Molecular and phylogenetic analysis of Anaplasma spp. in sheep and goats from six provinces of China

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Members of the genus Anaplasma are important emerging tick-borne pathogens in both humans and animals in tropical and subtropical areas. Here, we investigated the presence of Anaplasma spp. in 621 sheep and 710 goats from six provinces of China. Polymerase chain reaction (PCR) and DNA sequencing were conducted to determine the prevalence of Anaplasma (A.) phagocytophilum, A. ovis and A. bovis targeting the 16S ribosomal RNA or the major surface protein 4 gene. PCR revealed Anaplasma in 39.0% (240/621) of sheep and 45.5% (323/710) of goats. The most frequently detected species was A. ovis (88/621, 14.2% for sheep; 129/710, 18.2% for goats), followed by A. bovis (60/621, 9.7% for sheep; 74/710, 10.4% for goats) and A. phagocytophilum (33/621, 5.3% for sheep; 15/710, 2.1% for goats). Additionally, eight sheep and 20 goats were found to be infected with three pathogens simultaneously. DNA sequencing confirmed the presence of these three Anaplasma species in the investigated areas, and phylogenetic analysis indicated that there was geographic segregation to a certain extent, as well as a relationship between the host and cluster of A. ovis. The results of the present study provide valuable data that helps understand the epidemiology of anaplasmosis in ruminants from China.

Keywords: Anaplasma bovis, Anaplasma ovis, Anaplasma phagocytophilum, phylogenetic analysis, prevalence

Introduction

During the past few decades, the genus Anaplasma within the order Rickettsiales has attracted a great deal of interest because of its pathogenicity in farm animals and its ability to infect humans. Anaplasmosis, which is caused by various species of Anaplasma, is a tick-borne infectious disease that affects wild and domestic animals worldwide [17]. Many types of ruminants (deer, sheep, goat and cattle), wild animals, small mammals, rodents and humans have been shown to be susceptible to the disease, although there are some differences in severity and symptoms between them [10].

Members of the genus Anaplasma include A. phagocytophilum, A. marginale, A. bovis, A. ovis, A. platys and A. centrale, all of which are obligate intracellular bacteria that infect a variety of cell types [3]. A. phagocytophilum, which infects neutrophils in humans and animals, causes human granulocytic anaplasmosis and tick-borne fever in animals. A. ovis, A. marginale and A. centrale are intraerythrocytic pathogens of ruminants. A. bovis is another leukocyte pathogen of ruminants that is usually found in monocytes [16]. Finally, A. platys shows unique tropism for the platelets of dogs, leading to infectious canine cyclic thrombocytopenia [12].

In China, wild and domestic ruminants play active roles as Anaplasma carriers and as infection reservoirs. A. phagocytophilum has been detected in rodents, sheep, rabbits, cattle, goats and ticks in different areas of China [7,20-23]. In addition, the presence of A. bovis and A. ovis in sheep and goats, as well as A. marginale in cattle has been reported in China [7,20,25]. In a previous study, 262 field blood samples of goats collected from four cities in China were analyzed for the presence of A. phagocytophilum, A. bovis and A. ovis [7]. Molecular investigations have also been conducted in other provinces in China. In the current study, 1331 blood samples were collected from 22 counties in six provinces distributed across central, western and southwestern China. Here, we show that domestic ruminants in this area are commonly infected by distinct Anaplasma species.
Materials and Methods

Ethics statement
This study was conducted in accordance with the Chinese Laboratory Animal Administration Act of 1988. The research protocol was reviewed and approved by the Research Ethics Committee of Henan Agricultural University. The field studies did not involve endangered or protected species.

Sample collection and DNA extraction
A total of 1331 EDTA-K2 blood samples were collected from asymptomatic domestic ruminants (621 sheep and 710 goats) from 2011 to 2014 during June and October. Samples were collected from 22 randomly selected livestock farms or domestic animal owners interspersed across six provinces of China (Fig. 1). One blood sample was taken from the jugular vein of each animal. DNA was extracted from all samples using a TIANamp Blood DNA kit (TIANGEN biotech, China) according to the manufacturer’s instructions.

