Molecular detection of *Anaplasma* infections in ixodid ticks from the Qinghai-Tibet Plateau

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**Abstract**

*Anaplasma* species are tick-transmitted obligate intracellular bacteria that infect many wild and domestic animals and humans. The prevalence of *Anaplasma* spp. in ixodid ticks of Qinghai Province is poorly understood. In this study, a total of 1104 questing adult ticks were investigated for the infection of *Anaplasma* species. As a result, we demonstrated the total infection rates of 3.1, 11.1, 5.6, and 4.5% for *A. phagocytophilum*, *A. bovis*, *A. ovis* and *A. capra*, respectively. All of the tick samples were negative for *A. marginale*. The positive rates of *A. phagocytophilum*, *A. ovis* and *A. capra* in different tick species were significantly different. The positive rates of *A. capra* and *A. bovis* in the male ticks were significantly higher than that in the female ticks. Sequence analysis of *A. ovis* showed 99.5–100% identity to the previous reported isolates. The sequences of *A. phagocytophilum* had 100% identity to strains Ap-SHX21, JC3-3 and ZAM dog-181 from sheep, Mongolian gazelles, and dogs. Two genotypes of *A. capra* were found based on 16S rRNA, citrate synthase (*gltA*) gene and heat shock protein (*groEL*) gene analysis. In conclusion, *A. bovis*, *A. ovis*, *A. phagocytophilum*, and *A. capra* were present in the ticks in Qinghai Province. *Anaplasma* infection is associated with tick species, gender and distribution. These data will be helpful for understanding prevalence status of *Anaplasma* infections in ticks in Qinghai-Tibet Plateau.

**Keywords:** *Anaplasma*, Tick, Sequence analysis, Prevalence
known about the *Anaplasma* infection in the ticks in Qinghai Province.

Qinghai Province is located in the northeastern part of Qinghai-Tibet Plateau in western China. Qinghai has an average altitude of more than 3000 m with 54% of the total area being between 4000 m and 5000 m. The provincial climate is characterized by being relatively arid, windy, and cold. Qinghai contains significant amounts of pastures and is an important region for animal production. Qinghai has 33.45 million ha of grassland. The grassland meadows are classified as alpine, swamp, Gobi, forest, and prairie. Yaks, Tibetan sheep, sheep and goats are adapted for survival and growth on these grasslands. Ixodid ticks infestation of livestock is often found in Qinghai Province, including 54.5, 24.0, 36.1% infection rates of *A. ovis* [10], *Babesia* spp. in wild yaks [11], and *Theileria* spp. in yaks [12], respectively. However, very little is known about the *Anaplasma* infection in animals and ticks. In this study, we identified and analyzed the infections of *A. phagocytophilum*, *A. bovis*, *A. ovis*, *A. marginale* and *A. capra* in ticks. The data provide an overview of *Anaplasma* infections and the potential threats to both livestock and humans in the study areas.

**Methods**

**Sampling sites and tick collection**

Samples were collected in the Qinghai Province, the Qinghai-Tibetan Plateau at an average altitude of > 3000 m. From February to October in 2015–2017, a total of 1104 questing adult ticks were collected from vegetation on 22 counties of Qinghai by using the flagging method. All of the tick specimens were identified according to morphological criteria [13] and were confirmed by sequence analysis of a partial fragment of the 16S rRNA gene.

**DNA extraction, PCR amplification and sequencing**

DNA extraction of each individual ticks was conducted as described previously [2]. DNA samples were detected for the presence of the agents in the genus *Anaplasma* by PCR targeting the *msp4* gene for *A. ovis* and *A. marginale*, the 16S rRNA gene for *A. phagocytophilum* and *A. bovis*, and the citrate synthase (*gltA*) gene for *A. capra*, respectively. For further confirmation of the *A. capra*, the 16S rRNA gene and the heat-shock protein gene (*groEL*) were amplified from *A. capra* positive samples. The 16S rRNA gene was amplified for the molecular identification of the tick species. The PCR was carried out by using an automatic thermocycler (Bio-Rad, Hercules, USA). The reaction system for the PCRs was the same as described in our previous study [14] and the PCR primers and cycling conditions were shown in Table 1. The DNAs extracted from the animals infected with *A. ovis*, *A. marginale*, *A. phagocytophilum*, *A. bovis* and *A. capra* were used as positive controls, and double distilled water was used as a negative controls. The PCR products were visualized under UV illumination in a 1.2% agarose gel followed by electrophoresis and treated with GoldView I (Solarbio, Beijing, China).

