First molecular survey and identification of *Anaplasma* spp. in white yaks (*Bos grunniens*) in China

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SUMMARY

Anaplasmosis is caused by a group of obligate intracellular bacteria in the genus *Anaplasma*, which are transmitted by ticks and infect humans, domestic animals and wildlife. This study was conducted to determine the prevalence and molecular characterization of *Anaplasma* spp. in semi-wild white yaks sampled in Tianzhu Tibetan Autonomous County, northwest China. Out of 332 samples tested, 35 (10·9%) were positive for *Anaplasma* spp. The positive rates were 6·2% (20/322) and 5·3% (17/322) for *Anaplasma bovis* and *Anaplasma phagocytophilum* in white yaks, respectively. None of the sample was positive for *Anaplasma marginale*. Two (0·6%) samples were simultaneously positive to *A. bovis* and *A. phagocytophilum*. Sequence analysis of the 16S rRNA gene revealed two genotypes (ApG1 and ApG2) of *A. phagocytophilum* and two sequence types (ST1 and ST2) of *A. bovis* in white yaks. This study is the first to document the presence of *Anaplasma* in white yaks. Our findings extend the host range for *Anaplasma* species and provide more valuable information for the control and management of anaplasmosis in white yaks.

Key words: 16S rRNA gene, *Anaplasma phagocytophilum*, *Anaplasma bovis*, white yak, tick-borne disease.

INTRODUCTION

Anaplasmosis is a tick-borne infectious disease of a variety of wild and domestic animals and human beings throughout the world. It is caused by a group of obligate intracellular bacteria in the genus *Anaplasma* (Rickettsiales: *Anaplasmataceae*), including *Anaplasma phagocytophilum, Anaplasma marginale, Anaplasma ovis, Anaplasma bovis* and *Anaplasma platys* (Dumler *et al*. 2001). Among those, three *Anaplasma* species (*A. phagocytophilum, A. marginale* and *A. bovis*) have been detected in cattle and recognized as the agents of bovine anaplasmosis in China (Bai *et al*. 1987; Zhang *et al*. 2013; Yang *et al*. 2015). Recently, an *A. platys*-like pathogen was also identified in cattle from Xinjiang, Northwest China (Yang *et al*. 2015).

The white yak (*Bos grunniens*) is a semi-wild and endemic species in Tianzhu Tibetan Autonomous County (TTAC) that relies heavily on white yaks farming for milk, meat and local economy. These animals inhabit in the alpine steppe ecoregion at altitude over 3000 m, with a population of ~49 400. Previous studies have reported that the Tianzhu white yaks are infected with *Chlamydia abortus* (Qin *et al*. 2015*a*). Moreover, a high seroprevalence (17·76%) of tick-borne *Babesia bigemina* was also observed in them (Qin *et al*. 2015*b*). Apart from the above reports, information on the *Anaplasma* infection is currently not available. The objective of this study was to determine whether and what species of *Anaplasma* agents infect white yaks in TTAC in Gansu Province, Northwest China. The molecular characterization of the identified *Anaplasma* strains was further analysed.

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MATERIALS AND METHODS

Study sites

This study was carried out in TTAC in Gansu Province, Northwest China, with a total area of 7149 km^2. Sampling sites were located between longitude 102°07′–103°46′ east and latitude 36°31′–37°55′ north in Gansu Province, Northwest China. The annual average temperature here is −8 to 4 °C, having an obvious vertical distribution of temperature.

Blood sampling and DNA preparation

The surveillance was performed from March to July in 2015 during the peak season of tick in rural areas

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Table 1. Primers and PCR amplification conditions

Pathogen and target gene	Primer name	Primer sequence (5′–3′)	Annealing temp (°C)	Amplicon size (bp)
A. *phagocytophilum*, 16S rRNA	EC9	TACCTTGTTACGACTT	55	1462
	EC12a	TGATCCTGGCTCAGAACGAACG		
	SSAP2f	GCTGAATGTGGGGATAATTTAT	55	641
	SSAP2r	ATGGCTGCTTCCTTTCGGTTA		
A. *bovis*, 16S rRNA	EC9	TACCTTGTTACGACTT	55	1462
	EC12a	TGATCCTGGCTCAGAACGAACG		
	AB1f	CTCGTAGCTTGCTATGAGAAC	55	551
	AB1r	TCTCCCGGACTCCAGTCTG		
A. *marginale, msp4*	MSP45	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	55	869
	MSP43	CCGGATCCTTAGCTGAACAGGAATCTTGC		
A. *marginale, msp5*	AM1	TGTCTAACCTTCTGCTGTTCGTTGC	60	643
	AM2	ACACGAAACTGTACCACTGCCATGC		

in TTAC. EDTA whole-blood samples were taken from the jugular vein of 332 white yaks and collected in a sterile tube. Total DNA was extracted from 300 μL of blood using the Gentra Puregene DNA purification kit (Qiagen, Beijing, China) following the instructions of the manufacturer.