In summer of 2011 (July) and 2012 (May, June and July), a total of 31 adult female ticks were collected from naturally infected sheep at four localities in Henan province, China. After collection, ticks were identified morphologically by light microscopy, then preserved in absolute ethanol. Genomic DNA was extracted and purified from the entire body of adult ticks using a TIANamp Blood DNA kit (TIANGEN biotech, China) according to the manufacturer’s instructions.

PCR amplification
Nested polymerase chain reactions (PCRs) were applied to detect the presence of *A. phagocytophilum*, *A. bovis* and *A. ovis*. In the first round of the reaction, genus-specific primers EE1/2 were utilized to amplify the *Anaplasma* spp. 16S rRNA gene [1]. The products were then used as templates for the second round of PCR using the *A. phagocytophilum*-specific primers SSAP2f and SSAP2r, which generate a product of 641 bp, and the *A. bovis*-specific primers AB1f and AB1r, which generate a product of 551 bp [5]. For *A. ovis*, the major surface protein 4 (*msp4*) gene was amplified as previously described [2]. The primer pair T1B and T2A targeting the 16S rDNA gene of ticks was used to verify the results of microscopic observation. For the 60 kDa heat shock protein (groEL) of *A. phagocytophilum*, nested PCR containing two primer sets (groEL-1F: 5’-TATAGCTAGCATAATTACCCAGAGC-3’, groEL-1R: 5’-GGTTAGTCTGCTTTCGATGC-3’, groEL-2F: 5’-TTATGTCGTATGCGCCGTG-3’, groEL-2R: 5’-CGGACCTTGCCACATTTT-3’), which generates a product of 339 bp, was conducted. PCR amplification was performed as previously described [24]. The products were electrophoresed in a 0.8% agarose gel containing GelRed (Biotium, USA) and observed under UV light.

Sequence analysis
The PCR products of positive samples were sent for sequencing as previously described [24]. The sequence accuracy was verified by two-directional sequencing, and the
assembled sequences were analyzed by a BLASTn search (National Center for Biotechnology Information, USA). Phylogenetic analyses were performed using the MEGA 5.05 software [6]. Phylogenetic trees were constructed using previous methods [13,24].

**Results**

As shown in Table 1, of 621 sheep samples, 242 (39.0%) were positive for *Anaplasma*. The infection rates at different sampling sites varied from 36.2 to 78.1%. In Qinghai, only *A. ovis* infection (54.5%) was detected. The most commonly found co-infection was *A. bovis* and *A. phagocytophilum* combination, which reached 12.5% (4/32) in sheep from Shanxi, and had an overall infection rate of 6.4% (40/621). The simultaneous infection of three pathogens occurred in only 1.3% (8/621) of the sheep studied. Of 710 goats sampled from four provinces, *Anaplasma* DNA was identified in 323 (45.5%), among which only 15 (2.1%) were positive for *A. phagocytophilum* (Table 2). *A. ovis* and *A. phagocytophilum* co-infection was not detected in any goats. Simultaneously infection with three pathogens was found in 20 (2.8%) goats from Henan and Shaanxi. Among the 180 *A. phagocytophilum* 16SrRNA-positive animals, the *groEL* gene was amplified from 131 specimens (data not shown). Additionally, 31 tick samples (*Haemaphysalis longicornis*) were collected at the same time and tested for the presence of *A. phagocytophilum/A. bovis/A. ovis* DNA, and the results showed that 29% (9/31) were positive for *Anaplasma* (Table 3). Moreover, in all areas in which ticks positive for *Anaplasma* were reported, there were some positive cases of sheep/goats.

