/2014/313867
19. Mogg M, Wang HH, Baker A, Derouen Z, Borski J, Grant WE. Increased Incidence of *Ehrlichia chaffeensis* infections in the United States, 2012 through 2016. *Vector Borne Zoonotic Dis.* (2020) 20:547–50. doi: 10.1089/vbz.2019.2595
20. Seo MG, Ouh IO, Lee H, Geraldino PJL, Rhee MH, Kwon OD, et al. Differential identification of *Anaplasma* in cattle and potential of cattle to serve as reservoirs of *Anaplasma capra*, an emerging tick-borne zoonotic pathogen. *Vet Microbiol.* (2018) 226:15–22. doi: 10.1016/j.vetmic.2018.10.008
21. Jouglin M, Blanc B, De La Cotte N, Bastian S, Ortiz K, Malandrin L. First detection and molecular identification of the zoonotic *Anaplasma capra* in deer in France. *PLoS ONE.* (2019) 14:e0219184. doi: 10.1371/journal.pone.0219184
22. Chen Z, Yang X, Bu F, Yang X, Yang X, Liu J. Ticks (acari: ixodoidea: argasidae, ixodidae) of China. *Exp Appl Acarol.* (2010) 51:393–404. doi: 10.1007/s10493-010-9335-2
23. Han R, Yang JF, Mukhtar MU, Chen Z, Niu QL, Lin YQ, et al. Molecular detection of *Anaplasma* infections in ixodid ticks from the qinghai-tibet plateau. *Infect Dis Poverty.* (2019) 8:1–8. doi: 10.1186/s40249-019-0522-z
24. Barlough JE, Madigan JE, DeRock E, Bigornia L. Nested polymerase chain reaction for detection of *Ehrlichia equi* genomic DNA in horses and ticks (*Ixodes pacificus*). *Veterinary Parasitology.* (1996) 63:319–29. doi: 10.1016/0304-4017(95)00904-3
25. De La Fuente J, Atkinson MW, Naranjo V, de Mera IGF, Mangold AJ, Keating KA, et al. Sequence analysis of the msp4 gene of Anaplasma ovis strains. *Vet Microbiol.* (2007) 119:375–81. doi: 10.1016/j.vetmic.2006.09.011
26. 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. doi: 10.1186/s13071-016-1886-6
27. Dawson JE, Stallknecht DE, Howerth EW, Warner C, Biggie K, Davidson WR, et al. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to infection with *Ehrlichia chaffeensis*, the etiologic agent of human ehrlichiosis. *J Clin Microbiol.* (1994) 32:2725–8. doi: 10.1128/jcm.32.11.2725-2728.1994
28. Kumar S, Tamura K, Nei M, MEGA. Molecular evolutionary genetics analysis software for microcomputers. *Comput Appl Biosci.* (1994) 10:189–91. doi: 10.1093/bioinformatics/10.2.189
29. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* (1987) 4:406–25. doi: 10.1093/oxfordjournals.molbev.a040454
30. Shi Y, Yang J, Guan G, Liu Z, Luo J, Song M. Molecular investigation of *Anaplasma* species in sheep from Heilongjiang province, northeast China identified four *Anaplasma* species and a novel genotype of *Anaplasma capra*. *Parasitol Int.* (2020) 76:102072. doi: 10.1016/j.parint.2020.102072
31. Zhang QX, Wang Y, Li Y, Han SY, Wang B, Yuan GH, et al. Vector-borne pathogens with veterinary and public health significance in *Melophagus ovinus* (sheep ked) from the Qinghai-Tibet Plateau. *Pathogens.* (2021) 10:249. doi: 10.3390/pathogens10020249
32. Amer S, Kim S, Yun Y, Na KJ. Novel variants of the newly emerged *Anaplasma capra* from Korean water deer (*Hydropotes inermis argyropus*) in South Korea. *Parasit Vectors.* (2019) 12:365. doi: 10.1186/s13071-019-3622-5
33. Yang J, Han R, Niu Q, Liu Z, Guan G, Liu G, et al. Occurrence of four *Anaplasma* species with veterinary and public health significance in sheep, northwestern China. *Ticks Tick Borne Dis.* (2018) 9:82–5. doi: 10.1016/j.ttbdis.2017.10.005
34. 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) 11:101466. doi: 10.1016/j.ttbdis.2020.101466
35. 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. doi: 10.1186/s13071-018-2788-6
36. Chochlakis D, Ioannou I, Tselentis Y, Psaroulaki A. Human anaplasmosis and *Anaplasma ovis* variant. *Emerg Infect Dis.* (2010) 16:1031–2. doi: 10.3201/eid1606.090175

37. Yang J, Liu Z, Niu Q, Liu J, Guan G, Xie J, et al. First molecular survey and identification of *Anaplasma* spp. in white yaks (bos grunniens) in China. *Parasitology.* (2016) 143:686–91. doi: 10.1017/S003118201600041X
38. Zhang L, LY, Ni D, Li Q, Yu Y, Yu XJ, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA.* (2008) 300:2263–70. doi: 10.1001/jama.2008.626
