Short Communication

# Serological Evidence of Infection of *Anaplasma* and *Ehrlichia* in Domestic Animals in Xinjiang Uygur Autonomous Region Area, China.

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**Abstract**

Serological methods were utilized to detect *Anaplasma* and *Ehrlichia* infection in domestic animals in Xinjiang Uygur Autonomous Region, China. By using an indirect immunofluorescence assay, antibodies that reacted with *A. phagocytophilum* and *E. chaffeensis* were detected mainly in ruminants kept on pastureland in Altai, Ili and Kashgar area. Antibody titers up to 1:320 were recorded. These results indicate that ruminants kept in these areas may be infected with some species of *Anaplasma* and *Ehrlichia*.

Keywords. *Anaplasma*, *Ehrlichia*, domestic animals, seroepidemiology, Xinjiang Uygur Autonomous Region, China

## 1. **Introduction**

Ehrlichioses are important vector-borne diseases in both humans and animals. Both *Anaplasma* and *Ehrlichia* spp. are known to be transmitted by arthropod ticks and are distributed worldwide (Dumler et al., 1998). The genus *Anaplasma* includes *A. marginale, A. centrale, A. ovis, A. platys, A. phagocytophilum* and some unidentified species closely related to those pathogens. The genus *Ehrlichia* includes *E. canis, E. ewingii, E. chaffeensis, E. muris, E. ruminantium*, and some additional new *Ehrlichia* species. *A. phagocytophilum* and *E. chaffeensis* are two major zoonosis pathogens mainly reported in the United States and European countries (Foley et al., 2004; Parola, 2004). *A. phagocytophilum* can cause prevalent diseases in humans, ruminants and horses, and *E. chaffeensis* in both humans and dogs. Recently, both agents have also been reported in eastern Asia, including China and Korea (Cao et al., 2000; Cao et al., 2003; Kim et al., 2003). In China, DNA fragments of *A. phagocytophilum* have been detected in *Ixodes persulcatus* ticks in Heilongjiang Province in northeastern China (Cao et al., 2003). *E. chaffeensis* DNA were also detected by PCR from *Haemaphysalis yeni* and *Amblyomma testudinarium* in southern China (Cao et al., 2000). However, there has been little information available on ehrlichiosis in the western part of China. Xinjiang Uygur Autonomous Region is located in the western most area in China. The region has a cold and dry climate with high mountains and wide deserts. The animal grazing of ruminants on pastureland is one of the main industries of Xinjiang. Horses and donkeys are also important animals for use in transportation in this area. *Rickettsia sibilica* is the only known rickettsial pathogen that causes spotted fever in humans in Xinjiang (Ai et al., 1979; Fan et al., 1987), but it is not clear whether other tick-borne rickettsial diseases exist. The aim of this study was to determine whether pathogens of *Anaplasma* and *Ehrlichia* distribute in Xinjiang Uygur Autonomous Region. Thus the sero-prevalence of antibodies against *Anaplasma* and *Ehrlichia* in domestic animals, including cattle, sheep, goats, horses

and donkeys in this area were screened by using indirect immunofluorescence (IFA) test for *A. phagocytophilum* and *E. chaffeensis.*

## 2. Materials and Methods

### *2.1 Sera from animals*

Sera were collected from 146 cattle, 134 sheep, 133 goats, 85 horses and 100 donkeys in Xinjiang Uygur Autonomous Region from April to August in 2004. Histories and clinical symptoms of each animal were not recorded. Three areas, Altai, Ili and Kashgar, were selected for the survey (Fig.1). Altai is situated in the northern part of Xinjiang, and is bounded by Russia and the People's Republic of Mongolia. It is just south-west of the Altai Mountains. Ili is situated at the north-west border of Xinjiang, and is bounded by the Kazakhstan Republic, Russia. It is also north of the Tianshan Mountains. Kashgar is at the west end of Xinjiang, bordering the Taklamakan desert in the east and the Kunlun Range in the south. It is also the eastern neighbor of Kyrgys and Tajikistan. The numbers of sera from each area and type of animal are shown in Table 1. Samples were stored at –20°C until examined.