To investigate the genetic variability of *Anaplasma* spp. in

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**Table 1. Detection of Anaplasma* in sheep at various geographic sites**

| Site  | Number of tested | Number of positive | Number (%) of sheep infected with: |  |
|-------|------------------|--------------------|-----------------------------------|---|
|       |                  |                    | One pathogen                      | Two pathogens                  | Three pathogens                 |
|       |                  |                    | Anaplasma (A.) phagocytophilum     | A. bovis                       | A. ovis | A. ovis + A. phagocytophilum | A. ovis + A. bovis + A. phagocytophilum |
| Henan | 567              | 205 (36.2)         | 32 (5.6)                          | 55 (9.7)                       | 67 (11.8) | 2 (0.3) | 9 (1.6) | 36 (6.3) | 4 (0.7) |
| Shanxi| 32               | 25 (78.1)          | 1 (3.1)                           | 5 (15.6)                       | 9 (28.1) | 0 (0) | 2 (6.2) | 4 (12.5) | 4 (12.5) |
| Qinghai| 22              | 12 (54.5)          | 0 (0)                             | 0 (0)                          | 12 (54.5) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Total | 621              | 242 (39.0)         | 33 (5.3)                          | 60 (9.7)                       | 88 (14.2) | 2 (0.3) | 11 (1.8) | 40 (6.4) | 8 (1.3) |

*The 16S rRNA gene was used to detect the presence of *A. phagocytophilum* and *A. bovis*, while msp4 gene was used to detect the presence of *A. ovis*. |

**Table 2. Detection of Anaplasma in goats at various geographic sites**

| Site  | Number of tested | Number of positive | Number (%) of goats infected with: |  |
|-------|------------------|--------------------|-----------------------------------|---|
|       |                  |                    | One pathogen                      | Two pathogens                  | Three pathogens                 |
|       |                  |                    | A. phagocytophilum                | A. bovis | A. ovis | A. ovis + A. phagocytophilum | A. ovis + A. bovis + A. phagocytophilum |
| Henan | 251              | 105 (58.2)         | 9 (3.6)                           | 48 (19.1) | 11 (4.4) | 0 (0) | 1 (0.4) | 30 (11.9) | 6 (2.4) |
| Shaanxi| 123             | 52 (42.3)          | 3 (2.4)                           | 4 (3.2) | 0 (0) | 0 (0) | 1 (0.8) | 30 (24.4) | 14 (11.4) |
| Yunnan| 297              | 147 (49.5)         | 2 (0.7)                           | 10 (3.4) | 117 (39.4) | 0 (0) | 18 (6.1) | 0 (0) | 0 (0) |
| Guizhou| 39              | 19 (48.7)          | 1 (2.6)                           | 12 (30.8) | 1 (2.6) | 0 (0) | 3 (7.7) | 2 (5.1) | 0 (0) |
| Total | 710              | 323 (45.5)         | 15 (2.1)                          | 74 (10.4) | 129 (18.2) | 0 (0) | 23 (3.2) | 62 (8.7) | 20 (2.8) |

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Table 3. Detection of *Anaplasma* in ticks and animals

| Sampling time | Sampling site       | Number of sampled | Number of positive (%) | Infected *Anaplasma* | Animals infected *Anaplasma* in the same locality (number of positive/Number of sampled) |
|---------------|---------------------|-------------------|------------------------|----------------------|----------------------------------------------------------------------------------|
| July 10, 2011 | Luoyang Yiyang      | 5                 | 2 (40.0)               | *A. phagocytophilum*  | *A. phagocytophilum* (3/10), *A. bovis* (7/10), *A. ovis* (5/10)                  |
| May 14, 2012  | Luoyang Yiyang      | 10                | 3 (30.0)               | *A. bovis*           | *A. phagocytophilum* (3/65), *A. bovis* (32/65), *A. ovis* (1/65)                |
| June 13, 2012 | Nanyang Nanzhao     | 10                | 1 (10.0)               | *A. bovis*           | *A. phagocytophilum* (1/11), *A. bovis* (8/11)                                  |
| July 14, 2012 | Luoyang Yiyang      | 6                 | 3 (50.0)               | *A. bovis*           | *A. phagocytophilum* (16/20), *A. bovis* (19/20), *A. ovis* (7/20)              |
| Total         |                     | 31                | 9 (29.0)               |                      |                                                                                  |

Fig. 2. Phylogenetic tree of *Anaplasma* (*A.*) *phagocytophilum* based on the 16S rRNA partial gene sequence. A neighbor-joining tree was constructed using the Kimura two-parameter model in the MEGA 5.05 software. An alignment of 613 bp partial 16S rRNA sequences was used to construct this tree. The sequences in bold were obtained in the present study. Numbers on the branches indicate the percent of replicates that reproduced the topology for each clade.

