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*Full Length Research Paper*

# A preliminary survey on the ticks carrying *Ehrlichia* and *Anaplasma* in the Southern marginal zone of Gurbantunggut Desert

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**To understand the status of ticks carrying *Ehrlichia* and *Anaplasma* in the southern marginal zone of Gurbantunggut Desert, we collected and classified ticks from livestock in 11 locations of three different habitats in the southern marginal zone of Gurbantunggut Desert. Ticks were screened by nested polymerase chain reaction (PCR) and their 5' hypervariable regions of 16S rRNA genes were amplified by semi-nested PCR. The PCR products were sequenced and compared to DNA sequences in GenBank to identify the Anaplasmataceae species carried by the ticks. The collected 708 ticks (classified 236 specimens) were identified as 8 species of 4 genera. Among these specimens, 25 (10.59%) were positive for *Ehrlichia* and *Anaplasma*. *Ehrlichia chaffeensis*, *Ehrlichia canis*, *Anaplasma marginale* and *Anaplasma ovis* were found in *Hyalomma asiaticum*, *Rhipi cephalus sanguineus*, *Hyalomma detritum* and *Haemaphysalis longicornis*, respectively by sequence comparison. This study confirmed at molecular level that *E. chaffeensis*, *A. marginale*, *A. ovis* and *E. canis* are present in ticks carrying *Ehrlichia* and *Anaplasma* in the southern marginal zone of Gurbantunggut Desert.**

**Key words:** *Ehrlichia*, *Anaplasma*, tick-borne, southern marginal zone of Gurbantunggut Desert.

## INTRODUCTION

New or recurrent tick-borne rickettsial diseases such as Anaplasmosis and Ehrlichiosis are threatening human health and the development of livestock. (Walker and Dumler, 1996; Coetzee et al., 2010; Oliveira et al., 2011; Silaghi et al., 2011). Misdiagnosis and missed diagnosis of tick-borne rickettsial diseases are very common in China's hospitals due to lack of typical clinical presentation and diagnostic methods. In the veterinary area, the diseases have not yet received enough attentions. In order to prevent human and animal from Anaplasmataceae infection, it is of great significance to investigate and identify Anaplasmataceae species carried by different types of ticks in different regions.

China has a variety of ticks in its vast territory and diverse environments. Up to now, the DNA of *Ehrlichia canis*, *Ehrlichia chaffeensis*, human granulocyte *Ehrlichia* and *Ehrlichia tibet* from *Rhipicephalus sanguineeus, Haemaphysalis yeni*, *Ixodes persulcatus*, and Tibet *Boophilus microplus* have been detected (Cao et al., 2000a, 2000b; Wen et al., 2003; Zhou et al., 2010). However, systematic etiological investigation on the emerging Anaplasmosis and Ehrlichiosis in Gurbantunggut Desert has not been carried out. Here, we

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carried out the etiological investigation of local ticks collected from the southern marginal zone of Gurbantunggut Desert, hoping to explore the significance of ticks for veterinary and public health in transmission process of *Ehrlichia* and *Anaplasma*.

## MATERIALS AND METHODS

### Natural profile of sampling sites

Gurbantunggut Desert located in the hinterland of Junggar Basin is the largest semi-fixed desert and the second largest desert in China. It has latitude of 44°15' to 46°50'N, longitude of 84°50' to 91°20', altitude of 300 to 600 m and area of 48,800 $km^2$. Its southern marginal zone is connected with alluvial and flood fan zones originated from Tianshan district and constitute a natural vertical baseband from the northern slope of Tianshan to Gurbantunggut Basin. The region is a small tree desert mainly composed of white *Haloxylon*, *Haloxylon* and other desert plants, as well as short-lived and like short-lived plants. Because of its diverse natural habitats, there live a wide variety of domestic and wild animals, thus making it a habitat and good breeding ground for ticks.

### Sample collection, classification and identification

Ticks were collected from cattle, sheep, horses and dogs during April, 2010 to June, 2011 in a total of 11 regions of three different habitats at the southern marginal zone of Gurbantunggut Desert, Xinjiang, China. The collected ticks were stored in a glass test tube equipped with a wet cotton ball and sent back to laboratory. These ticks were identified to species based on the morphological classification criteria, grouped based on their species in a 5 ml sterile tube with three ticks in each group and immersed in 75% alcohol for future use.

