Anaplasma platys-Like Infection in Goats, Beijing, China

Wei Wei,^{1,2} Jie Li,² Ya-Wei Wang,² Bao-Gui Jiang,² Hong-Bo Liu,^{2,3} Ran Wei,² Rui-Ruo Jiang,^{2,4}
Xiao-Ming Cui,² Lian-Feng Li,^{2,5} Ting-Ting Yuan,^{2,6} Qian Wang,² Lin Zhao,² Luo-Yuan Xia,²
Jia-Fu Jiang,² Ye-Feng Qiu,⁷ Na Jia,² Wu-Chun Cao,² and Yan-Ling Hu¹

Abstract

As one of the important tick-borne zoonotic pathogens, *Anaplasma* has both veterinary and public health significance. Here, we performed a survey of *Anaplasma* infection in the goats from a farm in Beijing, China, and found 44.6% (41/92) were infected with *Anaplasma capra*, and 22.8% (21/92) were infected with *Anaplasma* sp. This *Anaplasma* sp. bacterium was close to a recently emerging *Anaplasma platys* strain based on *gltA* and *groEL* gene phylogenetic analysis. As to further understand the characteristics of *Anaplasma* sp., we raised a couple of positive goats ($n=2$) in the laboratory with tick-free settings. We observed inappetence, vomiting, high fever, and weakness of limbs in the goat's offspring ($n=3$). In addition, the blood samples from all offspring were all positive of this *Anaplasma* spp. We did not see any intracellular morulae in neutrophils, monocytes, and erythrocytes, but we identified some in the platelets of the blood smears from the positive goats by light microscopy. We named it *A. platys*-like and suggested it may infect platelets and be transmitted vertically through the placenta of goats. These findings deserve further evaluation.

Keywords: *Anaplasma platys*-like, goats, platelets, vertical transmission

Introduction

TICK-BORNE DISEASES (TBDs) have been an increasing threat on animal production, welfare, and human health worldwide (Torina et al. 2007, Perronne 2014). The *Anaplasma* spp. are tick-borne pathogens with mammalian hosts in temperate, subtropical, and tropical regions of the world (Geurden et al. 2008, Tay et al. 2014, Yang et al. 2018). *Anaplasma* is an intracellular gram-negative bacterium, which belongs to the *Anaplasmataceae* family and *Rickettsiales* order (Dumler et al. 2001). In recent years, the public health significance of *Anaplasma* as emerging pathogens has also been realized (Ismail et al. 2010, Botelho-Nevers et al. 2012, Stuenkel et al. 2013). *Anaplasma ovis*, *Anaplasma centrale*, *Anaplasma bovis*, *Anaplasma platys*, *Anaplasma phagocytophilum*, and *Anaplasma marginale* are recognized species contained in the genus *Anaplasma* (Dumler et al. 2001). Recently, *Anaplasma capra* (Li et al. 2015a) and *Anaplasma odocoilei* (Tate et al. 2013) species have

been included in the *Anaplasma* genus. The different species have diverse blood cell tropisms (Silaghi et al. 2017).

Anaplasmosis is one of the common tick-borne zoonotic diseases in domestic animals, such as sheep and goats on pastures. Clinical characteristics of infected animals depend on animal welfare and immune status (Čobádiová et al. 2013). The clinical symptoms of infected animals include inappetence, weight loss, sudden high fever, reduced milk production, abortions, and even death (Mathieson 1991, Stuenkel 2007, Liu et al. 2012, Yasini et al. 2012). Furthermore, infected animals are more susceptible to be coinfecting by other pathogens (Čobádiová et al. 2013). For example, *A. phagocytophilum* is not only an abortifacient but also associated with various bacterial opportunistic infections in sheep farms in northern Spain (García-Pérez et al. 2003). In addition, the human NY-18 *A. phagocytophilum* isolate can infect sheep and transmit to the lamb with some clinical symptoms (Reppert et al. 2013). Vertical transmission of *A. ovis* can cause death of neonatal elk calf (Hendrix et al. 2019).

¹Life Sciences Institute, Guangxi Medical University, Nanning, China.

²State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China.

³Center for Disease Control and Prevention of PLA, Beijing, China.

⁴Institute of NBC Defense, Yangfang, Beijing, China.