Sheep and goats from six provinces of China, positive samples representative of different hosts and geographic locations were sequenced, and 26 sequences including nine from *A. phagocytophilum*, six from *A. bovis* and 11 from *A. ovis* were obtained. A total of 613 bp of 16S rRNA of *A. phagocytophilum* and 541 bp of 16S rRNA of *A. bovis*, as well as 559 bp of the *msp4* gene of *A. ovis* were analyzed. The nine *A. phagocytophilum* 16S rRNA sequences grouped into three sequence clusters (Fig. 2), which were designated 1–3 in this study. The similarity among the five isolates (LY2, YY, YY10, YY19 and LY24) in cluster 1 ranged from 99.5% to 100%, while the three clusters were found to be more divergent, sharing 98.5% to 99.7% homology. Cluster 2 (JY, ZY and JC) was located in a separate clade with 99% identity to strain CE18 (GenBank accession No. GQ450278) that was detected in red deer (*Cervus elaphus*) from Poland. Cluster 3 had just one isolate, GY, which was most different from other isolates. The nine partial *groEL* gene sequences obtained in this study, which had only several basepair differences among them, grouped together on a separate clade (Fig. 3). In addition, the results of a BLASTn search of the NCBI showed the highest similarity (92%) with an uncultured *Anaplasma* sp. (GenBank accession No. JN055360) isolate, which was obtained from a Japanese sika deer (*Cervus nippon yasouensis*).

The 541 bp partial 16S rRNA genes of *A. bovis* were 100% identical and homologous with the sequences derived from several hosts (goat, cattle and deer) from different areas of China (GenBank Accession No. HQ913644, FJ169957 and KJ639883), as well as a deer from South Korea (GenBank accession No. EU682764) (Fig. 4). There was no evidence of
any geographic segregation of *A. bovis* in this study. The 11 sequences of the *msp4* gene of *A. ovis* formed three clusters, designated I–III (Fig. 5). The sequences within cluster I were 99.6% to 100% homologous with each other, while the sequences in cluster II showed 99.6% to 99.8% homology. The two isolates in cluster III showed 100% identity.

**Discussion**

Sheep and goats are natural reservoirs for a variety of pathogens including bacteria (*e.g.*, *A. phagocytophilum* and *Brucella abortus*) [19,21], parasites (*e.g.*, *Theileria spp.* and *Haemonchus contortus*) [4,15] and viruses (*e.g.*, peste-des-petits-ruminants virus) [14]. In China, the animal breeding industry and family farming are flourishing, which means that epidemiological investigations into *Anaplasma* spp. are of particular importance.

Previous studies on *A. phagocytophilum* infection in sheep and goats have been conducted in a forest area of northeastern China [22], four counties in southeastern China [7] and six counties in Xinjiang, northwest China [20]. The results of the present study in the six provinces of China showed that the
overall infection rate of *A. phagocytophilum* was 3.6%, which was much lower than that found in livestock from Changbai Mountain in Jilin Province, where there was a prevalence of 6.7% [22], as well as in sheep and goats in four counties in southeastern China, where there was a prevalence of 6.1% [7]. It was also far lower than the detection rate of sheep and cattle from Xinjiang (17.6%) [20]. This may have been due to the sampling sites in these three studies all being in mountainous areas, while the investigated sites in this study were mostly plain areas. Despite PCR detection of *A. phagocytophilum* in the study areas, no clinical symptoms were observed in any of the investigated animals. This was in agreement with the findings of Zhou et al. [25], indicating that subclinical infections caused by *A. phagocytophilum* occurred in sheep and goats. Phylogenetic analysis of *A. phagocytophilum* using the nine sequences in this study as well as five other sequences previously obtained from ruminants in different areas of the world indicated that the isolates in present study were located in three different clusters. Overall, five sequences within cluster 1 and three isolates in cluster 2 were obtained from different sampling sites. The groEL gene is often used for phylogenetic studies of *A. phagocytophilum* owing to its high genetic heterogeneity, and because two distinct lineages could be delineated in Europe by sequence analysis [8,11]. However, the partial groEL sequences obtained in this study appeared to be far from both two clusters mentioned above, implying a separate lineage or cluster may exist in China. Investigation of *A. bovis* infection revealed that it was the dominant pathogen in goats from all sampling sites except Yunnan (17.6%) [20]. This may have been due to the sampling sites in these three studies all being in mountainous areas, while the investigated sites in this study were mostly plain areas. Despite PCR detection of *A. phagocytophilum* in the study areas, no clinical symptoms were observed in any of the investigated animals. This was in agreement with the findings of Zhou et al. [25], indicating that subclinical infections caused by *A. phagocytophilum* occurred in sheep and goats. Phylogenetic analysis of *A. phagocytophilum* using the nine sequences in this study as well as five other sequences previously obtained from ruminants in different areas of the world indicated that the isolates in present study were located in three different clusters. Overall, five sequences within cluster 1 and three isolates in cluster 2 were obtained from different sampling sites.