### DNA extraction from ticks

After repeatedly washed with sterile water, ticks were grounded using a tube-type grinder. The slurry was used to extract DNA using a DNA extraction kit (Takara, Dalian, China) based on the manufacturer's instruction.

### Polymerase chain reaction (PCR) amplification,cloning and sequencing

The *Anaplasma* 16S rRNA genes are highly conservative and have a common specific sequence. Thus, a pair of external primers Eh-out1 (TTGAGAGTTTGA TCCTGGCTCAGAACG) and Eh-out2 (CACCTCTACACTAGGAATTCCGCT ATC) and internal primers Eh-gs1 (GTAATACTGTATAATCCCTG) and Eh-gs1 (GTACCGTCATTATCTTCCCTA) were used to screen ticks carrying *Anaplasma*. To obtain the 5' hypervariable region of *Anaplasma* 16S rRNA genes, the nested PCR products obtained with the external primers were used as templates in the semi-nested PCR with the internal primers. The first round of PCR were performed in a 25 µl system containing 1 µl Eh-out1, 1 µl Eh-out2, 2.5 µl 10× buffer, 5 µl DNA, 2 µl deoxynucleotide triphosphates (dNTPs) and 0.3 µl Taq at condition of 94°C for 5 min followed by 40 cycles of 94□C for 45 s, 55□C for 50 s and 72°C for 1 min and final 72°C for 5 min. The second round of PCR was performed at the same condition except using 1 µl each of primers Eh-gs1 and Eh-gs2 and 2 µl of the first round PCR products as templates. The samples with positive result in nested PCR were used to amplify the 5' hypervariable fragments of 16S rDNA in semi-nested PCR using 1 µl each of primers Eh-out1 and Eh-gs2 and 2 µl of the first PCR products (using Eh-out1 and Eh-out2 as primers) as templates. The PCR product was recovered and cloned into pMD18-T vector (Takara, Dalian, China). Three positive clones from each sample were randomly selected and sequenced by Shanghai Biological Engineering Technology Co.

### Alignment and phylogenetic analysis of 16S rRNA DNA sequences

DNA sequences were aligned and analyzed with DNASTAR software (Madison, WI, USA) and compared with *Anaplasma* DNA database in GenBank. The *Anaplasma* phylogenetic tree was constructed using Mega 5.0 software (http://www.megasoftware.net).

## RESULTS

The collected 708 ticks were divided into 236 specimens (the same species of three ticks is a specimen), which belong to 8 species of 4 genera namely *Hyalomma asiaticum, Hyalomma detritum*, *Hyalomma scupense*, *Dermacentor niveus*, *Dermacentor nuttalli*, *Haemaphysalis qinghaiensis*, *Haemaphysalis longicornis*, and *Rhipicephalus sanguineeus*. Among the identified ticks, *H. asiaticum* and *H. detritum* are the dominant species in the desert area of the southern marginal zone of Gurbantunggut Desert, *H. detritum* and *D. niveus* are the dominant species in the reclamation area, and *D. nuttalli* is the dominant species at forest-steppe zone in the foothills of the northern slope of Tianshan Mountain. Among all the ticks, *H. asiaticum*, *H. detritum, D. niveusand, D. nuttalli*, *R. sanguineeus*, *H. scupense* and *H. qinghaiensis* accounted for 21.47, 19.49, 18.08, 10.73, 9.60, 9.60, 6.50 and 4.52%, respectively.

Among the 236 tick samples, 25 ticks showed a DNA fragment in nested PCR. The fragment is about 280 bp as expected. Semi-nested PCR amplification of the 25 positive samples in nested PCR resulted in an about 440 bp fragment. *E. chaffeensis*, *E. canis, A**naplasma** marginale and A**naplasma** ovis* were detected from *H. asiaticum, R. sanguineeus, H. detritum* and *H. longicornis*, respectively.