PCR and sequence analysis

The total DNA was detected by nested PCR for the presence of *A*. *phagocytophilum* and *A*. *bovis* 16S rRNA gene and *A*. *marginale* major surface protein 4 (*msp4*) and major surface protein 5 (*msp5*) genes as previously described (de la Fuente *et al*. 2001; Kawahara *et al*. 2006; Zhou *et al*. 2007; Zhang *et al*. 2013). The PCR primers for 16S rRNA, *msp4* and *msp5* amplification were included in Table 1. The reaction was performed in an automatic thermocycler (Bio-Rad, Hercules, CA, USA) in a total volume of 25 μL containing 2·5 μL of 10 × PCR buffer (Mg^{2+} Plus), 2·0 μL of each dNTP at 2·5 mM, 1·25 U of *Taq* DNA polymerase (TaKaRa, Dalian, China), 2·0 μL of template DNA, 1·0 μL of each primer (20 pM) and 16·25 μL of distilled water. DNA extracted from whole blood of sheep infected with *A*. *phagocytophilum* and cattle infected with *A*. *marginale* and *A*. *bovis* was used as the positive control, and sterile water was used as the negative control for each run. Cycling conditions for PCR amplification were: 4 min of denaturation at 94 °C, 35 cycles at 94 °C for 30 s, annealing for 30 s (annealing temperatures of primers was listed in Table 1), and 72 °C for 1–1·5 min (dependent on the target gene), with a final extension step at 72 °C for 10 min. PCR products were determined by UV transillumination in a 1·0% agarose gel following electrophoresis and staining with ethidium bromide.

Positive PCR products were purified (TaKaRa Agarose Gel DNA purification Kit Ver.2·0, TaKaRa, China), cloned into pGEM-T Easy vector (Promega, USA) and transformed into *Escherichia coli* JM109 competent cells (TaKaRa, China). Two recombinant clones were selected for sequencing using BigDye Terminator Mix (Sangon, China). The obtained sequences were analysed by a BLASTn search in GenBank or by using the Clustal W method in the MegAlign software (DNAStar, Madison, WI). Phylogenetic trees were constructed based on the sequence distance method using the neighbour-joining (NJ) algorithm with the Kimura two-parameter model of the Mega 4·0 Software (Tamura *et al*. 2007).

Nucleotide sequence accession numbers

The GenBank accession numbers obtained in this study were as follows: KT824824–KT824833 (Ap20-a, Ap20-b, Ap31-a, Ap31-b, Ap54-a, Ap54-b, Ap5-a, Ap5-b, Ap12-a and Ap12-b) for *A*. *phagocytophilum* and KT824834–KT824851 (Ab20, Ab21-b, Ab21-c, Ab23-a, Ab23-b, Ab26-a, Ab26-b, Ab30-a, Ab30-b, Ab6-a, Ab6-b, Ab9-a, Ab9-b, Ab11-a, Ab11-b, Ab19-a, Ab19-b, Ab20-a) for *A*. *bovis*.

RESULTS

Out of 332 samples tested, 35 (10·9%) were positive for *Anaplasma* spp. The positive rates were 6·2% (20/322) and 5·3% (17/322) for *A*. *bovis* and *A*. *phagocytophilum* in white yaks, respectively. None of the sample was positive for *A*. *marginale*. Two (0·6%) samples were simultaneously positive to *A*. *bovis* and *A*. *phagocytophilum*.