| Target species | Target gene | Primer(5‘ → 3’) | Annealing temperature (°C) | No. of cycles | Expected size (bp) | References |
|---------------|-------------|----------------|-----------------------------|---------------|-------------------|------------|
| *Anaplasma* spp. | 16S rRNA | EE1: TCTCTGCTCAGAAAGACGCTGCGGC<br>EE2: AGTCATGACCACAAACTCTAAATGCTG | 55 | 35 | 1400 | [37] |
| *A. bovis* | 16S rRNA | AB1f: CTGCTAGCTGCTATGAGAAC<br>AB1r: TCTCCTCCTACTCTACGTTTCA | 55 | 35 | 551 | [26] |
| *A. phagocytophilum* | 16S rRNA | SSAP2f: GCTGTAATGTGGGGATAATTTAT<br>SSAP2r: ATGGCTGCTTCTTTCGTTA | 55 | 35 | 641 | [26] |
| *A. marginale* | *msp4* | Amargmsp4F: CTGAACCAGGGAGTAATGGG<br>Amargmsp4R: CCGATATTGCTGCCAGAGATCC | 60 | 30 | 344 | [38] |
| *A. ovis* | *msp4* | MSP43: CCGGATACCTCTAGCTGAACAGAATCTTG<br>MSP45: GGGAGCTCCTATGAATTACAGAGAATTGTTTAC | 60 | 35 | 869 | [39] |
| *A. capra* | *gltA* | gltAouterF: GCGATTTTAGAGTGYGGAGATTG<br>gltAouterR: TACAATACCGGAGTAAAAGTCA | 55 | 35 | 1031 | [1] |
| | | gltAinnerF: GCGATTTTAGAGTGYGGAGATTG<br>gltAinnerR: GCGATTTTAGAGTGYGGAGATTG | 60 | 35 | 594 | |
| 16S rRNA | Forward: GCAAGTCCCAAGCCCAACCTTGTT<br>Reverse: CCACGATATTGCTGCCAGAGATCC | 58 | 35 | 1261 | [35] |
| *groEL* | Forward: TGAAGAGCATCAAACCCGAAG<br>Reverse: CTGCTGCTGATGCTATCGG | 55 | 35 | 874 | [35] |
| Tick | 16S rRNA | 16SrRNA-F: CTGCTCAATGATTTTTTAAATTGCTG<br>16SrRNA-R: CCGGTCTGAACTCAGATCAAGT | 55 | 35 | 450 | Designed for this study |
The PCR products were purified with the TaKaRa Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa, Dalian, China). Purified PCR products were cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA), and then transformed into Escherichia coli JM109 competent cells (TaKaRa, Dalian, China). Three positive colonies from each sample were subjected to sequencing. The obtained sequences were used to conduct BLAST search in GenBank® of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis
The data were grouped into three variables in terms of tick species, tick gender and the altitude of the sampling sites, respectively. Differences in each group were statistically calculated using a Chi-square test in Predictive for Analytics Software Statistics 18 (PASW, SPSS Inc., Chicago, IL, USA). A $P$-value of $<0.05$ was considered significant.

Results
Identification of the tick species
A total of 1104 questing adult ticks (512 female, 592 male) were collected from vegetation in 22 counties of Qinghai Province. The ticks included seven species in three genera. There were 454 Haemaphysalis qinghaiensis, 263 D. nuttalli, 246 D. silvarum, 42 H. danieli, 3 Ixode crenulatus and two H. tibetensis respectively (Fig. 1). The species of ticks identified by morphology and supported by sequence analysis. The 16S rRNA sequence of H. qinghaiensis showed 100% identity to H. qinghaiensis isolate HY21 (GenBank accession number: MF629877) from Huangyuan in Qinghai; D. nuttalli and D. silvarum showed 99% similarity to D. nuttalli isolate HBS5 (GenBank accession number: KU558731) and D. silvarum isolate Hebei (GenBank accession number: JF979379) from Hebei Province in China. The 16S rRNA sequences of H. danieli, H. tibetensis, D. abaensis and I. crenulatus were obtained for the first time.