Compared with the corresponding gene fragments isolated from different Anaplasmataceae strains of different genera (Figure 1), the sequence of 9 fragments isolated form *H. asiaticum* is identical to that of *E. chaffeensis* Arkansas and was named *E. chaffeensis* XJ1 (GenBank accession number, JN187090); the sequence of 4 fragments from *R. sanguineeus* is identical to that of *E. canis* Oklahoma and named *E. canis* XJ2 (JN187091); the sequence of 7 fragments from *H. detritum* is identical to that of Veld strain of *A. marginale* and named *A. maginale* XJ3 (JN187092), the sequence of 5 fragments from *H. longicornis* is identical to that of *A. ovis* and

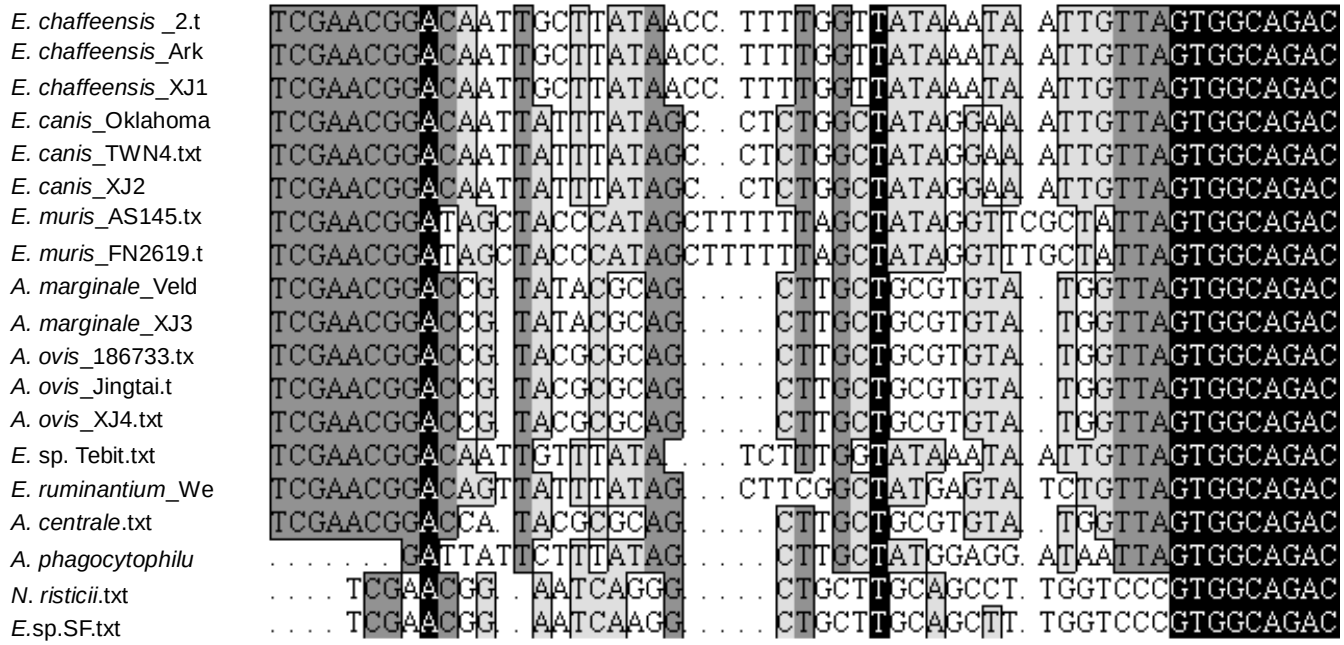


**Figure 1.** Sequence alignment and comparison of the 5' hypervariable region of 16S rRNA genes from different species of *Anaplasma*. The GenBank accession numbers of the aligned sequences are shown as N187090, JN187091, JN187092 and JN187093 for *E. chaffeensis* XJ1, *E. canis* XJ2, *A. maginale* and *A. ovis* XJ4, respectively.

named *A. ovis* XJ4 (JN187093) (Table 1).