⁵School of Information and Management, Guangxi Medical University, Nanning, China.

⁶Shanghai Institute for Emerging and Re-emerging Infectious Diseases, Shanghai Public Health Clinical Center, Shanghai, China.

⁷Laboratory Animal Center, Academy of Military Medical Sciences, Beijing, China.

The previous study has indicated that there was a high incidence of anaplasmosis in the goats in the suburban areas of Beijing, China (Zhang et al. 2012). In 2012–2013, we performed a survey and found *A. capra* infections among the goats from a farm in Fangshan District, another suburban region of Beijing. The goats in this farm were usually grazing on the mountains, where *Haemaphysalis longicornis* had a high density in the peak season (Li et al. 2015a). In this study, we continued the survey in this farm and identified *A. capra* and a new *Anaplasma* spp. To understand the characteristics of this new species, we brought a couple of positives from this farm to the laboratory for further investigation.

Materials and Methods

Investigation and sample collection

The investigation was conducted on a herd of goats ($n=92$) from a farm in Dayuan village, Qinglonghu Town, Fangshan District, Beijing (E116°3' N39°45'36"), in October 2016. To assess the *Anaplasma* infection on the goats, 92 ethylenediaminetetraacetic acid (EDTA)-anticoagulant blood samples of the goats were collected and detected by PCR targeting 16S rRNA and *gltA* genes. After initial screening, two adult positive goats (G1 [male], G2 [female]) were further raised in the laboratory with tick-free settings. Soon after, the couple naturally bore their offspring (G3 [female], G4 [male]), and G1 and G3 gave birth to G5 [male]). Their relationship is described in Figure 1. Those five goats were raised in the laboratory of Beijing Institute of Microbiology and Epidemiology, following the animal ethics authority regulations (IACUC-13-2016-004).

FIG. 1. G1 and G2 gave birth to G3 and G4, G1 and G3 gave birth to G5. F, female; M, male.

Molecular detection and amplification of *Anaplasma* sp. genes

Total DNA was extracted from all samples using the TIANamp Genomic DNA Kit (DP304; TIANGEN (Beijing) Biotech, Beijing, China) according to the kit recommended protocol. The DNA specimens were diluted into 80 μ L nucleic acid eluant and stored at -20°C . All DNA samples were tested by nested PCR using the primer pairs, targeting the 16S rRNA and citrate synthase (*gltA*) genes for first screening of *Anaplasma*. The identification of *A. capra* was followed by our previously described methods (Li et al. 2015a). To obtain the *Anaplasma* sp. gene sequences, DNAs of the goats' family (goat 1–goat 5, G1–G5) were amplified for the near-complete 16S rRNA, and partial *gltA* and *groEL* genes. To obtain the 16S rRNA gene, the primer pair Eh-out1/Eh-3-17 was used in the first round, and the primer pairs Eh-out1/Eh-out2/Eh-out2/Eh-3-17 were used in the second round reaction. Seminested PCR was used to amplify the *gltA* and *groEL* genes, and the primers are shown in Table 1. Nuclease-free water was used as a negative control, and $2\times$ DreamTaq Master Mix (Thermo Scientific, USA) including DNA polymerase was used in the PCR. To avoid cross-contamination, the experimental operations of DNA extraction, PCR preparation, DNA amplification, and agarose gel electrophoresis were in separate rooms; in addition, the template isolation and amplification were performed with the dedicated pipettes.

Sequencing of PCR products and phylogenetic analysis

All PCR products were sent to Sangon Biotech (Shanghai, China) for sequencing. The sequences obtained were checked and assembled by CLC Main Workbench 5, and were compared with the registered sequences in GenBank using the BLAST program (NCBI, Bethesda, MD) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic organization of *Anaplasma* strain revealed in this study with other referenced *Anaplasma* strains was constructed using the program MEGA version 5.0 software based on the DNA sequence variation of partial 16S rRNA, *gltA*, and *groEL*, respectively (Tamura et al. 2011, Li et al. 2015a).

Morphological examination

The blood smears of the goats' family (G1–G5) were prepared by air-drying and were further fixed in absolute formaldehyde and acetone 1:1 for 10 min. Wright–Giemsa staining was then performed. The slides were rinsed with water slowly, dried, examined under the ordinary light microscope (Olympus BX41, Japan).