Detection of the Anaplasma spp. in ticks
Five Anaplasma species were investigated in the ticks. Of the 1104 samples tested, the average infection rates were 3.1, 11.1, 5.6, and 4.5% for A. phagocytophilum, A. bovis, A. ovis, and A. capra, respectively. All of the samples were negative for A. marginale. A. phagocytophilum was detected in four tick species from ten sampling sites, and it was detected for the first time in D. abaensis, D. nuttalli, and H. danieli. A. bovis was detected in five tick species from 14 sampling sites, whereas A. ovis was detected in three tick species from nine sampling sites. Three tick species including H. qinghaiensis, D. abaensis

Fig. 1 Map of the Sampling sites and the distribution of the collected tick species in Qinghai Province
and *D. nuttalli* were infected by *A. capra*. The prevalence of *Anaplasma* spp. in each sampling site is shown in Table 2.

Molecular characterization was based on the partial sequences of 16S rRNA gene (642 and 551 bp) for *A. phagocytophilum* and *A. bovis*, *msp4* gene (869 bp) for *A. ovis*, 16S rRNA, *gltA* and *groEL* genes (1261 bp, 594 bp and 874 bp) for *A. capra*. These sequences were generated from positive samples representing the different sampling sites. As listed in Table 3, *A. ovis* were grouped into four genotypes. *A. phagocytophilum* were classified into three genotypes, and they were 100% identical to sequences of strains Ap-SHX21, JC3–3, and ZAM dog-181 from sheep, Mongolian gazelles, and dogs, respectively. *A. bovis* were classified into five genotypes. The 16S rRNA gene sequences of *A. capra* showed 99.8–100% similarity to strain S62b from sheep and strain 9-13a from goat, and the *groEL* gene sequences were identical with strain tick102/China/2013 and M141a, respectively. These sequences showed a close relation to the sequences of strain HLJ-14 from a patient. In addition, two genotypes of *gltA* gene sequences of *A. capra* were obtained in this study.

### Risk factors for *Anaplasma* infection to in the tick species
Risk factors, including tick species, gender, and altitude of sampling sites, were used as variables for statistical analysis of the infection patterns of *Anaplasma* spp. (Table 4).