Phylogenetic analysis based on the 5' hypervariable region of *Anaplasma* 16S rRNA gene showed that the 18 *Anaplasma* gene sequences can be divided into three major branches, namely *Ehrlichia*, *Anaplasma* and *Neorickettsia*. Among them, XJ1, XJ2, XJ3 and XJ4 are clustered with *E. chaffeensis*, *E. canis*, *A. marginale* and *A. ovis*, respectively (Figure 2).

## DISCUSSION

Gurbantunggut Desert is located in the hinterland of Junggar Basin, Xinjiang, China. Its southern marginal zone is mostly farming-pastoral area and has a wide range of domestic and wild animals, therefore ecologically suitable for tick survival. Currently, a total 42 tick species in 9 genera of 2 classes have been found in the region, accounting for 1/3 more ticks known in China. Etiological investigation found more than 10 tick-borne infectious diseases including Russian spring-summer encephalitis, tick-borne relapsing fever, Xingjiang hemorrhagie fever, Lymedisease and Theileria annulata, which have become a serious threat to the public health and livestock production.

Tick-borne rickettsia infection is tended to grow in the world in recent years, (Coetzee et al., 2010; Castellaw et al., 2011, Dergousoff and Chilton, 2011; Pfitzer et al., 2011; Silaghi et al., 2011; Simuunza et al., 2011) and becomes an increasingly serious threat to human beings and animals. Many studies showed that different ticks can carry different *Anaplasma* species in China (Cao et al., 2000a; Wen et al., 2003; Zhan et al., 2010; Jiang et al., 2011). In this study, 236 tick samples from 8 species of 4 genera were detected by nested PCR. The results showed that 25 samples (10.59%) were positive. Sequences analysis found that *H. asiaticum, R. sanguineeus, H. detritum,* and *H. longicornis* carry *E. chaffeensis*, *E. canis*, *A. marginale*, and *A. ovis*, respectively, suggesting that different ticks carry different *Anaplasma* species.

Sequences of 5' hypervariable region of 16S rDNA are characteristics of bacterial species, thus can be used to identify bacterial species (Wen et al., 1995; Wen et al., 1996; Wen et al., 2003; Zhou et al., 2010). In our study, the full-length sequence alignment of 16S rRNA gene (1430 to 1490 bp) from different *Anaplasma* species, found four genetically hypervariable regions, of which the largest variable region is in the first 60 to 110 bp, which varies greatly among genera, but more conservative among species. Therefore, this region was used to identify *Anaplasma* species..

In conclusion, we preliminarily investigated *Ehrlichia* and *Anaplasma* in livestock ticks in the southern marginal zone of Gurbantunggut Desert in this study. Among the 236 tick samples collected from cattle, sheep, horses and dogs, 8 species of 4 genera, *E. chaffeensis*, *E. canis*, *A. marginale* and *A. ovis* were detected in *H. asiaticum*, *R. sanguineeus*, *H. detritum* and *H. longicornis*, respectively, which confirmed at molecular level that ticks carry