Results

We have tested the whole-blood samples from the 92 goats by PCR, and found that the infection rate of *A. capra* was 44.6% (41/92), and the positive rate of *Anaplasma* sp. was 22.8% (21/92). *Anaplasma* sp. infection was found in the goat couples (G1, G2) and goats G3–G5. We have obtained the sequences of near-complete 16S rRNA gene (~ 1470 bp) and partial *gltA* (~ 920 bp) and *groEL* genes (~ 850 bp) for genetic identification of this *Anaplasma* sp. Based on the evolutionary analysis in the 16S rRNA phylogenetic tree, the

TABLE 1. PRIMER SEQUENCES USED IN THIS STUDY

Target genes	Primers	Oligonucleotide sequences (5'-3')	Target fragment (bp)
16S rRNA	Eh-out1 Eh-out2 Eh-3-17	TTGAGAGTTTGATCCTGGCTCAGAACG CACCTCTACACTAGGAATTCCGCTATC TAAGGTGGTAATCCAGC	1470
<i>gltA</i>	AP1SPglF1 AP1168SPglR1 APJ10F2	ATGBTAGAAAAARGCTGTTTTRGMGTGT TCATACCATTGMGATRCCCCATCC AAKGCTGTTTTAGCGTGTGGTGATCTT	920
<i>groEL</i>	APJ932R2 Gro240F Gro712R Gro481F Gro1059R	ATTTTCGCCCTCGGGTCGTGA ATTAGYAAGCCTTATGGGTC CCGCGATCAAACCTGCATACC TGCACAGGTTGCTACTAT CDGARCTACTATCRACAC	850

Anaplasma sp. in our study and the *Anaplasma* sp. in the Wen-Ping Guo study formed a cluster, and close to *A. phagocytophilum*, *A. platys*, and *A. platys*-like strains identified in Ankang (Fig. 2a) (Guo et al. 2019). The partial *gltA* gene sequence of our *Anaplasma* sp. formed a cluster with the

Anaplasma sp. identified in Xi'an, and this cluster was close to *A. platys*-like strain identified in *Rhipicephalus microplus* in Ankang, northwest China (Fig. 2b) (Guo et al. 2018, 2019). For the *groEL* gene, *Anaplasma* sp. found in our research was also close to the *Anaplasma* spp. identified in Xi'an, and it

FIG. 2. Phylogenetic analyses based on nucleotide sequences of the 16S rRNA (1170 bp) (a), citrate synthase (*gltA*) (532 bp) (b), and heat-shock protein (*groEL*) (815 bp) genes (c). The phylogenetic trees were constructed by the ML method (bootstrap replications: 1000) with Kimura 2-parameter plus Gamma rate model. The scale bar indicated the estimation of evolutionary distance. The taxa marked by *bold font* depict the sequences obtained in the study, and GenBank accession number is provided at the end of each strain name. ML, maximum likelihood.

(continued)

FIG. 2. (Continued).

(continued)

was genetically related to the *A. platys* and *A. platys*-like strains in Ankang (Fig. 2c) (Guo et al. 2018, 2019).

The couple of positive goats infected with *Anaplasma* sp. gave birth in our laboratory settings. We found that all the family members of the goats (G1–G5) were infected with this *Anaplasma* unexpectedly (Fig. 2), although there were some gene variations. The almost full-length 16S rRNA sequences shared 100% nucleotide identities with each other and 99.93% identities with the *Anaplasma* strain detected in the goats from Xi'an, China (Guo et al. 2018) (Fig. 2a). The sequences of the partial *gltA* gene showed 98.5–100% similarity with each other and exhibited 99.07–99.87% identities with *Anaplasma* sp. found in Xi'an (Fig. 2b). For the partial sequences of *groEL* gene, they showed 98.9–100% nucleotide similarity with each other and presented 98.03–99.64% identities with *Anaplasma* spp. identified in Xi'an (Fig. 2c). We only got the short-length gene sequence of 16S rRNA of G1 and G2 but not the complete one; thus, we did not include them in the phylogenetic tree. We observed the offspring G3–G5 with some symptoms of anaplasmosis, including inap-

petence, high fever, vomiting, and weakness of limbs. During the examination, morulae were found in the platelet of the goats' family (G1–G5) in the blood smears by Wright–Giemsa staining, but not in monocytes, neutrophils, and red blood cells (Fig. 3). Consequently, we named this bacterium *A. platys*-like.