### Table 2 Detection of *Anaplasma* spp. in the ticks collected from 22 counties in Qinghai Province

| County/Average altitude | Tick species | Number of tested | Number of infected (n)/Infection rate (%) |
|-------------------------|--------------|-----------------|------------------------------------------|
|                         |              |                 | *A. phagocytophilum* | *A. bovis* | *A. ovis* | *A. capra* |
| Ledu/2000 m             | *H. qinghaiensis* | 57              | 0                         | 0          | 10/17.5   | 17/29.8     |
| Huangzhong/2645 m       | *H. qinghaiensis* | 57              | 7/12.3                    | 14/24.6    | 5/8.8     | 14/24.6     |
| Qumalai/4223 m          | *D. abaensis*  | 51              | 0                         | 2/3.9      | 0         | 5/9.8       |
| Yushu/4493 m            | *D. abaensis*  | 55              | 0                         | 16/29.1    | 0         | 7/12.7      |
| Maduo/4300 m            | *H. qinghaiensis* | 48             | 1/2.1                     | 9/18.8     | 0         | 1/2.1       |
|                         | *D. abaensis*  | 3               | 0                         | 0          | 0         | 0           |
| Maqin/3730 m            | *D. abaensis*  | 38              | 2/5.3                     | 7/18.6     | 3/7.9     | 1/2.6       |
|                         | *H. qinghaiensis* | 5               | 0                         | 1/33.3     | 0         | 0           |
| Mengyuan/2880 m         | *H. qinghaiensis* | 58             | 1/1.7                     | 19/32.8    | 0         | 0           |
|                         | *D. silvarum*  | 1               | 0                         | 0          | 0         | 0           |
| Tianjun/3180 m          | *D. silvarum*  | 54              | 0                         | 0          | 0         | 0           |
| Delingha/2980 m         | *D. silvarum*  | 39              | 0                         | 0          | 0         | 0           |
| Chengduo/4500 m         | *D. nuttalli*  | 16              | 0                         | 0          | 0         | 2/12.5      |
|                         | *H. qinghaiensis* | 30             | 5/16.7                    | 4/15.2     | 0         | 0           |
|                         | *H. tibetensis* | 2               | 0                         | 0          | 0         | 0           |
|                         | *I. crenulatus* | 3               | 0                         | 1/33.3     | 0         | 0           |
| Banma/3560 m            | *H. qinghaiensis* | 43             | 0                         | 0          | 0         | 0           |
| Gangcha/3300 m          | *D. nuttalli*  | 29              | 0                         | 0          | 0         | 0           |
| Huangyuan/2666 m        | *H. qinghaiensis* | 56             | 8/14.3                    | 11/19.6    | 0         | 1/1.8       |
| Qilian/2810 m           | *D. abaensis*  | 66              | 0                         | 2/3.0      | 6/9.1     | 0           |
|                         | *D. nuttalli*  | 17              | 0                         | 0          | 0         | 0           |
| Dulan/3180 m            | *D. nuttalli*  | 31              | 1/3.2                     | 0          | 4/12.9    | 0           |
| Guinan/3100 m           | *D. nuttalli*  | 51              | 0                         | 1/2.0      | 14/27.5   | 0           |
| Huzhu/2520 m            | *H. qinghaiensis* | 50             | 0                         | 5/10.0     | 0         | 0           |
| Zaduo/4200 m            | *D. abaensis*  | 50              | 4/8.0                     | 5/10.0     | 0         | 0           |
| Guide/2200 m            | *H. danieli*  | 42              | 4/8.5                     | 5/100      | 0         | 0           |
| Henanxian/3600 m        | *D. nuttalli*  | 51              | 1/2.0                     | 20/39.2    | 11/21.6   | 2/3.9       |
| Minhe/1650 m            | *H. qinghaiensis* | 50             | 0                         | 0          | 3/6.0     | 0           |
| Geermu/2800 m           | *D. nuttalli*  | 51              | 0                         | 0          | 6/11.8    | 0           |
| Total                   |               | 1104            | 34/3.1                    | 122/11.1   | 62/5.6    | 50/4.5      |
The presence of *A. phagocytophilum*, *A. capra*, and *A. ovis*. *H. danieli* had a higher risk than other tick species to be infected with *A. phagocytophilum*. *D. nuttalli* had a higher risk to be infected with *A. ovis*. *H. qinghaiensis* was most likely to be infected by *A. capra*. Male ticks were more likely to be infected by *A. bovis* or *A. capra* than female ticks. Altitude was a risk factor to *A. phagocytophilum*, *A. bovis* and *A. capra* infections. Ticks collected below 3000 m areas had a higher risk for being infected by *A. phagocytophilum* and *A. capra* than in the ticks collected at elevations greater than 3000 m. *A. bovis* infection rates in ticks collected above 4000 m were higher than in the ticks collected below 4000 m.

**Discussion**

Qinghai is one of the five largest animal grazing regions in China. Grazing animal production is a supporting industry in this region. The Qinghai ecosystem is very suitable for ixodid tick infestation and 25 tick species in six genera has been reported [15]. In this study we collected seven tick species from three genera. These were *H. qinghaiensis*, *H. tibetensis*, *H. danieli*, *D. nuttalli*, *D. silvarum*, and *I. crenulatus*. *H. qinghaiensis* is common in northwestern China, and it has been the dominant tick species in Qinghai since it was initially discovered in Huangyuan County [13]. In the present study, 41.1% of the collected ticks were *H. qinghaiensis*. Three *Dermacentor* spp. ticks (*D. abaensis*, *D. nuttalli* and *D. silvarum*) were frequently encountered on grazing livestock in high altitude areas (2800 to 4300 m), whereas *I. crenulatus* and *H. tibetensis* were rare. To verify the morphological identification of the tick species, the 16S rRNA gene sequences were analyzed. The sequences from *H. qinghaiensis*, *D. nuttalli*, and *D. silvarum* were compared with our reference sequences (data unpublished) because of the lack of the reference sequences in GenBank.

**Table 3** Genotyping of *Anaplasma* spp. in the ticks in Qinghai Province

| Anaplasma spp. | Gene marker | Number of obtained sequences | Number of genotypes | GenBank accession numbers of obtained sequences | Reference sequences from GenBank |
|---------------|-------------|------------------------------|---------------------|-----------------------------------------------|----------------------------------|
| *A. ovis*     | 16S rRNA    | 47                           | 4                   | MG940865, MG940866, MG940868, MG940867         | MF071305, HQ456347, EF067541, HQ456350 |
| *A. phagocytophilum* | 16S rRNA | 56                           | 3                   | MG940877, MG940878, MG940879                    | KU321304, KM186948, LC269823       |
| *A. bovis*    | 16S rRNA    | 40                           | 5                   | MG940884, MG940881, MG940880, MG940882, MG940883 | KU509990, HQ913645, EU682764, KJ639885, KF465981 |
| *A. capra*    | 16S rRNA    | 28                           | 2                   | MG940874, MG940873                             | MFO66917, KX417196                  |
|               | groEL       | 20                           | 2                   | MG940875, MG940876                             | KR261634, KX685888                  |
|               | gltA        | 18                           | 2                   | MG940871, MG940872                             | KX417308, KX685885                  |

