Identification of *Anaplasma* spp. in Tian Shan wapiti deer (*Cervus elaphus songaricus*) in Xinjiang, China

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**A R T I C L E   I N F O**

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- Phylogenetic analysis
- Tian Shan wapiti
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**A B S T R A C T**

*Anaplasma* spp. are important zoonotic tick-borne pathogens that impact on human health. There are few reports on the prevalence and molecular genetic characteristics of *Cervidae* species in China. The purpose of this study, therefore, was to investigate the presence of *Anaplasma* spp. in blood samples of Tian Shan wapiti (*Cervus elaphus songaricus*) in the Xinjiang Uyghur Autonomous Region of China, and conduct phylogenetic analyses. A total of 50 blood samples (wild deer n = 26, and captive deer n = 24) were collected from the deer. PCR was used to detect *Anaplasma* spp. in the blood samples. Forty percent (20) of the samples were found to contain *Anaplasma* spp. Three *Anaplasma* species DNA were detected in blood deer samples: *A. bovis* (n = 13), *A. ovis* (n = 18), and *A. phagocytophilum* (n = 11). Among the 20 *Anaplasma* spp. positive samples, 14 were mixed infection of two or three pathogens. The prevalence of *Anaplasma* species in wild deer was significantly higher than that of captive deer, 73.1% (19) vs 4.2% (1) respectively, (p < 0.01). Two *A. ovis* sequence types (AB1, and AB2), three *A. ovis* sequence types (AO1-AO3), and one *A. phagocytophilum* sequence type (AP1) were obtained in this study. The sequences of AO1 shared 100% identity with a human isolate from Cyprus. Our results suggest that wild deer are more likely to become infected with *Anaplasma* spp. than captive individuals, and thus, could potentially transmit pathogens to humans.

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1. **Introduction**

*Anaplasma* spp. are transmitted by ticks and are obligate intracellular bacteria that infect a variety of cell types. The genus *Anaplasma* has attracted much attention because of its pathogenicity in animals, and its ability to infect humans (Yang et al., 2017a; Li et al., 2016; Park et al., 2020). Infection of stock animals with *Anaplasma* spp. can cause considerable economic losses within the farming industry, as well as raising serious public health concerns (Cui et al., 2018; Schotthoefer et al., 2018).

Over the last few decades, members of the genus *Anaplasma* have been found in unexpected hosts, for example, in several domestic livestock species (sheep, cattle, and goats), and wild ruminants (Snorre et al., 2013). Some of have also been found to occur in humans, such as *Anaplasma phagocytophilum* (Dumler et al., 2005), *A. ovis* (Chochlakis et al., 2010), and *A. capra* (Hao et al., 2015) have been reported in roe deer (*Capreolus pygargus*), red deer (*Cervus elaphus*) and sika deer in China (Li et al., 2016; Wang et al., 2019). *A. bovis* was found as the causative agent for anaplasmosis, detected in red deer and roe deer in southern Norway (Razanske et al., 2019). *A. platys* was predominantly detected in dogs, but has a single case of detection in deer in China (Li et al., 2016).

More recently, a large number of global surveys on *Anaplasma* spp. infection in deer shows a high prevalence. In France, *A. phagocytophilum* was identified in 14 of 59 red deer (23.7%) (Jouglin et al., 2019). For water deer in South Korea, the overall infection rate of *A. capra* was 17.8% (35 of 198) (Amer et al., 2019). However, few studies have investigated *Anaplasma* spp. infections in deer in China (Wang et al., 2019; Yang et al., 2017b).

The Tian Shan wapiti is a national second-class protected species,
with less than 10,000 remaining in the wild, and only about 10,000 in captivity. The deer are found predominantly in the Tian Shan Mountains in the Xinjiang Uygur Autonomous Region (hereafter referred to as Xinjiang) of China. The purpose was to identify the prevalence and genetic characteristics of *Anaplasma* spp. in Tian Shan wapiti in Xinjiang.

### 2. Material and method

#### 2.1. Ethics statement

Permission was obtained from the Tian Shan wapiti Breeder Association for the collection of captive deer blood samples. The blood sampling protocol was reviewed and approved by the Ethics Committee of Tarim University, Xinjiang, China.

#### 2.2. Blood sampling and DNA extraction

Fifty blood samples were collected from individual Tian Shan wapiti from June to September 2019. All samples were collected from adult males and females Tian Shan wapiti, some of which were held in captivity (n = 24) in Changji city, while others were wild (n = 26) in Tacheng county. Genomic DNA was extracted from 200 μL of each of the blood samples, using a Blood DNA Extraction Kit (Lifefeng, Shanghai, China) following the manufacturer’s instructions. The extracted DNA was re-suspended in TE buffer and stored at –20 °C.