To characterize the *Anaplasma* spp. detected in yaks, positive samples were sequenced. Twenty-eight sequences were obtained in this study: 10 for *A*. *phagocytophilum* and 18 for *A*. *bovis*. The partial 16S rRNA gene sequences of *A*. *phagocytophilum* (599 bp) and *A*. *bovis* (511 bp) were analysed. Alignment of these sequences revealed two genotypes of *A*. *phagocytophilum* in white yaks (ApG1

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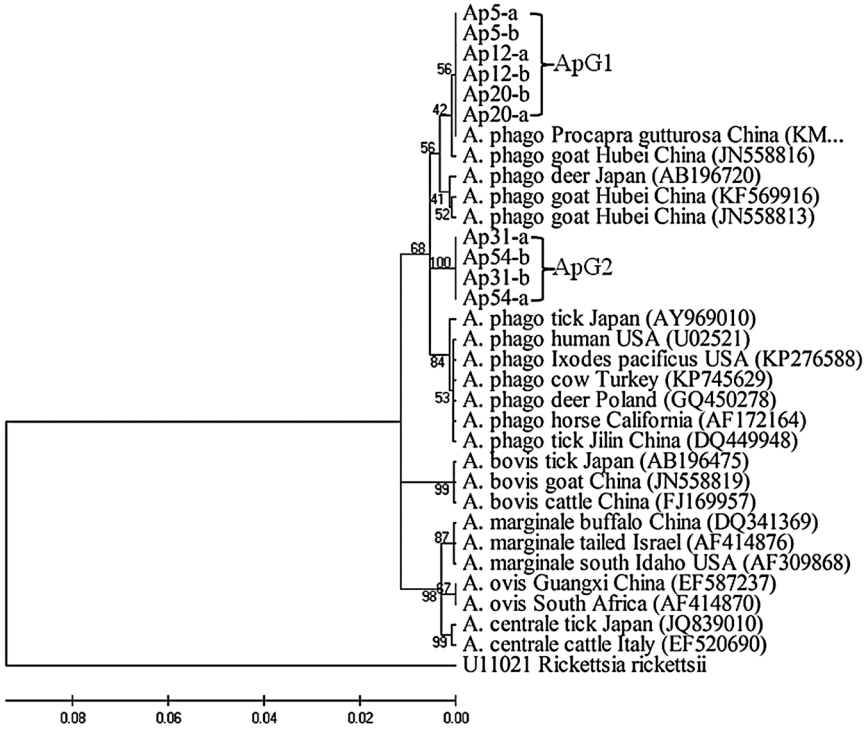

Fig. 1. Phylogenetic analysis of *A. phagocytophilum* (*A. phago*) based on 16S rRNA gene partial sequences. An alignment of 16S rRNA sequences from position 694 to 1334 of the sequence (based on strain ES34, GenBank accession no. AB196720) was used to construct this tree. *Rickettsia rickettsii* is used as an outgroup.

and ApG2). The similarity between ApG1 (Ap54-a, Ap54-b, Ap31-a and Ap 31-b) and ApG2 (Ap5-1, Ap5-b, Ap12-a, Ap12-b, Ap20-a and Ap20-b) was 98·5%. ApG1 were 98·8% identical to strains MR-23 (GenBank accession no. KP276588) isolated from *Ixodes pacificus* in the USA and Trbrt45 (GenBank accession no. KP745629) isolated from cow in Turkey. ApG2 were 100% identical to strain JC3-3 (GenBank accession no. KM186948) that was detected in the Mongolian gazelle from China. The 16S rRNA of *A. bovis* sequences identified in yaks were 99·6–100% identical to each other and to strain Ab4a (GenBank accession no. KJ639885) identified in red deer from Qilian-Mountain in Northwest China.

Phylogenetic analysis of 16S rRNA gene was conducted with *A. phagocytophilum* and *A. bovis* sequences in this study and selected sequences of *Anaplasma* spp. deposited in GenBank (Figs 1 and 2). The results revealed that ApG1 clustered independently from all known *A. phagocytophilum* sequences, ApG2 displayed a close relationship with the sequence amplified from Mongolian gazelle found in China (GenBank accession no. KM186948) (Fig. 1). All *A. bovis* strains from white yaks were classified into *A. bovis* cluster and contained two sequence types (ST1 and ST2) (Fig. 2).

DISCUSSION

In the present study, molecular survey and characterization of *Anaplasma* pathogens was performed in white yaks. Our findings clearly demonstrated that the presence of *A. phagocytophilum* and *A. bovis* in white yaks from TTAC.