**Table 4** Patterns of *Anaplasma* spp. prevalence in the ticks, grouped by tick species, tick gender and the altitude of the sampling sites

| Group | Number of tested | Number of infected (n)/Infection rate (%) | A. phagocytophilum P-value | A. bovis P-value | A. ovis P-value | A. capra P-value |
|-------|------------------|--------------------------------------------|-----------------------------|------------------|-----------------|------------------|
| Tick  | H. qinghaiensis  | 454                                        | 22/4.8                      | 0.0032           | 63/13.9         | 0.230            | 18/4.0           | 0.000057         | 33/7.3           | 0.0056           |
|       | H. tibetensis    | 2                                          | 0                           |                  | 1               | 0                | 0                | 0                |
|       | H. danieli       | 42                                         | 4/9.5                       | 5/11.9           | 0               | 0                | 0                | 0                |
|       | D. abaensis      | 263                                        | 6/2.3                       | 32/12.2          | 0               | 0                | 0                | 0                |
|       | D. silvarum      | 94                                         | 0                           | 0                | 0               | 0                | 0                | 0                |
|       | D. nuttalli      | 246                                        | 2/0.8                       | 21/8.5           | 35/14.2         | 0.312            | 4/1.6            | 13/4.9           |
|       | I. crenulatus    | 3                                          | 0                           | 1/33.3           | 0               | 0                | 0                | 0                |
| Gender| Female           | 512                                        | 16/3.1                      | 0.935            | 47/9.2          | 0.045            | 23/4.5           | 0.312            | 14/2.7           | 0.0077           |
|       | Male             | 592                                        | 18/3.0                      | 75/12.7          | 39/6.6          | 36/6.1           | 36/6.1           | 36/6.1           | 36/6.1           |
| Altitude| ≤ 3000 m     | 461                                        | 20/4.3                      | 0.015            | 54/11.7         | 0.037            | 30/6.5           | 0.316            | 32/6.9           | 0.000066         |
|       | 3000–3900 m     | 385                                        | 4/1.0                       | 31/8.1           | 32/8.3          | 3/0.8            | 3/0.8            | 3/0.8            |
|       | ≥ 4000 m        | 258                                        | 10/3.9                      | 37/14.3          | 0               | 0                | 15/5.8           | 15/5.8           | 15/5.8           |
Aanaplasma prevalence in ticks demonstrated a wide distribution of A. phagocytophilum, A. bovis, A. ovis and A. capra. Among the Anaplasm species, A. phagocytophilum is an emerging tick-borne zoonotic pathogen of public health significance [16], and it has been detected in many tick species, including H. qinghaiensis, H. concinna, H. longicornis, I. persulcatus, and D. silvarum in China [17–20]. We detected A. phagocytophilum in H. qinghaiensis, and, for the first time, found it in D. abaensis, D. nuttalli, and H. danieli. The 16S rRNA gene sequences represented three genotypes, which showed high identities to the sequences found in goats from Central and Southern China [21], these genotypes were different from the genotype identified from human samples. Therefore, the significance of these genotypes to public health needs further investigation. A. bovis was initially found as a pathogen of cattle but has also been reported in sheep, goats, wild deer, and dogs [5, 22, 23], indicating this agent has a broad host range. We detected A. bovis in five tick species (H. qinghaiensis, D. abaensis, D. nuttalli, I. crenulatus, and H. danieli) from 14 sampling sites and it has the highest infection rate when compared with A. phagocytophilum, A. ovis and A. capra. Five genotypes of A. bovis were found, demonstrating its diversity in the ticks of Qinghai. A. bovis can be found in many tick species, such as H. longicornis in China [24], Korea [25] and Japan [26]. A. bovis was also found in H. lagrangei in Thailand [27], H. concinna in Russia [28], H. megaspinosa in Japan [29]; Amblyomma variegatum and R. appendiculatus in Africa [30], Rhipicephalus evertsi in South Africa [31], and R. turaicus in Israel [32]. We found A. bovis in H. qinghaiensis, D. abaensis, D. nuttalli, I. crenulatus, and H. danieli ticks. Statistics analysis indicated that A. bovis was more likely to infect male ticks and ticks at altitude above 4000 m. This result may be related to the distribution of its mammal hosts, since the majority of the yak population lives at altitudes more than 4000 m.

