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

Tao Li [a], Yanyan Cui [b], Jinxiu Xiao [a], Yuxi Jiang [a], Changshen Ning [a,c], Meng Qi [a,*], Dayong Tao [a,**]

[a] College of Animal Science, Tarim University, Alar, Xinjiang, 843300, PR China
[b] School of Biotechnology and Food, Shangqiu Normal University, Shangqiu, 476000, PR China
[c] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan, 450002, PR China

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ABSTRACT

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 Uygur 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 deer blood 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.

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,

* Corresponding author. College of Animal Science, Tarim University, Tarim Road 1487, Alar, Xinjiang, 843300, China.
** Corresponding author. College of Animal Science, Tarim University, Tarim Road 1487, Alar, Xinjiang, 843300, China.
E-mail addresses: 33304095@qq.com (T. Li), 914170313@qq.com (Y. Cui), 455768554@qq.com (J. Xiao), 1069338742@qq.com (Y. Jiang), nnl1986@163.com (C. Ning), qimengdz@163.com (M. Qi), tdydky@126.com (D. Tao).

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Table 1
Primers and PCR conditions used in this study.

Pathogen	Target gene	Primer name	Primer sequence (5′–3′)	Amplicon size (bp)	References
A.phagocytophilum[a]	16S rRNA	EE1	CCTGGCTCAGAACGAACGCTGGCGGC	1430	(Barlough et al., 1996)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG		
		SSAP2f	GCT GAATGTGGGGATAATTTAT	641	(Kawahara et al., 2006)
		SSAP2r	ATGGCTGCTTCCTTTCGGTTA		
A. bovis[b]	16S rRNA	EE1	CCTGGCTCAGAACGAACGCTGGCGGC	1430	(Barlough et al., 1996)
		EE2	AGTCACTGACCCAACCTTAAATGGCTG		
		AB1f	CTCGTAGCTTGCTATGAGAAC	551	(Kawahara et al., 2006)
		AB1r	TCTCCCGGACTCCAGTCTG		
A. ovis[c]	msp4	AMOf	GCTCCCTACTTGTTAGTGG	794	(Guiqiang, 2007)
		AMOr	TTAGCTGAACAGGAATCTTG		
		MSP4f	CAAGCAGAGAGACCTCGTAT	584	(Guiqiang, 2007)
		MSP4r	GGCTTTTGCTTCTCCGGG		
A. platy[d]		8F	AGTTTGATCATGGCTCAG	1440	(Almazán et al., 2016)
		1448R	CCATGGCGTGACGGGCAGTGTG		
		PLATYS	GATTTTTGTCGTAGCTTGCTATG	678	(Silva et al., 2016)
		EHR16SR	TAGCACTCATCGTTTACAGC		
A. capra[e]	gltA	acgltA1	GCGATTTTAGAGTGYGGAGATTG	1031	(Yang et al., 2016)
		acgltA2	TACAATACCGGAGTAAAAGTCAA		
		acgltA3	TCATCTCCTGTTGCACGGTGCCC	594	(Yang et al., 2016)
		acgltA4	CTCTGAATGAACATGCCCACCCT		

a, b, c, d, e: *A naplasma phagocytophilum, Anaplasma bovis, Anaplasma ovis, Anaplasma platy, Anaplasma capra.*

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 (ddH_2O). 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* app.

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. QuickCalcs software (GraphPad Software Inc., La Jolla, CA) was used for analysis.

Table 2
The prevelence of *Anaplasm* spp. under different farming mode.

Sampling Site	farming mode	No. positive/No. examined (%)	No. infected/(%)				
			One pathogen		Two pathogen		Three pathogen
			A. bovis[a]	A. ovis[b]	A. bovis + A. ovis	*A. ovis* + A. phagocytophilum[c]	A. bovis + A. ovis+A. phagocytophilum
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)			
Total		20/50 (40.0)	2 (4.0)	4 (8.0)	3 (6.0)	3 (6.0)	8 (16.0)

a b and c: Anaplasma bovis, Anaplasma ovis and A naplasma phagocytophilum.

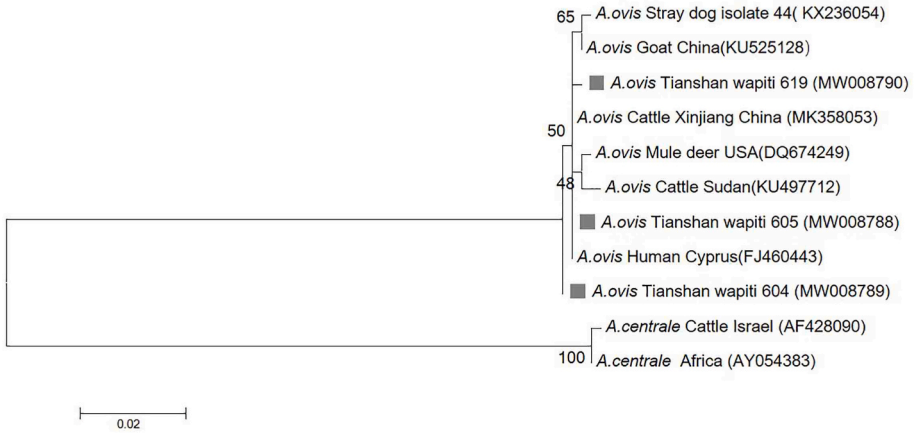

Fig. 1. Phylogenetic tree of *A. ovis* based on the msp4 partial gene sequences. A neighbor-joining tree was constructed using the Kimura two-parameter model in the Mega 5.1 software. An alignment of 584 bp partial msp4 gene sequences was used to construct this tree. Numbers on the branches indicate the percent of replicates that reproduced the topology for each clade. Gray square indicates sequences obtained from the study.

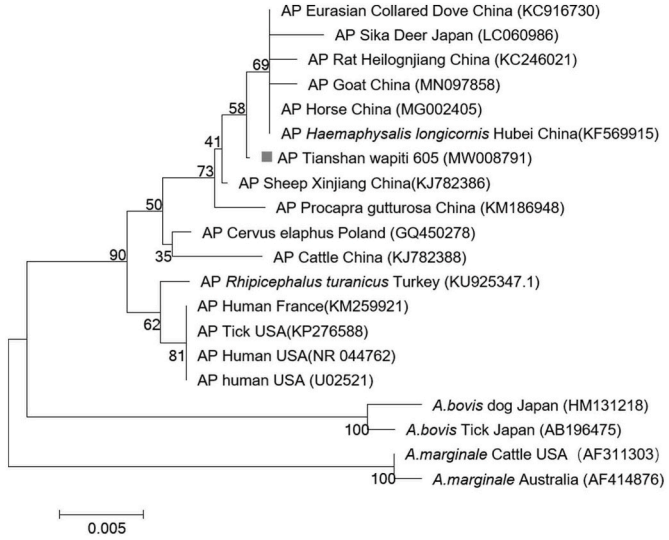

Fig. 2. Phylogenetic tree of *A. phagocytophilum* based on the 16S rRNA partial gene sequences. A neighbor-joining tree was constructed using the Kimura two-parameter model in the Mega 5.1 software. An alignment of 641 bp partial 16S rRNA sequence was used to construct this tree. Numbers on the branches indicate the percent of replicates that reproduced the topology for each clade. Gray square indicates sequences obtained from the study.

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 *Anaplasma* 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 (Hornok 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 (Chen et al., 2010). 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 Xinxiang, 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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