Anaplasma phagocytophilum has been recognized as an emerging pathogen of veterinary and human health significance (Chen *et al*. 1994). In addition to humans, *A. phagocytophilum* infection has been reported in some domestic animals, such as sheep, goats and cattle (Aktas *et al*. 2011, 2012; Altay *et al*. 2014). *Anaplasma phagocytophilum* is a wide spread tick-borne infection causing human granulocytic anaplasmosis and tick-borne fever in domestic ruminants, responsible for serious economic loss to sheep and cattle industry (Stuen, 2007). Several molecular surveys of *A. phagocytophilum* have been performed in ticks and domestic ruminants in China. In this study, *A. phagocytophilum* infection was reported in white yaks. The positive rate of *A. phagocytophilum* in white yaks (17/322, 5·3%) was much lower than in black yaks (51/158, 32·3%) and cattle-yaks (7/20, 35·0%) conducted in Gannan Tibetan Autonomous Prefecture in Gansu Province, and was slightly lower than that in cattle (8/125, 6·4%) in Xinjiang Province (Yang *et al*. 2013, 2015). In general, *A. phagocytophilum* is associated with *Ixodes* ticks, including *I. pacificus, Ixodes dentatus* and *Ixodes scapularis* in the USA (Goethert and Telford, 2003; Teglas and Foley, 2006); *Ixodes ricinus* and *Ixodes trianguliceps* in Europe (Bown *et al*. 2008; Aktas *et al*. 2010); *Ixodes persulcatus* in Asia (Cao *et al*. 2003). However, the information

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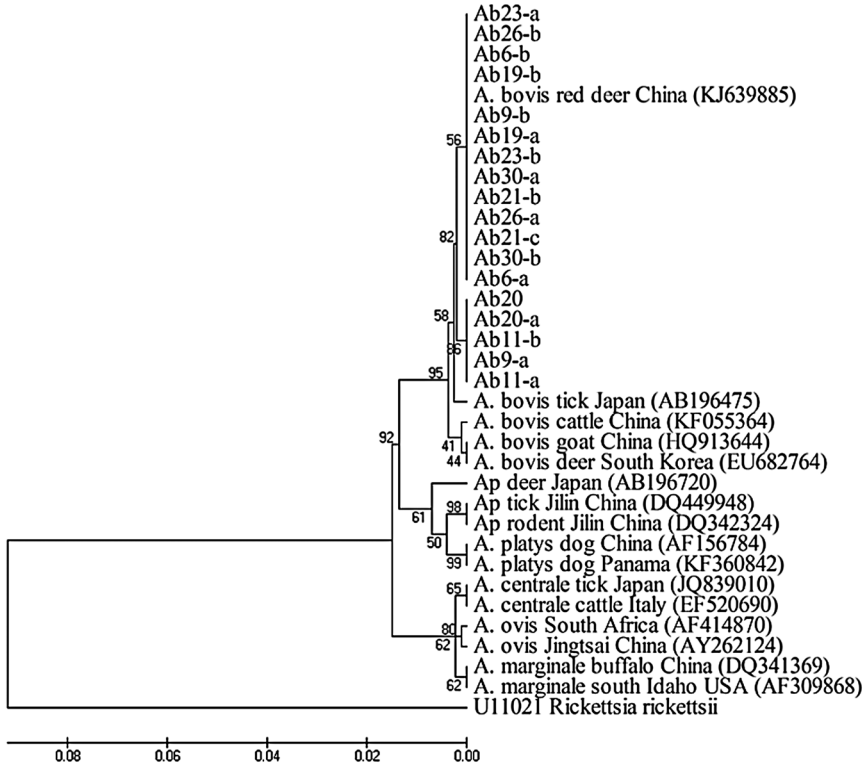

Fig. 2. Phylogenetic analysis of *A. bovis* based on 16S rRNA gene partial sequences. An alignment of 16S rRNA sequences from position 60 to 610 of the sequence (based on strain ES1019, GenBank accession no. HQ913644) was used to construct this tree. *Rickettsia rickettsii* is used as an outgroup.