### *2.2. IFA test*

Antigens for IFA test were kindly given by Dr. P. Brouqui (Unité des Rickettsies, Université de la Méditerranée, Marseille, France). *A. phagocytophilum* (HGE agent Webster strain, originally supplied by Dr. J. S. Dumler, The Johns Hopkins University School of Medicine, Baltimore, MD, USA) and *E. chaffeensis* (Arkansas strain, originally supplied by Dr. J. E. Dawson, Center for Diseases Control and Prevention, Atlanta, GA, USA) were used as antigens in the IFA test as previously described (Brouqui et al., 1994). Sera from mice that were experimentally infected with *A. phagocytophilum* and *E. chaffeensis* were used as

positive controls. Sera from healthy animals kept in Japan were used as negative controls. Sera were screened at a 1:20 dilution in phosphate-buffered saline (pH 7.4), Tween 0.5% (PBST) and an optimized dilution (1:160 to 1:200) of fluorescein isothiocyanate-labelled anti-IgG conjugate (anti-cattle IgG; ICN Pharmaceuticals Inc., U.S.A., anti-sheep IgG; ICN Pharmaceuticals Inc., U.S.A., Capple, anti-goat IgG; ICN Pharmaceuticals Inc., U.S.A., anti-horse IgG, MP Biomedicals, Inc., U.S.A., or anti-donkey IgG; Santa Cruz Biotechnology, U.S.A.) in PBST was used as the second antibody. Reactive antibodies were then detected using a fluorescence light microscope. Antibody levels of test samples were determined by comparison with the appropriate positive and negative controls. Those samples that reacted with any of the antigens at the screening dilution were then titrated using serial twofold dilutions to determine end titers.

**3. Results and Discussion**

The results are summarized in Table 2. A total of 7 cattle serum samples among 47 (14.9 %) in Altai, 6 among 50 (12.0 %) in Ili, and 2 among 49 (4.1 %) in Kashgar reacted with at least one of the antigens at a dilution of 1:40 or more. Dual positivity was occasionally seen, but most samples reacted more strongly with one of the two antigens. In Altai, all of the 7 positive cattle reacted with *E. chaffeensis* with titers of 1:40 to 1:320, and showed weak reaction with *A. phagocytophilum* with titers of 1:20 or less. Five cattle serum samples in Ili showed higher titers against *E. chaffeensis* (1:40 to 1:160), while 1 showed a higher titer against *A. phagocytophilum* (1:80). In contrast, the 2 positive cattle in Kashgar showed higher titers against *A. phagocytophilum* (1:40 and 1:160) than those against *E. chaffeensis*. A total of 6 sheep serum samples among 37 (16.2 %) in Altai, 11 among 50 (22.0 %) in Ili and 8 among 47 (14.9 %) in Kashgar, reacted with *A. phagocytophilum* or *E. chaffeensis* at a dilution of 1:40 or more. In Altai, 3 of the 6 sheep sera showed higher titers

against *A. phagocytophilum* (with titers of 1:40 to 1:160) than those against *E. chaffeensis*, while the other 3 samples showed the same titers (of 1:40 or 1:80) against *A. phagocytophilum* and *E. chaffeensis*. In Ili, 4 sheep samples showed higher titers against *E. chaffeensis* (1:80 to 1:160), and 1 against *A. phagocytophilum* (1:40), while the other 6 showed the same titers against both antigens. In Kashgar, 3 positive sheep sera showed higher titers against *A. phagocytophilum* (1:40, 1:80 and 1:320), 3 showed higher titers against *E. chaffeensis*, and the other 2 showed equal titers (of 1:40 and 1:80) against both antigens. None of the goat sera in Altai showed any positive reaction, while a total of 3 goat serum samples among 50 (6.0 %) in Kashgar and 1 among 33 (3.0 %) in Ili reacted with *A. phagocytophilum* or *E. chaffeensis* at a dilution of 1:40 or more. In Ili, the only positive sample showed a higher titer against *E. chaffeensis*, with titer of 1:40. In Kashgar, 2 positive goat sera showed higher titers against *A. phagocytophilum* (1:40 and 1:80) and 1 showed a higher titer against *E. chaffeensis* (1:40).

In the present study, antibodies that reacted with *A. phagocytophilum* and *E. chaffeensis* were detected in ruminants in Xinjiang. However, the relationship between pathogenesis and antibodies against these agents was not analyzed, because the histories and clinical symptoms were not recorded in this study. In Altai, cattle showed higher titers against *A. phagocytophilum*, while sheep showed higher titers against *E. chaffeensis*. This may reflect the differences of location of where the examined animals were kept. It was impossible to examine the existence of *A. phagocytophilum* and *E. chaffeensis* in these areas, because cross reaction of antibodies is commonly seen for antigens among the same genus. The positive reaction might have resulted from infection of species closely related to *A. phagocytophilum* and *E. chaffeensis*. Higher titers may be associated with multiple exposure to individual animals or recent exposure, although some younger animals also showed higher titers. In Xinjiang, most ruminants are kept on pastureland, and are usually infested with

many ticks from spring to autumn. Ticks may transmit the ehrlichial pathogens to animals.

All the horse serum samples were obtained in Altai, and none of there sera reacted with any of the antigens. The only positive serum of donkey was obtained from an animal in Kashgar. The titer against *A. phagocytophilum* was 1:40. Most of the horses and donkeys examined in this study were not kept on pastureland, but lived near the farm houses and were used for transportation. Thus, tick infestation of horses and donkeys is less likely than that of ruminants.