Discussion

In this study, the goat couples (G1, G2) were fed in a tick-free environment, and the goats G3–G5 were found to be all infected with *A. platys*-like bacterium. This suggests the vertical transmission of *A. platys*-like in goats. We also observed some clinical symptoms in the offspring, which might be caused by *A. platys*-like. By morphological examination of blood smears from the infected goats, we suggest that this *Anaplasma* might infect platelets.

In previous research, there are six species of the genus *Anaplasma*, which infect mammals and particular cell types of hosts such as erythrocytes, neutrophils, platelets, and

FIG. 2. (Continued).

monocytes (Dumler et al. 2001). Currently, *A. platys* is the only classified rickettsial species known to infect platelets, and caused cyclic thrombocytopenia in dogs (Dumler et al. 2001); this pathogen has also been found in cats (Lima et al. 2010), cattle, *Camelus bactrianus*, goats, *Cervus elaphus*, and African buffalo (Chochlakis et al. 2009, Dahmani et al. 2015, Li et al. 2015b, Lorusso et al. 2016a, 2016b, Machado et al. 2016). The investigation from Italy indicated that the prevalence of *A. platys*-like infection was higher in the goats than in the calves and sheep (Zobba et al. 2014), and it was also lower in the cattle in Algeria than in the calves in Italy (Dahmani et al. 2015). Through pathogen detection and symptom observation of the goat offspring (G3–G5), we suggest that there is a high probability that *A. platys*-like strain might impact goat husbandry, which deserves further epidemiological investigations and surveillance.

Up to date, there are two *Anaplasma* spp. that are recognized as human pathogens. For instance, *A. phagocytophilum* was the agent of human granulocytic anaplasmosis (Chen et al. 1994). *A. capra* was identified as a human pathogen in China recently (Li et al. 2015a). *A. platys* and *A. ovis* have

been identified as the potential human pathogens (Chochlakis et al. 2010, Arraga-Alvarado et al. 2014). Whether *A. platys*-like identified in our study could infect human is an important issue to be clarified, however, we did not successfully isolate the bacterium. Further studies should be carried out to determine the pathogenicity of *Anaplasma* infected with platelets to humans.

To date, molecular and pathogenic identifications are still the significant methods of detecting *Anaplasma*, and serological testing is also commonly used as a supplementary detection. In our study, we identified the infection with *Anaplasma* by PCR and blood smear, but not by the antibody detection because *A. platys*-like strain isolate is not available. Based on the previous comparison on diagnosis methods, PCR was highly sensitive and endorsed for blood parasite diagnosis (Rucksaken et al. 2019, Wardrop et al. 2016). In addition, for PCR detection, it is worth noting that only targeting the 16S rRNA gene is not sufficient for the identification of *Anaplasma*. Phylogenetic analysis based on the sequences of 16S rRNA, *gltA*, and *groEL* indicated that *A. platys*-like in our study is similar to *Anaplasma* sp. identified

FIG. 3. The blood smear of *Anaplasma platys*-like-infected goat was stained with the Wright–Giemsa staining method, and the inclusion is identified with arrowhead as blue-purple stained bodies within the platelet cytoplasm.

from goats in Xi'an and is related to *A. platys* strains and *A. platys*-like in *R. microplus* in Ankang. With the phylogenetic analysis, the *gltA* and *groEL* sequences from the five goats (G1–G5) were not completely identical, which suggested that the G3–G5 might be coinfecting with other *Anaplasma* sp. However, we performed molecular detection and blood smear observation, and found no other *Anaplasma* infection.