A. ovis is widely distributed in Asia, Europe, Africa and North American. Several msp4 gene variants of A. ovis have been identified in sheep and goats in northwest regions of China [14, 33, 34]. D. nuttalli, Hyalomma asiaticum and Rhipicephalus pumilio are vectors of A. ovis in China [9]. We detected A. ovis in D. abaensis, D. nuttalli, H. tibetensis, and four msp4 gene variants were identified in ticks. These variants showed high similarities to those from Chinese and Spanish strains, indicating diversity of A. ovis in the study ticks.

A. capra was initially identified in goats, and was subsequently considered to be an emerging human pathogen [1]. A. capra was previously identified in H. qinghaiensis in Gansu Province, in H. longicornis in Shandong Province, and in I. persulcatus in Heilongjiang Province [35, 36]. We detected A. capra in H. qinghaiensis, D. abaensis, and D. nuttalli, and two genotypes were identified on the basis of gltA, 16S rRNA, groEL gene analysis. One genotype showed high sequence identity to the A. capra HLJ-14 strain, which had been reported in both goats and humans in China [1]. Another genotype showed low sequence identity to the strain HLJ-14 of A. capra, but high identity to an A. capra-like bacteria from H. qinghaiensis ticks [35]. Additionally, H. qinghaiensis is the dominant tick species for the infection of A. capra, and high prevalence occurs in the ticks found at altitudes less than 3000 m.

Although the present study has revealed the current status of ixodid tick infestation with Anaplasma spp. in the investigated areas, the specific biological vector for the individual Anaplasma species need to be further studied by transmission experiments. In addition, the infections of Anaplasma species in animals or humans should be investigated to understand the true impact of anaplasmiosis in Qinghai Province.

Conclusions

We demonstrated the prevalence of A. bovis, A. ovis, A. phagocytophilum, and A. capra in ticks from 22 counties of Qinghai Province. Anaplasma infection in ticks is associated with the species, gender and distribution of the ticks. The prevalence of A. capra in ticks may be a threat to public health in Qinghai Province.

Additional file

Additional file 1: Multilingual abstracts in the five official working languages of the United Nations. (PDF 726 kb)

Abbreviations

gltA: Citrate synthase; groEL: Heat shock protein

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Availability of data and materials

The datasets used or analyzed for this study are available in the corresponding author.

Authors’ contributions

HY and Z-JL designed this study and critically revised the manuscript. RH participated in study design, coordination, and manuscript revision. RH, Q-LN, and YQ-L participated in sample collection. RH, YJ, M-UM, ZC-QLN, and G-YL performed the experiments, data analysis, and drafted the manuscript. All of the authors read and approved the final manuscript.
Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Consent for publication

All of the authors of this manuscript declare that we have seen and approved the submitted version of this manuscript. Not applicable any individual persons data.

Competing interests

The authors declare that they have no competing interests.

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References

1. Li H, Zheng YC, Ma L, Ji N, Jiang BG, Jiang RR, et al. Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. Lancet Infect Dis. 2015;15:663–70.

2. Dumler JS, Barbert AF, Bekker CPJ, 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 ‘YKE agent’ as subjective synonyms of Ehrlichia phagocytophila. Int J Syst Evol Microbiol. 2001;51:1245–65.

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

4. Ben SM, Belkahia H, Messadi L. New Anaplasma species combinations in ruminants, rodents and ticks in Tunisia. Vet Microbiol. 2015;179:322–30.

5. Yang J, Liu Z, Niu Q, Liu J, Han R, Liu G, et al. Prevalence of Anaplasma phagocytophilum infection in ticks, China-Russia border. Emerg Infect Dis. 2011;17:922–4.

6. Zhang Y, Lv Y, Zhang F, Zhang W, Wang J, Cui Y, et al. Molecular and genetic identification of Anaplasma phagocytophilum DNA from Haemaphysalis hirudinacea ticks from northern China. Am J Trop Med Hyg. 2003;68:547–50.