on the epidemiology of the potential tick vectors is unclear in the study sites, therefore the investigation of tick species transmitting *A. phagocytophilum* in TTAC should be further carried out. To date, no transovarial transmission of *A. phagocytophilum* was described, except for *Dermacentor albipictus* ticks (abnormal feeding systems) (Baldridge *et al*. 2009). Identification of reservoir hosts is important to prevent and control of *A. phagocytophilum*, that play a critical role in the maintenance of the agent in nature. Our results indicated that white yaks are part of the natural maintenance cycle of *A. phagocytophilum*. *Anaplasma phagocytophilum* can cause persistent infection in ruminants and other animals for several years (Brown and Barbet, 2016). The white yak serve as reservoir host may facilitate further spread of infection. In this study, sequence analysis showed two 16S rRNA genotypes of *A. phagocytophilum* (ApG1 and ApG2) existed in white yaks (Fig. 1). Considerable strain variation of *A. phagocytophilum* has been reported in different hosts or geographic locations, and four geographically dispersed ecotypes were identified based on *groESL* gene and showed significantly different host ranges in Europe (Jahfari *et al*. 2014). It has been showed that the *A. phagocytophilum* ecotypes I in cattle were in the same group as those that infected human beings (Jahfari *et al*. 2014). Although no human case was currently reported in TTAC, *A. phagocytophilum* infection in white yaks warrants further investigation.

Historically, *A. bovis* was usually reported in buffalo and cattle from South America and Africa (Ooshiro *et al*. 2008). Recently, it has also been reported in cattle in continental Europe (Ceci *et al*. 2014; Aktas and Ozubek, 2015). Aside from the aforementioned study, the agent was identified in other ruminant and non-ruminant animals such as sheep, goats, deer, cats, dogs and rabbits (Kawahara *et al*. 2006; Ooshiro *et al*. 2008; Sakamoto *et al*. 2010; Liu *et al*. 2012; Tateno *et al*. 2013; Ben Said *et al*. 2015). In China, molecular evidence for the presence of *A. bovis* was reported in several wild and domestic animals including Reeves' muntjac, Mongolian gazelle, red deer, sika deer, cattle and goats (Liu *et al*. 2012; Yang *et al*. 2014, 2015; Li *et al*. 2015). In the present study, *A. bovis* infection was found in white yaks. The infection rate (6·2%, 20/322) was significantly lower than the 49·6% prevalence in goats in central and Southern China (Liu *et al*. 2012), and 42·7% in sheep and 23·8% in goats in Tunisia (Ben Said *et al*. 2015). However, it is almost comparable with 4·8% prevalence in cattle in Xinjiang, Northwest China (Yang *et al*. 2015). Sequences analysis revealed that *A. bovis* identified in this study were divided into two sequence types, indicating two different strains infected white yaks in TTAC. The ST1 has been identified in red deer in the Qilian-Mountain area that is near the study site, indicating *A. bovis* ST1 circulates in this region and has deer

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and white yaks as hosts. Previous report suggested that infection with *A. phagocytophilum* strain precludes infection with other strains; this could help to maintain the host tropism among strains (Rejmanek *et al*. 2012). *Anaplasma bovis* has a wide range of hosts, and the host tropism of *A. bovis* strains has not been demonstrated. However, both ST1 and ST2 of *A. bovis* were found in a white yak in this study (Ab9-a and Ab9-b) (Fig. 2).

Furthermore, *A. marginale* was known to cause severe disease in Northern China (Bai *et al*. 1987). However, none of the white yaks in TTAC were positive for *A. marginale* using two PCR assays based on *msp4* and *msp5* genes. More samples should be investigated to determine the ability of white yak as the host for *A. marginale* in the future study. In addition, coinfection of *A. phagocytophilum* and *A. bovis* occurred in two (0·6%) of the sampled animals. Although *Anaplasma* species infect different cell types (Dumler *et al*. 2001; Rar and Golovljova, 2011), coinfection increases the difficulties in diagnosis and treatment of bovine anaplasmosis.

In summary, this study is the first report to document the presence of *Anaplasma* in white yaks. Our findings extend the host range for *A. phagocytophilum* and *A. bovis* and demonstrate that white yaks play an important part in the natural life cycles of *Anaplasma* spp. Further studies are needed to investigate the potential tick vectors of these pathogens in this alpine steppe ecoregion.

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REFERENCES

Aktas, M. and Ozubek, S. (2015). Bovine anaplasmosis in Turkey: first laboratory confirmed clinical cases caused by *Anaplasma phagocytophilum*. *Veterinary Microbiology* **178**, 246–251.

Aktas, M., Vatansever, Z., Altay, K., Aydin, M. F. and Dumanli, N. (2010). Molecular evidence for *Anaplasma phagocytophilum* in *Ixodes ricinus* from Turkey. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 10–15.