The previous reports also identified *Anaplasma* infection in domestic animals in northern China including Beijing, Inner Mongolia, Shanxi, and Hebei. The investigations in rural areas of Beijing showed that 48.9% (44/90) of goats, 23.9% (17/71) of cattle, and 0% (0/2) of dogs were infected with *A. phagocytophilum* by PCR amplification (Zhang et al. 2012). In Shanxi Province, 78.1% (25/32) of sheep were infected with *Anaplasma* spp., and 8.8% (30/338) of goats were identified as *A. capra* positive (Li et al. 2012, Zhang et al. 2016, Peng et al. 2018). The study from Hebei indicated that *A. capra* was detected in 51.1% (23/45) of sheep (Yang et al. 2017). The infection rate of *A. capra* in the goats in Inner Mongolia was 10% (4/40) (Peng et al. 2018). The seroprevalence of *A. phagocytophilum* was higher in rural residents from Tianjin (41.8%) than from Beijing (13.6%) (Zhang et al. 2014). According to the data described above, the infection rate of *A. capra* in the goats in our study was 44.6% (41/92), which was lower than in the sheep in Hebei (51.1%). Until now, there has not an *A. platys* infection report in northern China.

Ticks play an important role in the transmission of *Anaplasma* (Rar et al. 2011). *Rhipicephalus sanguineus* is considered to be the primary vector of *A. platys* transmission (Simpson et al. 1991, Cicuttin et al. 2015, Li et al. 2015b). *Rhipicephalus turanicus* and *Rhipicephalus microplus* were reported to be infected with *A. platys*-like strains (Harrus et al. 2011, Lu et al. 2017), and *A. capra* (Guo et al. 2019).

The limitations of this study were as follows. Although we have observed that *A. platys*-like-infected goats had some symptoms, the veterinary significance of this bacterium should be further evaluated with a larger sample size. In addition, we found the possibility of vertical transmission, however, this observation was beyond our initial plan, and thus, no detailed procedure was designed to describe this transmission. Many critical characteristics were not available, such as what time was the offspring found positive after its birth. This should be evaluated in further animal models. Finally, the infection rate of *A. platys*-like in ticks is unknown, specially around the farm where we got goat samples.

Conclusions

In recent years, due to frequent contact between the rural and urban populations, the threat of TBDs to human health is not limited to rural areas. In our study, we detected *A. platys*-like in goats in Fangshan district, Beijing, which might infect platelets of goats and be a risk of vertical transmission although its pathogenicity to human was uncertain.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This work was supported by the Natural Science Foundation of China (81773492), State Key Research Development Program of China (2019YFC1200401, 2019YFC1200202, 2019YFC1200501), Guangxi scientific and technological research (2020AB39264), and Special Program for Prevention and Control of Infectious Diseases in China (2018ZX10712001-018-001).

References

- Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, et al. Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. *Am J Trop Med Hyg* 2014; 91: 1161–1165.
- Botelho-Nevers E, Socolovschi C, Raoult D, Parola P. Treatment of *Rickettsia* spp. infections: A review. *Expert Rev Anti Infect Ther* 2012; 10:1425–1437.
- Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994; 32:589–595.
- Chochlakis D, Ioannou I, Sharif L, Kokkini S, et al. Prevalence of *Anaplasma* sp. in goats and sheep in Cyprus. *Vector Borne Zoonotic Dis* 2009; 9:457–463.
- Chochlakis D, Ioannou I, Tselentis Y, Psaroulaki A. Human anaplasmosis and *Anaplasma ovis* variant. *Emerg Infect Dis* 2010; 16:1031–1032.
- Cicuttin GL, Tarragona EL, De Salvo MN, Mangold AJ, et al. Infection with *Ehrlichia canis* and *Anaplasma platys* (Rickettsiales: Anaplasmataceae) in two lineages of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) from Argentina. *Ticks Tick Borne Dis* 2015; 6:724–729.
- Čobádiová A, Reiterová K, Derdákova M, Špilovská S, et al. *Toxoplasma gondii*, *Neospora caninum* and tick-transmitted