7. Yang J, Liu Z, Ni Q, Liu J, Han 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.

8. Ben Said M, Belkahia H, Karacou M, Bousnini M, Yahiaou M, Daoulal-Jedidi M, et al. First molecular survey of Anaplasma bovis in small ruminants from Tunisia. Vet Microbiol. 2015;173:322–6.

9. Atif FA. Alpha proteobacteria of genus Anaplasma (Rickettsiales: Ehrlichioidea): epidemiology and characteristics of Anaplasma species related to veterinary and public health importance. Parasitology. 2016;143:659–85.

10. Qin XR, Han FJ, Luo LM, Zhao FM, Han HJ, Zhang ZT, et al. Anaplasma species detected in Haemaphysalis longicornis tick from China. Ticks Tick Borne Dis. 2018;9:840–3.

11. Lee MJ, Chae JS. Molecular detection of Ehrlichia chaffeensis and Anaplasma bovis in the salivary glands from Haemaphysalis longicornis ticks. Vector Borne Zoonotic Dis. 2010;10:411–3.

12. Mawata M, Kikihisa Y, Lin Q, Isogai T, Iwata H, Ito K, et al. Novel genetic variants of Anaplasma phagocytophilum, Anaplasma bovis, Anaplasma centrale, and a novel Ehrlichia spp. in wild deer and ticks on two major islands in Japan. Appl Environ Microbiol. 2006;72:1102–9.

13. Parola P, Cerreti JP, Sanogo YO, Miller RS, Thien HV, Gonzalez JP, et al. Detection of Ehrlichia spp., Anaplasma spp., Rickettsia spp, and other Eu报记者 in ticks from the Thai-Myanmar border and Vietnam. J Clin Microbiol. 2003;41:1600–5.

14. Shpyrno S, Fournier PE, Rudakov N, Tarasevich I, Raoult D. Detection of members of the genera Rickettsia, Anaplasma, and Ehrlichia in ticks collected in the Asiatic part of Russia. Ann N Y Acad Sci. 2006;1078:378–83.

15. Yoshimoto K, Matsuyama Y, Matsuda H, Sakamoto L, Matsumoto K, Yokoyama N, et al. Detection of Anaplasma phagocytophilum DNA from Haemaphysalis megaspinosa in Hokkaido, Japan. Vet Parasitol. 2010;168:170–2.

16. Sunpomporn K, Scott GR, Coetzter JAW, Tustin RC. Lesser-known rickettsias infecting livestock. ABC Press. 2004;2:536–49.

17. Tonetti N, Berggoetz M, Ruhle C, Pretorius AM, Gern L. Ticks and tick-borne pathogens from Israel. Clin Microbiol Infect. 2011;17:459–69.

18. Harrus S, Perlman-Avraham M, Murmucoiu Ky, Morick D, Eyal O, Baneth G. Molecular detection of Ehrlichia canis, Anaplasma bovis, Anaplasma pluts,Candidatus Midliclorna mitochondri and Babesia canis vogeli in ticks from Israel. Clin Microbiol Infect. 2011;17:459–63.

19. Han R, Yang J, Liu Z, Gao S, Ni Q, Hassan MA, et al. Characterization of Anaplasma ovis strains using the major surface protein 1a repeat sequences. Parasit Vectors. 2017;10:447.

20. Rennekke S, Abdo J, Sall DE, Karacou T, Biligh C, Torina A, et al. Can Anaplasma ovis in small ruminants be neglected any longer? Transbound Emerg Dis. 2013;60(Suppl 2):105–12.

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

22. Sun XF, Zhao L, Wen HJ, Luo LM, Yu XJ. Anaplasma species in China. Ticks Tick Borne Dis. 2015;15:1263–4.

23. Barlough JE, Madigan JE, Deroch E, Bigornia L. Nested polymerase chain reaction for detection of Ehrlichia equi genomic DNA in horses and ticks (Ixodes pacificus). Vet Parasitol. 1996;63:319–29.
38. Torina A, Agnone A, Blanda V, Alongi A, D’Agostino R, Caracappa S, et al. Development and validation of two PCR tests for the detection of and differentiation between Anaplasma ovis and Anaplasma marginale. Ticks Tick Borne Dis. 2012;3:283–7.
39. de la Fuente J, Atkinson MW, Naranjo V, Fernández de Mera IG, Mangold AJ, Keating KA, et al. Sequence analysis of the msp4 gene of Anaplasma ovis strains. Vet Microbiol. 2007;119:375–81.