Anaplasma ovis and Anaplasma phagocytophilum Infection in Sheep and Wild Rodents from Northern Xinjiang, Northwest China

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INTRODUCTION

Xinjiang Uygur Autonomous Region (XUAR) covers 1.66 million square kilometers, which is located in the hinterland of the Eurasian continent is geographically divided into two parts by Tianshan Mountain, namely northern XUAR and southern XUAR [1]. In northern XUAR, which is composed of the desert, Gobi desert, saline beach and...
patched oases. There is a great diversity of tick species in XUAR, owing to the variability of geographical landscape and the availability of multiple vertebrate host species for these parasites. Ticks were reported to transmit bacterial, viral and parasitic diseases to animals and humans [2]. Anaplasmosis is a tick-borne disease and considered emerging or reemerging pathogens with serious public health implications, which are obligate intracellular pathogens that infect humans and animals [3]. Although these agents could infect humans through various routes, animal hosts play an important role in transmission.

Since the first case was reported in the USA in 1990, Anaplasma species have been described in both Europe and Asia [4-7]. The major Anaplasma species that impact animal and human health include Anaplasma marginale, Anaplasma ovis, Anaplasma centrale, Anaplasma bovis, Anaplasma phagocytophilum, and Anaplasma platys [9]. Among them, A. phagocytophilum is distributed worldwide and infects a variety of hosts [9,10]. A. ovis is the main erythrocytic pathogens of ovine, which are responsible for ovine anaplasmosis in tropical and subtropical areas [9]. Although studies on Anaplasma species have been carried out in part of China [9,12] information is scarce on the animal reservoirs of Anaplasma spp. in northern XUAR. Therefore, the aim of this study was to identify A. ovis and A. phagocytophilum infection in wild and domestic hosts in different districts of northern XUAR, Northwest China.

MATERIAL and METHODS

During 2015-2016, blood samples were collected from 137 sheep in Ili Kazakh Autonomous Prefecture (Ili), 79 sheep in Bole Mongol Autonomous Prefecture (Bole) and 71 sheep in Shihezi City. Spleen samples were collected from 356 great gerbils (Rhombomys opimus) in Urumqi City and 134 R. opimus in Bole in northern XUAR, Northwest China. The study area ranged between latitude 43°39'31.42''N - 44°54'18.04''N and longitude 81°31'27.78'' - 87°36'50.35''E. In accordance with the different types of landscapes, 1-4 sampling sites were selected in each county or city. The sheep bloods were sampled at different intervals under the owner agreements. As to wild rodents (i.e. the great gerbils (R. opimus)), their carcasses were submitted for postmortem examination to the Xinjiang Uygur Autonomous Region Wildlife Management Office, and then sent to our laboratory for scientific research. This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2015-01).

Genomic DNA was extracted from blood or dissected tissues/organs of the animals (including sheep and the great gerbil) (i.e. spleen in this study) by using the 96 flux automatic nucleic acid extraction instrument with a matching commercial kit (Cell & Tissue Kit, Biotek, Beijing, China) according to the manufacturer’s instructions, cloned into the pBS-T vector, and used for the transformation of One-shot® Top10 Escherichia coli cells. To prevent contamination problems, as negative control, used purified sterile water, were tested after every sample in our PCR.

To determine genetic variability and regional differences of A. phagocytophilum and A. ovis, all samples were examined targeting 16s rRNA genes by polymerase chain reaction (PCR) according to previous descriptions [13,14]. An approximately 400bp fragment was amplified using primers 16s rRNA-Outer for the first round, then 16s rRNA-Inner for the second round PCR. Each amplified product was repeatedly sequenced three times. Sequences were compared with GenBank data using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [15]. Evolutionary analyses were conducted in MEGA7 [16].

RESULTS

In this study, A. ovis was found in the blood samples from sheep in Ili (21/137, 15.3%), Bole (18/79, 22.8%) and Shihezi City (13/71, 18.3%) in northern XUAR, respectively. In addition to the above results, DNA from A. phagocytophilum was detected in spleen samples from great gerbils (R. opimus) in Urumqi City (37/356, 10.4%) and Bole (21/134, 15.7%), northern XUAR. Interestingly, co-infection A. ovis and A. phagocytophilum in blood of 9/137 sheep (6.7%) from Ili, northwest China (Table 1) was detected.

Based on the analysis of BLAST and phylogenetic tree, six Anaplasma genotypes (A. ovis and A. phagocytophilum) were detected (Fig. 1). The sequences of 16S rRNA fragments amplified from the three A. ovis isolates were 100% identical in our study (GenBank No. MK260043, MK260044