- bacterium *Anaplasma phagocytophilum* infections in one selected goat farm in Slovakia. *Acta Parasitol* 2013; 58:541–546.
- Dahmani M, Davoust B, Benterki MS, Fenollar F, et al. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. *Comp Immunol Microbiol Infect Dis* 2015; 39:39–45.
- Dumler JS, Barbet AF, Bekker CP, Dasch GA, 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; 51:2145–2165.
- Garcia-Perez AL, Barandika J, Oporto B, Povedano I, et al. *Anaplasma phagocytophila* as an Abortifacient Agent in Sheep Farms from Northern Spain. *Ann N Y Acad Sci* 2003; 990:429–432.
- Geurden T, Somers R, Thanh NT, Vien LV, et al. Parasitic infections in dairy cattle around Hanoi, Northern Vietnam. *Vet Parasitol* 2008; 153:384–388.
- Guo WP, Huang B, Zhao Q, Xu G, et al. Human-pathogenic *Anaplasma* spp., and *Rickettsia* spp. in animals in Xi'an, China. *PLoS Negl Trop Dis* 2018; 12:e0006916.
- Guo WP, Zhang B, Wang YH, Xu G, et al. Molecular identification and Characterization of *Anaplasma capra* and *Anaplasma platys*-like in *Rhipicephalus microplus* in Ankang, Northwest China. *BMC Infect Dis* 2019; 19:434.
- Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, et al. Molecular detection of *Ehrlichia canis*, *Anaplasma bovis*, *Anaplasma platys*, *Candidatus Midichloria mitochondrii* and *Babesia canis vogeli* in ticks from Israel. *Clin Microbiol Infect* 2011; 17:459–463.
- Hendrix GK, Brayton KA, Burcham GN. *Anaplasma ovis* as the suspected cause of mortality in a neonatal elk calf. *J Vet Diagn Invest* 2019; 31:267–270.
- Ismail N, Bloch KC, McBride JW. Human ehrlichiosis and anaplasmosis. *Clin Lab Med* 2010; 30:261–292.
- Li GH, Yu Q, Zhang QX, Pan L, et al. [Molecular epidemiology regarding *Anaplasma phagocytophilum* in *Dermacentor silvarum* in Ningwu county, Shanxi province]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2012; 33:642–643.
- Li H, Zheng YC, Ma L, Jia N, et al. Human infection with a novel tick-borne *Anaplasma* species in China: A surveillance study. *Lancet Infect Dis* 2015a; 15:663–670.
- Li Y, Yang J, Chen Z, Qin G, et al. *Anaplasma* infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. *Parasit Vectors* 2015b; 8:313.
- Lima ML, Soares PT, Ramos CA, Araújo FR, et al. Molecular detection of *Anaplasma platys* in a naturally-infected cat in Brazil. *Braz J Microbiol* 2010; 41:381–385.
- Liu Z, Ma M, Wang Z, Wang J, et al. Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Appl Environ Microbiol* 2012; 78:464–470.
- Lorusso V, Wijnveld M, Latrofa MS, Fajinmi A, et al. Canine and ovine tick-borne pathogens in camels, Nigeria. *Vet Parasitol* 2016a; 228:90–92.
- Lorusso V, Wijnveld M, Majekodunmi AO, Dongkum C, et al. Tick-borne pathogens of zoonotic and veterinary importance in Nigerian cattle. *Parasit Vectors* 2016b; 9:217.
- Lu M, Tian JH, Yu B, Guo WP, et al. Extensive diversity of rickettsiales bacteria in ticks from Wuhan, China. *Ticks Tick Borne Dis* 2017; 8:574–580.
- Machado RZ, Teixeira MM, Rodrigues AC, André MR, et al. Molecular diagnosis and genetic diversity of tick-borne Anaplasmataceae agents infecting the African buffalo *Syncerus caffer* from Marromeu Reserve in Mozambique. *Parasit Vectors* 2016; 9:454.
- Mathieson AO. Ectoparasites and their control. In: Martin WB, Aitken ID, eds. *Diseases of Sheep*, 2nd ed. Oxford: Blackwell Science Publication, 1991:284–289.
- Peng Y, Wang K, Zhao S, Yan Y, et al. Detection and phylogenetic characterization of: An emerging pathogen in sheep and goats in China. *Front Cell Infect Microbiol* 2018; 8:283.
- Perronne C. Lyme and associated tick-borne diseases: Global challenges in the context of a public health threat. *Front Cell Infect Microbiol* 2014; 4:74.
- Rar V, Golovljova I. *Anaplasma*, *Ehrlichia*, and “*Candidatus Neoehrlichia*” bacteria: Pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infect Genet Evol* 2011; 11:1842–1861.
- Reppert E, Galindo RC, Breshears MA, Kocan KM, et al. Demonstration of transplacental transmission of a human isolate of *Anaplasma phagocytophilum* in an experimentally infected sheep. *Transbound Emerg Dis* 2013; 60 (Suppl.): 93–96.
- Rucksaken R, Maneeruttanarungroj C, Maswanna T, Sussadee M, et al. Comparison of conventional polymerase chain reaction and routine blood smear for the detection of *Babesia canis*, *Hepatozoon canis*, *Ehrlichia canis*, and *Anaplasma platys* in Buriram Province, Thailand. *Vet World* 2019; 12:700–705.
- Silaghi C, Santos AS, Gomes J, Christova I, et al. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. *Vector Borne Zoonotic Dis* 2017; 17:12–22.
- Simpson RM, Gaunt SD, Hair JA, Kocan KM, et al. Evaluation of *Rhipicephalus sanguineus* as a potential biologic vector of *Ehrlichia platys*. *Am J Vet Res* 1991; 52:1537–1541.
- Stuen S. *Anaplasma phagocytophilum*—The most widespread tick-borne infection in animals in Europe. *Vet Res Commun* 2007; 31(Suppl.):79–84.
- Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum* a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol* 2013; 3:31.
- Tamura K, Peterson D, Peterson N, Stecher G, et al. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; 28:2731–2739.
- Tate CM, Howerth EW, Mead DG, Dugan VG, et al. *Anaplasma odocoilei* sp. Nov.(family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*). *Ticks Tick Borne Dis* 2013; 4:110–119.
- Tay ST, Koh FX, Kho KL, Ong BL. Molecular survey and sequence analysis of *Anaplasma* spp. in cattle and ticks in a Malaysian farm. *Trop Biomed* 2014; 31:769–776.
- Torina A, Vicente J, Alongi A, Scimeca S, et al. Observed prevalence of tick-borne pathogens in domestic animals in Sicily, Italy during 2003–2005. *Zoonoses Public Health* 2007; 54:8–15.
- Wardrop KJ, Birkenheuer A, Blais MC, Callan MB, et al. Update on canine and feline blood donor screening for blood-borne pathogens. *J Vet Intern Med* 2016; 30:15–35.