**Fig. 5.** Phylogenetic tree of *A. ovis* based on the msp4 partial gene sequence.

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Investigation of *A. bovis* infection revealed that it was the dominant pathogen in goats from all sampling sites except Yunnan province. The overall infection rates in sheep and goats were 9.7% and 10.4%, respectively, which were similar to the prevalence of *A. bovis* in deer (9.0%) and *Haemaphysalis longicornis* ticks (12.0%) in Japan, but much lower than that of goats in China (49.6%) and Japanese cattle (53.5%) [5,7,9]. Phylogenetic analysis indicated that the six sequences obtained in the present study were 100% homologous and formed a clade with seven previously obtained sequences derived from different hosts and areas. The phylogenetic trees of *A. phagocytophilum* and *A. bovis* showed no relationship between host and cluster. There was also no evidence of any geographic segregation of the two organisms in this study.

The average infection rates of *A. ovis* were relatively high in both sheep (14.2%) and goats (18.2%); however, they were lower than that of sheep in Xinjiang [7,20]. Half of the sheep from Qinghai were infected with *A. ovis*, and none of these were positive for two other *Anaplasma* species. Infection with *A. ovis* was confirmed by sequencing of the msp4 gene, which has been shown to be reliable for phylogenetic studies of *A. ovis* [2]. The 11 sequences analyzed in the present study fell into three clusters. Additionally, three of the four isolates in cluster I from sheep and goats in central China fell into a clade with another isolate previously obtained from Hebei, which is also a central province of China (GenBank accession No. HQ456350). The sequences in cluster II were all obtained from goats. The two sequences in cluster III, which were derived from two goats in Yunnan, fell into a separate clade. Additionally, the results revealed geographic segregation to a certain extent, as well as a relationship between the host and cluster of *A. ovis* in the present study.

Co-infection of two *Anaplasma* species was detected in nearly all investigated sites. *A. ovis* was found to be dominant in sheep, while *A. bovis* was the dominant *Anaplasma* species found in goats, which was consistent with the results reported by Liu et al. [7]. Furthermore, 40 (6.4%) sheep and 62 (8.7%) goats were found to be positive for both *A. phagocytophilum* and *A. bovis*, which was much more common than other combinations. It is still not clear if this could be explained by the exclusion of different *Anaplasma* species; however, similar phenomena occurred among genotypes of *A. phagocytophilum* [18].
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Conflict of Interest

There is no conflict of interest.

References

1. 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*). Vet Parasitol 1996, 63, 319-329.

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

3. Dumler JS, Barlough JE, Madigan JE, DeRock E, Bigornia L. *Ehrlichia* and *Anaplasma* of the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasmataceae*, *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 Int J Syst Evol Microbiol 2001, 51, 2145-2165.

4. Hassan MA, Raoofi A, Lotfollahzadeh S, Javanbakht J. Clinical and cytological characteristics and prognostic implications on sheep and goat *Theileria* infection in north of Iran. J Parasit Dis 2015, 39, 190-193.

5. Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki AK, Hiramitsu Y, Tajima T. 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, 1102-1109.

6. Kumar S, Tamura K, Nei M. MEGA: Molecular Evolutionary Genetics Analysis software for microcomputers. Comput Appl Biosci 1994, 10, 189-191.

7. Liu Z, Ma M, Wang Z, Wang J, Peng Y, Li Y, Guan G, Luo J, Yin H. Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. Appl Environ Microbiol 2012, 78, 464-470.

8. Lommano E, Dvořák C, Vallotton L, Jenni L, Gern L. Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland. Ticks Tissue Borne Dis 2014, 5, 871-882.

9. Ooshiro M, Zakimi S, Matsukawa Y, Katagiri Y, Inokuma H. Detection of *Anaplasma bovis* and *Anaplasma phagocytophilum* from cattle on Yonaguni Island, Okinawa, Japan. Vet Parasitol 2008, 154, 360-364.

10. Rav V, Golovljova L. *Anaplasma*, *Ehrlichia*, and "Candidatus Neoehrlichia" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect Genet Evol 2011, 11, 1842-1861.

11. Rymaszewska A. Genotyping of *Anaplasma phagocytophilum* strains from Poland for selected genes. Folia Biol (Krakow) 2014, 62, 37-48.

12. Sainz A, Amusategui I, Tesouro MA. *Ehrlichia phagocytophilum* infection and disease in dogs in Spain. J Vet Diagn Invest 1999, 11, 382-384.

13. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987, 4, 406-425.

14. Şevik M, Sait A. Genetic characterization of peste des petits ruminants virus, Turkey, 2009-2013. Res Vet Sci 2015, 101, 187-195.

15. Singh G, Singh R, Verma PK, Anand A. Anthelmintic efficacy of aqueous extract of *Butea monosperma* (Lam.) Kuntze against *Haemonchus contortus* of sheep and goats. J Parasit Dis 2015, 39, 200-205.

16. Sreekumar C, Anandan R, Balasundaram S, Rajavelu G. Morphology and staining characteristics of *Ehrlichia bovis*. Comp Immunol Microbiol Infect Dis 1996, 19, 79-83.

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

18. Stuen S, Whist SK, Bergström K, Moom T. Possible exclusion of genotypes in *Anaplasma phagocytophilum*-infected lambs. Vet Rec 2005, 156, 518-520.

19. Wärth G, Melzer F, Tomaso H, Roesler U, Neubauer H. Detection of *Brucella abortus* DNA in aborted goats and sheep in Egypt by real-time PCR. BMC Res Notes 2015, 8, 212.

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

21. Zhan L, Cao WC, Jiang JF, Zhang XA, Liu YX, Wu XM, Zhang WY, Zhang PH, Buien CL, Dumler JS, Yang H, Zuo SQ, Chu CY, Liu W, Richardus JH, Habbema JD. *Anaplasma phagocytophilum* from Rodents and Sheep, China. Emerg Infect Dis 2010, 16, 764-768.

22. Zhan L, Cao WC, Jiang JF, Zhang XA, Wu XM, Zhang WY, Liu W, Zuo SQ, Cao ZW, Yang H, Richardus JH, Habbema JD. *Anaplasma phagocytophilum* in livestock and small rodents. Vet Microbiol 2010, 144, 405-408.

23. Zhan L, Chu CY, Zuo SQ, Wu XM, Dumler JS, Jia N, Jiang BG, Yang H, Cao WC. *Anaplasma phagocytophilum* and *Borreliia burgdorferi* in rabbits from southeastern China. Vet Parasitol 2009, 162, 354-356.

24. Zhang Y, Li Y, Cui Y, Wang J, Cao S, Jian F, Wang R, Zhang L, Ning C. First molecular evidence for the presence of *Anaplasma* DNA in milk from sheep and goats in China. Parasitol Res 2016, 115, 2789-2795.

25. Zhou Z, Nie K, Tang C, Wang Z, Zhou R, Hu S, Zhang Z. Phylogenetic analysis of the genus *Anaplasma* in southwestern China based on 16S rRNA sequence. Res Vet Sci 2010, 89, 262-265.