Aktas, M., Altay, K. and Dumanli, N. (2011). Molecular detection and identification of *Anaplasma* and *Ehrlichia* species in cattle from Turkey. *Ticks and Tick-borne Diseases* **2**, 62–65.

Aktas, M., Altay, K., Ozubek, S. and Dumanli, N. (2012). A survey of *ixodid* ticks feeding on cattle and prevalence of tick-borne pathogens in the Black Sea region of Turkey. *Veterinary Parasitology* **187**, 567–571.

Altay, K., Dumanlı, N., Aktaş, M. and Özübek, S. (2014). Survey of anaplasma infections in small ruminants from East Part of Turkey. *Kafkas Universitesi Veteriner Fakultesi Dergisi* **20**, 1–4.

Bai, Q., Chen, Z., Ying, S., Liu, G. and Zhou, J. (1987). Study on isolation and preservation of single species of haematocytozoon in bovine: separation of bovine *A. marginale* single isolate. *Chinese Journal of Veterinary Science and Technology* **3**, 12–15.

Baldridge, G. D., Scoles, G. A., Burkhardt, N. Y., Schloeder, B., Kurtti, T. J. and Munderloh, U. G. (2009). Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). *Journal of Medical Entomology* **46**, 625–632.

Ben Said, M., Belkahia, H., Karaoud, M., Bousrih, M., Yahiaoui, M., Daaloul-Jedidi, M. and Messadi, L. (2015). First molecular survey of *Anaplasma bovis* in small ruminants from Tunisia. *Veterinary Microbiology* **179**, 322–326.

Bown, K. J., Lambin, X., Telford, G. R., Ogden, N. H., Telfer, S., Woldehiwet, Z. and Birtles, R. J. (2008). Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. *Applied and Environmental Microbiology* **74**, 7118–7125.

Brown, W. C. and Barbet, A. F. (2016). Persistent infections and immunity in ruminants to arthropod-borne bacteria in the family Anaplasmataceae. *Annual Review of Animal Biosciences* **4**, 177–197.

Cao, W. C., Zhao, Q. M., Zhang, P. H., Yang, H., Wu, X. M., Wen, B. H., Zhang, X. T. and Habbema, J. D. (2003). Prevalence of *Anaplasma phagocytophila* and *Borrelia burgdorferi* in *Ixodes persulcatus* ticks from northeastern China. *American Journal of Tropical Medicine and Hygiene* **68**, 547–550.

Ceci, L., Iarussi, F., Greco, B., Lacinio, R., Fornelli, S. and Carelli, G. (2014). Retrospective study of hemoparasites in cattle in southern Italy by reverse line blot hybridization. *Journal of Veterinary Medical Science* **76**, 869–875.

Chen, S. M., Dumler, J. S., Bakken, J. S. and Walker, D. H. (1994). Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *Journal of Clinical Microbiology* **32**, 589–595.

de la Fuente, J., Van Den Bussche, R. A. and Kocan, K. M. (2001). Molecular phylogeny and biogeography of North American isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae). *Veterinary Parasitology* **97**, 65–76.

Dumler, J. S., Barbet, A. F., Bekker, C. P., Dasch, G. A., Palmer, G. H., Ray, S. C., Rikihisa, Y. and Rurangirwa, F. R. (2001). 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*. *International Journal of Systematic and Evolutionary Microbiology* **51**, 2145–2165.

Goethert, H. K. and Telford, S. R., III (2003). Enzootic transmission of the agent of human granulocytic ehrlichiosis among cottontail rabbits. *American Journal of Tropical Medicine and Hygiene* **68**, 633–637.

Jahfari, S., Coipan, E. C., Fonville, M., van Leeuwen, A. D., Hengeveld, P., Heylen, D., Heyman, P., van Maanen, C., Butler, C. M., Foldvari, G., Szekeres, S., van Duijvendijk, G., Tack, W., Rijks, J. M., van der Giessen, J., Takken, W., van Wieren, S. E., Takumi, K. and Sprong, H. (2014). Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. *Parasites and Vectors* **7**, 365.

Kawahara, M., Rikihisa, Y., Lin, Q., Isogai, E., Tahara, K., Itagaki, A., Hiramitsu, Y. and Tajima, T. (2006). 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. *Applied and Environmental Microbiology* **72**, 1102–1109.

Li, Y., Chen, Z., Liu, Z., Liu, J., Yang, J., Li, Q., Luo, J. and Yin, H. (2015). Molecular Survey of *Anaplasma* and *Ehrlichia* of Red Deer and Sika Deer in Gansu, China in 2013. *Transboundary and Emerging Diseases*, in press.