39. Zhang L, Wang G, Liu Q, Chen C, Li J, Long B, et al. Molecular analysis of *Anaplasma phagocytophilum* isolated from patients with febrile diseases of unknown etiology in China. *PLoS ONE.* (2013) 8:e57155. doi: 10.1371/journal.pone.0057155
40. Negi T, Kandari LS, Arunachalam K. Update on prevalence and distribution pattern of tick-borne diseases among humans in India: a review. *Parasitol Res.* (2021) 120:1523–39. doi: 10.1007/s00436-021-07114-x
41. Noaman V. Epidemiological study on *Anaplasma phagocytophilum* in cattle: molecular prevalence and risk factors assessment in different ecological zones in Iran. *Prev Vet Med.* (2020) 183:105118. doi: 10.1016/j.prevetmed.2020.105118
42. Shi K, Li J, Yan Y, Chen Q, Wang K, Zhou Y, et al. Dogs as new hosts for the emerging zoonotic pathogen *Anaplasma capra* in China. *Front Cell Infect Microbiol.* (2019) 9:394. doi: 10.3389/fcimb.2019.00394
43. Seo HJ, Jin BC, Kim KH, Yoo MS, Seong KW, Jeong SJ, et al. Molecular detection and phylogenetic analysis of *Anaplasma* spp. in Korean native goats from ulsan metropolitan city, Korea. *Vector Borne Zoonotic Dis.* (2019) 19:773–6. doi: 10.1089/vbz.2018.2374
44. Peng Y, Lu C, Yan Y, Song J, Pei Z, Gong P, et al. The novel zoonotic pathogen, *Anaplasma capra*, infects human erythrocytes, HL-60, and TF-1 cells in vitro. *Pathogens.* (2021) 10:600. doi: 10.3390/pathogens10050600
45. Hou J, Ling F, Liu Y, Zhang R, Song X, Huang R, et al. A molecular survey of *Anaplasma*, *Ehrlichia*, *Bartonella* and *Theileria* in ticks collected from southeastern China. *Exp Appl Acarol.* (2019) 79:125–35. doi: 10.1007/s10493-019-00411-2
46. Chen Z, Liu Q, Liu JQ, Xu BL, Lv S, Xia S, et al. Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. *Parasit Vectors.* (2014) 7:237. doi: 10.1186/1756-3305-7-237
47. Cao WC, Gao YM, Zhang PH, Zhang XT, Dai QH, Dumler JS, et al. Identification of *Ehrlichia chaffeensis* by nested PCR in ticks from southern China. *J Clin Microbiol.* (2000) 38:2778–80. doi: 10.1128/JCM.38.7.2778-2780.2000
48. Dong T, Qu Z, Zhang L. Detection of A phagocytophilum and E chaffeensis in patient and mouse blood and ticks by a duplex real-time PCR assay. *PLoS ONE.* (2013) 8:e74796. doi: 10.1371/journal.pone.0074796
49. Li Y, Chen Z, Liu Z, Liu J, Yang J, Li Q, et al. Molecular survey of *Anaplasma* and *Ehrlichia* of red deer and sika deer in gansu, China in 2013. *Transbound Emerg Dis.* (2016) 63:e228–36. doi: 10.1111/tbed.12335
50. Mengfan Q, Lixia W, Ying L, Yan R, Kuojun C, Jinsheng Z, et al. Molecular detection and genetic variability of *Ehrlichia canis* in pet dogs in Xinjiang, China. *Vet World.* (2020) 13:916–22. doi: 10.14202/vetworld.2020.916-922
51. Yang J, Liu Z, Niu Q, Tian Z, Liu J, Guan G, et al. Tick-borne zoonotic pathogens in birds in Guangxi, Southwest China. *Parasit Vectors.* (2015) 8:637. doi: 10.1186/s13071-015-1249-8
52. Du CH, Liu HB, Wei R, Jongejan F, Gao ZH, Shao ZT, et al. Investigation on *Ehrlichia* infection in small mammals and ticks from tengchong, Yunnan Province, Southern China. *Vector Borne Zoonotic Dis.* (2018) 18:563–6. doi: 10.1089/vbz.2017.2205
53. Miranda EA, Han SW, Cho YK, Choi KS, Chae JS. Co-infection with *Anaplasma* species and novel genetic variants detected in cattle and goats in the Republic of Korea. *Pathogens.* (2021) 10:28. doi: 10.3390/pathogens10010028
54. Chatanga E, Kainga H, Maganga E, Hayashida K, Katakura K, Sugimoto C, et al. Molecular identification and genetic characterization of tick-borne pathogens in sheep and goats at two farms in the central and southern regions of Malawi. *Ticks Tick Borne Dis.* (2021) 12:101629. doi: 10.1016/j.ttbdis.2020.101629
55. Sazmand A, Harl J, Eigner B, Hodzic A, Beck R, Hekmatimoghaddam S, et al. Vector-borne bacteria in blood of camels in Iran: new data and literature review. *Comp Immunol Microbiol Infect Dis.* (2019) 65:48–53. doi: 10.1016/j.cimid.2019.04.004
56. Ringo AE, Aboge GO, Adjou Moumouni PF, Lee SH, Jirapattharasate C, Liu M, et al. Molecular detection and genetic characterisation of pathogenic *Theileria*, *Anaplasma* and *Ehrlichia* species among apparently healthy sheep in central and western Kenya. *Onderstepoort J Vet Res.* (2019) 86:e1–8. doi: 10.4102/ojvr.v86i1.1630

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.