Recently, several new ehrlichial species were detected by molecular methods or isolated around China. *Ehrlichia muris* and a new *Ehrlichia* species closely related to *E. chaffeensis* were isolated in Japan (Shibata et al., 2000; Wen et al., 1995). *E. muris* DNA has also been detected from ticks in central Russia near Xinjiang (Shpynov et al., 2004). Another novel *Ehrlichia* DNA closely related to *E. ewingii* was detected in Tibet, Myanmar and Japan (Inokuma et al., 2004; Parola et al., 2003; Wen et al., 2002). It is possible that domestic animals in Xinjiang have been infected with some new ehrlichial pathogens and showed positive antibodies against *A. phagocytophilum* and *E. chaffeensis.* Isolation and characterization of the pathogens will be required for the next step of this study.

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Figure Captions

Fig.1. A map of Xinjiang. The three study sites, Altai, Ili and Kashgar, are indicated in the figure. Urmuqui is the capital city of Xinjiang Uygur Autonomous Region.

Table 1. Information of sera examined from each area

| Area | Animals | Numbers of animals examined | Age (Range, years old) |
|---|---|---|---|
| Altai | Cattle | 47 | 5-9 |
| | Sheep | 37 | 3-5 |
| | Goat | 50 | 3-5 |
| | Horse | 85 | 2-20 |
| | Donkey | 50 | |
| Kashgar | Cattle | 49 | 0-4 |
| | Sheep | 47 | 0-6 |
| | Goat | 50 | 0-6 |
| | Donkey | 50 | |
| Ili | Cattle | 50 | 1-9 |
| | Sheep | 50 | 0-2 |
| | Goat | 33 | 0-4 |

Table 2. Antibody titers against *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* of domestic animals that showed titers of 1:40 or more against any of the agents

| Animals | Area | No. | Age (years old) | Titers | |
|---|---|---|---|---|---|
| | | | | *A. phagocytophilum* | *E. chaffeensis* |
| Cattle | Altai | 12 | 5 | 20 | 40 |
| | | 13 | 6 | <20 | 80 |
| | | 26 | 6 | <20 | 160 |
| | | 28 | 6 | <20 | 80 |
| | | 29 | 6 | <20 | 80 |
| | | 30 | 5 | <20 | 80 |
| | | 31 | 6 | <20 | 320 |
| | Ili | 4 | 5 | 20 | 40 |
| | | 6 | 3 | <20 | 80 |
| | | 11 | 2 | 80 | 160 |
| | | 22 | 4 | 20 | 80 |
| | | 24 | 2 | 40 | 80 |
| | | 36 | 2 | 80 | 40 |
| | Kashgar | 34 | 2 | 160 | <20 |
| | | 42 | 2 | 40 | <20 |
| Sheep | Altai | 10 | 3-5* | 80 | 80 |
| | | 11 | 3-5* | 80 | 80 |
| | | 12 | 3-5* | 160 | 80 |
| | | 23 | 3-5* | 40 | <20 |
| | | 44 | 3-5* | 40 | 40 |
| | | 49 | 3-5* | 40 | 20 |
| | Ili | 6 | 0 | 40 | 40 |
| | | 13 | 2 | 40 | 160 |
| | | 14 | 2 | 40 | 20 |
| | | 19 | 0 | 40 | 80 |
| | | 26 | 0 | 40 | 40 |
| | | 28 | 0 | 160 | 160 |
| | | 32 | 2 | 40 | 80 |
| | | 33 | 2 | <20 | 80 |
| | | 38 | 1 | 160 | 160 |
| | | 42 | 2 | 80 | 80 |
| | | 45 | 0 | 80 | 80 |
| | Kashgar | 8 | 0 | 320 | 80 |
| | | 12 | 2 | 40 | 80 |
| | | 13 | 2 | 20 | 40 |
| | | 17 | 2 | 80 | 40 |
| | | 18 | 2 | 80 | 80 |
| | | 24 | 0 | 40 | 80 |
| | | 33 | 2 | 40 | 40 |
| | | 44 | 4 | 40 | 20 |
| Goats | Ili | 27 | 3 | 20 | 40 |
| | Kashgar | 8 | 2 | 80 | 20 |
| | | 22 | 0 | 40 | 20 |
| | | 39 | 2 | 20 | 40 |
| Donkeys | Kashgar | 50 | 8 | 40 | 20 |

*: Age of individual sheep in Altai was not recorded.


Russia
Kazakhstan
Altai
Mongolia
Kyrgys
Ili
Urmuqui
Kashgar
Tajikistan
Xinjiang
Pakistan
Tibet highland
India
Map of China

Fig.1