#### 2.3. PCR amplification

Nested PCR (nPCR) was used to identify the presence of *A. phagocytophilum*, *A. bovis*, *A. ovis*, *A. platys*, and *A. capra* in the DNA samples (Table 1) (Barlough et al., 1996; Kawahara et al., 2006; de la Fuente et al., 2007; Silva et al., 2016; Guiqiang, 2007; Yang et al., 2016; Almazán et al., 2016). To avoid false-positive results, nPCR was conducted at least twice with a negative control (ddH2O). DNA amplification was evaluated using electrophoresis in 1.5% agarose gel.

#### 2.4. Sequencing and phylogenetic analysis

PCR amplicons of the correct size were DNA sequenced by GENEWIZ (Suzhou, China). Nucleotide sequences were confirmed by bidirectional sequencing, which was then compared with reference sequences downloaded from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/), using MEGA 5.1 software (http://www.megasoftware.net/) to identify new variant strains of *Anaplasma* spp.

Phylogenetic trees were constructed using the Neighbor-Joining (NJ) algorithm, based on a matrix of evolutionary distances, calculated using the Kimura 2-parameter model in MEGA 6.0 (http://www.megasoftware.net/) (Cui et al., 2017). Confidence in the NJ tree was estimated using a bootstrap analysis with 1000 replicates.

The nucleotide sequences reported in this paper have been submitted to the GenBank database at the NCBI under the following accession numbers: MW008784, MW008785, and MW008788-MW008791.

#### 2.5. Statistical analysis

Infection rates were compared using the chi-square test and differences considered statistically significant when P-values were <0.05. QuickCals software (GraphPad Software Inc., La Jolla, CA) was used for analysis.

### Table 1

| Site      | No. positive/No. examined (%) | One pathogen | Two pathogen | Three pathogen |
|-----------|------------------------------|--------------|--------------|----------------|
|           |                              | A. bovis<sup>a</sup> | A. ovis<sup>b</sup> | A. bovis + A. ovis | A. phagocytophilum<sup>c</sup> | A. bovis + A. ovis + A. phagocytophilum<sup>d</sup> |
| Tacheng   | 19/26 (73.1)                 | 2 (7.7)      | 3 (11.5)     | 3 (11.5)       | 3 (11.5)       | 8 (30.8)     |
| Changji   | 1/24 (4.2)                   | 1 (4.2)      | 3 (12.5)     | 1 (4.2)        | 3 (11.5)       | 8 (30.8)     |
| Total     | 20/50 (40.0)                 | 2 (4.0)      | 4 (8.0)      | 3 (6.0)        | 3 (6.0)        | 8 (16.0)     |

<sup>a</sup> A. bovis,
<sup>b</sup> A. ovis,
<sup>c</sup> A. phagocytophilum,
<sup>d</sup> A. bovis + A. ovis + A. phagocytophilum.

### Table 2

The prevalence of Anaplasm spp. under different farming mode.

| Sampling Site | Farming mode | No. positive/No. examined (%) | One pathogen | Two pathogen | Three pathogen |
|---------------|--------------|------------------------------|--------------|--------------|----------------|
|               |              |                              | A. bovis<sup>a</sup> | A. ovis<sup>b</sup> | A. bovis + A. ovis | A. phagocytophilum<sup>c</sup> | A. bovis + A. ovis + A. phagocytophilum<sup>d</sup> |
| Tacheng       | Wild         | 19/26 (73.1)                 | 2 (7.7)      | 3 (11.5)     | 3 (11.5)       | 3 (11.5)       | 8 (30.8)     |
| Changji       | Captive      | 1/24 (4.2)                   | 1 (4.2)      | 3 (12.5)     | 1 (4.2)        | 3 (11.5)       | 8 (30.8)     |
| Total         |              | 20/50 (40.0)                 | 2 (4.0)      | 4 (8.0)      | 3 (6.0)        | 3 (6.0)        | 8 (16.0)     |

<sup>a</sup> A. bovis,
<sup>b</sup> A. ovis,
<sup>c</sup> A. phagocytophilum,
<sup>d</sup> A. bovis + A. ovis + A. phagocytophilum.
3. Results

3.1. PCR positive rates of Anaplasma spp

From the 50 Tian Shan wapiti blood samples, 19 (38.0%) wild deers and 1 (2%) captivity deer were positive for Anaplasma spp., respectively. Three Anaplasma species were detected: including A. bovis (n = 13), A. ovis (n = 18), and A. phagocytophilum (n = 11) (Table 2). A. platys and A. capra were not found in any of the samples. Of the 50 samples, two (4.0%) were found to have a single infection of A. bovis, and four (8.0%) were found to have a single infection of A. ovis. A. ovis and A. bovis DNA were simultaneously detected in 6% (3) of the samples, while A. ovis and A. phagocytophilum co-infections were also detected in 6% (3) of the samples. Three pathogens (A. phagocytophilum, A. ovis, and A. bovis) were detected in eight wild Tian Shan wapiti samples.