- Yang J, Han R, Niu Q, Liu Z, et al. Occurrence of four *Anaplasma* species with veterinary and public health significance in sheep, northwestern China. *Ticks Tick Borne Dis* 2018; 9: 82–85.
- Yang J, Liu Z, Niu Q, Liu J, et al. A novel zoonotic *Anaplasma* species is prevalent in small ruminants: Potential public health implications. *Parasit Vectors* 2017; 10:264.
- Yasini S, Khaki Z, Rahbari S, Kazemi B, et al. Hematologic and clinical aspects of experimental ovine anaplasmosis caused by *Anaplasma ovis* in Iran. *Iran J Parasitol* 2012; 7: 91–98.
- Zhang L, Liu H, Xu B, Zhang Z, 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.
- Zhang XC, Zhang LX, Li WH, Wang SW, et al. Ehrlichiosis and zoonotic anaplasmosis in suburban areas of Beijing, China. *Vector Borne Zoonotic Dis* 2012; 12:932–937.
- Zhang Y, Lv Y, Zhang F, Zhang W, et al. Molecular and phylogenetic analysis of *Anaplasma* spp. in sheep and goats from six provinces of China. *J Vet Sci* 2016; 17:523–529.
- Zobba R, Anfossi AG, Pinna P, Pappalardo ML, Dore GM, et al. Molecular investigation and phylogeny of *Anaplasma* spp. in Mediterranean ruminants reveal the presence of neutrophil-tropic strains closely related to *A. platys*. *Appl Environ Microbiol* 2014; 80:271–280.

Address correspondence to:
 Yan-Ling Hu
 Life Sciences Institute
 Guangxi Medical University
 22 Shuangyong Street
 Nanning, Guangxi 530021
 China

E-mail: huyanling@gxmu.edu.cn

Na Jia
 State Key Laboratory of Pathogen and Biosecurity
 Beijing Institute of Microbiology and Epidemiology
 20 Dong-Da Street
 Fengtai, Beijing 100071
 China

E-mail: jiana7941@hotmail.com

Wu-Chun Cao
 State Key Laboratory of Pathogen and Biosecurity
 Beijing Institute of Microbiology and Epidemiology
 20 Dong-Da Street
 Fengtai, Beijing 100071
 China

E-mail: caowc@bmi.ac.cn