Liu, Z., Ma, M., Wang, Z., Wang, J., Peng, Y., Li, Y., Guan, G., Luo, J. and Yin, H. (2012). Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Applied and Environmental Microbiology* **78**, 464–470.

Ooshiro, M., Zakimi, S., Matsukawa, Y., Katagiri, Y. and Inokuma, H. (2008). Detection of *Anaplasma bovis* and *Anaplasma phagocytophilum* from cattle on Yonaguni Island, Okinawa, Japan. *Veterinary Parasitology* **154**, 360–364.

Qin, S. Y., Huang, S. Y., Yin, M. Y., Tan, Q. D., Liu, G. X., Zhou, D. H., Zhu, X. Q., Zhou, J. Z. and Qian, A. D. (2015*a*). Seroprevalence and risk factors of *Chlamydia abortus* infection in free-ranging white yaks in China. *BMC Veterinary Research* **11**, 8.

Qin, S. Y., Wang, J. L., Ning, H. R., Tan, Q. D., Yin, M. Y., Zhang, X. X., Zhou, D. H. and Zhu, X. Q. (2015*b*). First report of *Babesia bigemina* infection in white yaks in China. *Acta Tropica* **145**, 52–54.

Rar, V. and Golovljova, I. (2011). *Anaplasma*, *Ehrlichia*, and "*Candidatus Neoehrlichia*" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infection, Genetics and Evolution* **11**, 1842–1861.

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Rejmanek, D., Bradburd, G. and Foley, J. (2012). Molecular characterization reveals distinct genospecies of *Anaplasma phagocytophilum* from diverse North American hosts. *Journal of Medical Microbiology* **61**, 204–212.

Sakamoto, L., Ichikawa, Y., Sakata, Y., Matsumoto, K. and Inokuma, H. (2010). Detection of *Anaplasma bovis* DNA in the peripheral blood of domestic dogs in Japan. *Japanese Journal of Infectious Diseases* **63**, 349–352.

Stuen, S. (2007). *Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe. *Veterinary Research Communications* **31**, 79–84.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.

Tateno, M., Nishio, T., Sakuma, M., Nakanishi, N., Izawa, M., Asari, Y., Okamura, M., Maruyama, S., Miyama, T. S., Setoguchi, A. and Endo, Y. (2013). Molecular epidemiologic survey of *Bartonella, Ehrlichia*, and *Anaplasma* infections in Japanese Iriomote and Tsushima leopard cats. *Journal of Wildlife Diseases* **49**, 646–652.

Teglas, M. B. and Foley, J. (2006). Differences in the transmissibility of two *Anaplasma phagocytophilum* strains by the North American tick vector species, *Ixodes pacificus* and *Ixodes scapularis* (Acari: Ixodidae). *Experimental and Applied Acarology* **38**, 47–58.

Yang, J., Liu, Z., Guan, G., Liu, Q., Li, Y., Chen, Z., Ma, M., Liu, A., Ren, Q., Luo, J. and Yin, H. (2013). Prevalence of *Anaplasma phagocytophilum* in ruminants, rodents and ticks in Gansu, north-western China. *Journal of Medical Microbiology* **62**, 254–258.

Yang, J., Li, Y., Liu, Z., Guan, G., Chen, Z., Luo, J., Wang, X. and Yin, H. (2014). Molecular evidence for *Anaplasma bovis* infection in wild Reeves' muntjac (*Muntiacus reevesi*), southwest China. *Journal of Wildlife Diseases* **50**, 982–985.

Yang, J., Li, Y., Liu, Z., Liu, J., Niu, Q., Ren, Q., Chen, Z., Guan, G., Luo, J. and Yin, H. (2015). Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. *Parasites and Vectors* **8**, 108.

Zhang, L., Wang, Y., Cai, D., He, G., Cheng, Z., Liu, J., Meng, K., Yang, D. and Wang, S. (2013). Detection of *Anaplasma marginale* in *Hyalomma asiaticum* ticks by PCR assay. *Parasitology Research* **112**, 2697–2702.

Zhou, Y., Hou, X. and Piao, F. Z. (2007). Detection of dairy cattle anaplasmosis and its application by PCR. *Chinese Journal of Preventive Veterinary Medicine* **29**, 718–722.

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