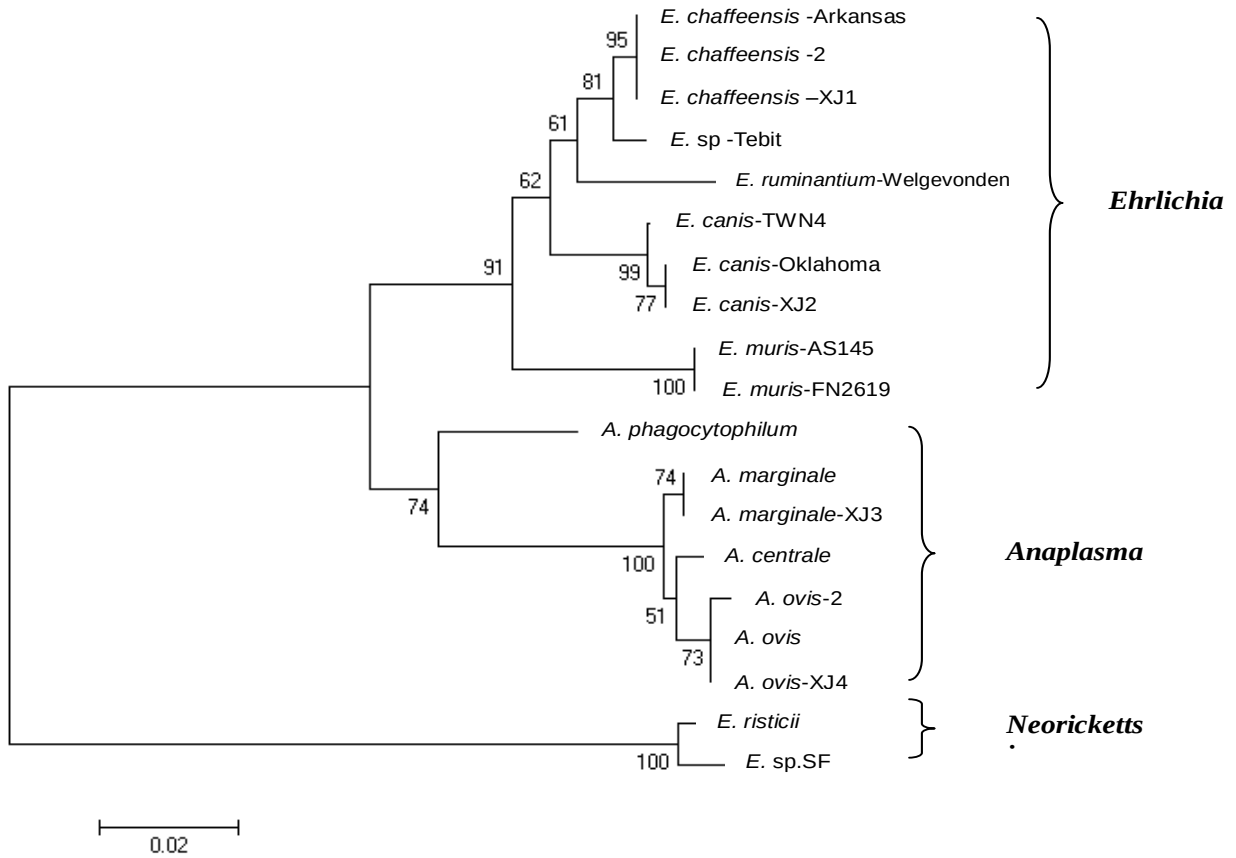


**Figure 2.** Phylogenetic analysis of 5' hypervariable region of 16S rRNA genes from different *Anaplasma* species. The GenBank accession numbers of the aligned sequences are as follows: *E. chaffeensis* (M73222), *E. canis* (M73221), *E. muris* (U15527), *E. ewingii* (U96436), *E. bovis* (U03775), *E. ruminantium* (NC_005295), *E. risticii* (M21290), *Ehrlichia sp.* SF (U34280), *Ehrlichia sp.* Tibet (AF414399), *A. phagocytophila* (M73220), *A. marginale* (AF414873), *A. bovis* (U03775 ), *A. centrale* (AF414869), *A. ovis* (AF441131) , *A. platys* (AF286699), *E. chaffeensis* XJ1( JN187090), *E. canis* XJ2 (JN187091), *A. maginale* XJ3 (JN187092) and *A. ovis* XJ4 (JN187093), respectively. Sequence alignment was conducted using Neighbor-Joining method. Bootstrap values were calculated after repeated 1000 times.

**Table 1.** Ticks carrying different *Ehrlichia* spp. or *Anaplasma* spp. in the southern marginal zone of Gurbantunggut Desert.

| Genera | Ticks species | ***Ehrlichia* spp. and *Anaplasma* spp.** |
|---|---|---|
| *Hyalomma* | *H. asiaticum* | *E. chaffeensis* |
| | *H. detritum* | *A. marginale* |
| | *H. scupense* | *E. canis* |
| *Dermacentor* | *D. nuttalli* | - |
| | *D. niveus* | |
| *Haemaphysalis* | *H. qinghaiensis* | - |
| | *H. longicornis* | *A. ovis* |
| *Rhipicephalus* | *R.sanguineeus* | - |
| Total | 8 | 4 |

"-", Negative.

*Ehrlichia* and *Anaplasma* in the southern marginal zone of Gurbantunggut Desert.

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