<table>
<thead>
<tr>
<th>District</th>
<th>Host</th>
<th>Anaplasma ovis (%)</th>
<th>Anaplasma phagocytophilum (%)</th>
<th>Coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ili</td>
<td>sheep</td>
<td>21/137 (15.3%)</td>
<td>9/137 (6.6%)</td>
<td>43°58'33.93''N 81°31'27.78''E</td>
</tr>
<tr>
<td>Bole</td>
<td>sheep</td>
<td>18/79 (22.8%)</td>
<td>-</td>
<td>44°54'18.04''N 82°03'48.45''E</td>
</tr>
<tr>
<td></td>
<td>the great gerbil (Rhombomys opimus)</td>
<td>-</td>
<td>21/134 (15.7%)</td>
<td></td>
</tr>
<tr>
<td>Urumqi</td>
<td>the great gerbil (Rhombomys opimus)</td>
<td>-</td>
<td>37/356 (10.4%)</td>
<td>43°49'31.42''N 87°36'50.35''E</td>
</tr>
<tr>
<td>Shihezi</td>
<td>sheep</td>
<td>13/71 (18.3%)</td>
<td>-</td>
<td>44°18'19.04''N 85°18'19.04''E</td>
</tr>
</tbody>
</table>
and MK260045], and varied from all known A. ovis sequences in GenBank, with 99.2%-99.7% nucleotide identity from sheep, yaks and ticks in other countries [GenBank No. KF293716 (Italy), LC194134 (Mongolia), KU569702 (Kenya) and KC484562 (Russia), respectively], and separated into different clusters in the phylogenetic tree. A. phagocytophilum from sheep and great gerbils (R. opimus) in this work, which had 97.5%-98.8% similarity with the corresponding sequences of A. phagocytophilum originated from sheep, muntjac, tick and malebank voles (Clethrionomys glareolus) derived from different areas [GenBank No. KX279357 (China), KX197408 (China), KF293673 (Italy), KC916734 (China), AY082656 (United Kingdom), AF470699 and KF458570 (South Korea), respectively]. Interestingly, the sequence divergence between A. phagocytophilum from sheep and great gerbils (R. opimus) was 2.53% from different regions in our study. The phylogenetic analysis confirmed these results: the separation of genus Anaplasma genotypes in the study area were distinct from previously reported in other continents, geographic and host-associated cluster was strongly supported (Fig. 1). All obtained sequences were deposited in GenBank (A. ovis: MK260043-MK260045; A. phagocytophilum: MK260046-MK260047), but the number has not been released.

**DISCUSSION**

Since it was first recognised, anaplasmosis caused by A. ovis and A. phagocytophilum is considered to have a worldwide distribution [4-7]. Studies about the extent of its occurrence in animals and humans have been detected mostly in Europe, the USA and part of Asia [4,6,7]. Here, we isolated four strains of A. ovis and A. phagocytophilum from sheep, and two strains of A. phagocytophilum originated from rodents and used molecular methods to investigate the occurrence of A. ovis and A. phagocytophilum in northern XUAR, Northwest China.

Ovine anaplasmosis is caused by A. ovis in sheep, goats and ticks, which is widely distributed in different regions of the world [1]. The sequences, named as SHZ, YL and BL in XUAR were clustered together, and close to A. ovis genotypes from sheep in Kenya and Italy, sheep and yaks in Mongolia and ticks in Russia (Fig. 1). The phylogenetic analysis indicated that A. ovis genotype exist for sheep in Italy, Kenya and Mongolia, for yaks in Mongolia and for ticks in Russia, but the geographical differences for each species was not distinguished yet. Although these results add new information on the reservoirs of this disease agent we still need to have more sequence information to identify the differences of every A. ovis species.

Anaplasma phagocytophilum is reportedly maintained in various animal reservoirs, such as white-footed mice, goats, sheep, ticks and horses [4,17,18]. The sequence variation in the 16S rRNA gene among different A. phagocytophilum strains confirmed that three genotypes of A. phagocytophilum were detected in this study (Fig. 1). A. phagocytophilum infection in sheep from Ili compared with another two
A. phagocytophilum genotypes derived from different geographical regions in XUAR showed high diversity to A. phagocytophilum of the sheep, raccoon dogs, ticks, Clethrionomys glareolus and muntjacs from Italy, South Korea, United Kingdom and other China areas, respectively. These results showed A. phagocytophilum genotypes and displayed a high degree of genetic diversity, geographical and host tropisms, and the results coincides with Barakova et al.[19] report. To determine the level of infectivity in rodents as well as domestic animals, further studies are needed.

In northern XUAR, tick species distributed widespread, it is important to mention that animals located in this area suffer from heavy infestations by ticks. Here, we concluded that the emerging tick-borne A. ovis and A. phagocytophilum infection is already prevalent in different areas of ChinaThese results thus add new information on the reservoirs of those disease agents. Despite the existence of the biggest livestock industry in northwest China, there is still a considerable gap in our knowledge regarding the distribution of these pathogens and its economical relevance. In the future, it is important to mention that animals located in this geographical regions in XUAR showed high diversity to A. phagocytophilum of the sheep, raccoon dogs, ticks, Clethrionomys glareolus and muntjacs from Italy, South Korea, United Kingdom and other China areas, respectively. These results showed A. phagocytophilum genotypes and displayed a high degree of genetic diversity, geographical and host tropisms, and the results coincides with Barakova et al.[19] report. To determine the level of infectivity in rodents as well as domestic animals, further studies are needed.

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CONFLICT OF INTEREST

These authors have no conflict of interest related with this study.

REFERENCES