The prevalence of Anaplasm a species in wild deer was 73.1% (19/26), which was significantly higher than in captive deer (4.2%, 1/24), \( (\chi^2 = 24.69, p < 0.01) \). The prevalence of A. ovis was 65.4% (17/26), which was higher than that of A. bovis (50.0%, 13/26), and A. phagocytophilum (42.3%, 11/26) among wild deer. Only one captive deer was positive for A. ovis.

3.2. Phylogenetic analysis

The two A. bovis sequence types (named AB1 and AB2) differed from each other at two nucleotide positions, and differed by three bases from reference sequences obtained from a red deer (KJ639885) in China. Among the three A. ovis sequence types (AO1-AO3), AO1 was from the captive individual, while AO2 and AO3 were from wild deers, with a homology ranging from 99.7% to 99.8%. Phylogenetic analysis of the AO2 sequence type showed 100.0% identity with a human strain (FJ460443) from the Cyprus States and fell into the same phylogenetic group (Fig. 1).

The A. phagocytophilum sequence type (AP1) showed 98.6% identity with a human strain (NR044762) from the United States, and a close relationship with the sequences from Chinese Eurasian collared dove, rat, goat, horse, tick, and Japanese Sika Deer (Fig. 2).

4. Discussion

Previous studies found that wild animal hosts were significantly more positive for tick-borne pathogens than captive animals (Giangaspero et al., 2015; Swai et al., 2005). The wild grazing of animals, away from direct observation, can delay detection of infection and treatment. In this study, the prevalence of Anaplasma spp. in wild deer was 73.1% (19/26), which was much higher than in captive deer (4.2%, 1/24). The overall prevalence of Anaplasma spp. in wild deer in this study was much higher in wild deer (26%) in Japan (Kawahara et al., 2006), while it was much lower than that in red deer (88.1%) from Norway (Razanske et al., 2019) and in cervids (96.1%) from Slovakia (Horwik et al., 2018). Ticks can carry and transmit a variety of pathogens, including Anaplasma spp (Hai-Yan and Jin-Lin, 2005). Xinjiang, as the largest province in China, contains the majority of China’s arid areas, which includes an abundance of tick species (Sheng et al., 2019). Studies have shown that more than 1/3 (42 species) of China’s tick species occur in Xinjiang, and the range of China’s tick species in Xinjiang can include Anaplasma species (Hai-Yan and Jin-Lin, 2005). Wild Tian Shan wapiti are therefore more exposed to ticks and tick bites than captive Tian Shan wapiti, due to the far less intensive stock management practices.

Some Anaplasma species have been identified as having zoonotic pathogens that can impact human health. For example, A. phagocytophilum has been identified in people in the USA as the agent for human granulocytic anaplasmosis (HGA), which is now being increasingly detected worldwide (Battilani et al., 2017). Additionally,
A. ovis was detected in blood from a 27-year-old woman in Cyprus (Chochlakis et al., 2010). Both A. phagocytophilum and A. ovis were detected in Tian Shan wapiti in this study. Furthermore, the AO1 sequence showed 100% sequence identity with a human strain from the Cyprus States and fell into the same phylogenetic group. Although A. phagocytophilum and A. ovis are known zoonotic pathogens, their pathogenicity in cervidae animals was previously unknown. However, there being no direct evidence deer-tick-human transmission of pathogens *Anaplasma* spp. of potential risk as humans encroach on deer habitat.

5. Conclusion

To our knowledge, this is the first report of *Anaplasma* spp. in Tian Shan wapiti in Xinjiang, China. Wild Tian Shan wapiti are more likely to become infected with these pathogens than captive deer. Our findings are important for Tian Shan wapiti herders for controlling tick-borne diseases in wild-farmed stock in Xinjiang, China, to ensure the healthy and sustainable development of the Tian Shan wapiti farming industry, and to minimize risks to human health.

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Declaration of competing interest

The authors declare there are no conflict of interest.

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