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ORIGINAL PAPER

Molecular detection and characterization of *Theileria* infection in cattle and yaks from Tibet Plateau Region, China

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Abstract Theileriosis continues to threaten the livestock industry worldwide, but comprehensive epidemiological surveys for this disease have not been conducted in the Tibet Plateau Region, China. In this study, we screened 154 cattle blood samples from the Tibet Plateau Region (Lhasa, Lhoka, and Tianzhu), China, for detection of *Theileria* pathogens by polymerase chain reaction (PCR) with species-specific primers. The results revealed that the prevalence was 6.9 % (2/29) for *Theileria orientalis* and 27.6 % (8/29) for *Theileria sinensis* in Lhasa, 0 % (0/30) for *T. orientalis* and 26.7 % (8/30) for *T. sinensis* in Lhoka, and 0 % (0/95) for *T. orientalis* and 30.5 % (29/95) for *T. sinensis* in Tianzhu. Interestingly, *Theileria luwenshuni*, which was a previously reported pathogenic *Theileria* sp. in sheep and goats, was detected in blood samples from cattle and yaks for the first time, with a prevalence of 10 % (3/30) in Lhoka and 1.1 % (1/95) in Tianzhu. No other *Theileria* sp. was detected in these samples. *T. sinensis* and *T. orientalis* infections were detected in cattle and yaks, and *T. luwenshuni* was discovered for the first time in cattle and yaks in the Tibet Plateau Region, China.

Keywords *Theileria* · Detection · Cattle · Yak · Tibet Plateau

Introduction

Bovine theileriosis is a tick-borne disease caused by *Theileria* spp., causing clinical infections in domestic and wild bovine species that lead to heavy economic losses (Dolan 1989). Of all bovine *Theileria* spp., only three (*Theileria annulata*, *Theileria orientalis*, and *Theileria sinensis*) have been found in China (Bai et al. 2002; Luo and Lu 1997; Liu et al. 2010). *T. annulata* is the most pathogenic etiological agent of theileriosis worldwide (Bilgic et al. 2010). It is prevalent in the Ningxia, Xinjiang, Inner Mongolia, and Gansu province (Luo and Lu 1997; Lü et al. 1995), and its average infection

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rate is up to 57.7 %. *T. orientalis* has caused outbreaks of mild theileriosis in many countries (McFadden et al. 2011; Eamens et al. 2013); it is distributed throughout Henan, Hebei, Yunnan, Guizhou, Jiangsu, Hunan, Liaoning, Jilin, Guangdong, Guangxi, Qinghai, Gansu, Heilongjiang, etc. (Liu et al. 2010, 2013; Lü et al. 1995). *T. sinensis* has been previously reported in cattle and yaks in Gansu, China, but its level of pathogenicity is unclear (Bai et al. 2002).

There are many reports providing epidemiological data on bovine theileriosis in China, but there are no data on bovine theileriosis in the Tibet Plateau Region, China. The aim of this study was to detect and identify the *Theileria* spp. infecting cattle and yaks in Tibet Plateau Region, China.

Material and methods

Blood sampling and DNA extraction

A total of 154 blood samples were collected into Vacutainer tubes with ethylene diamine tetraacetic acid (EDTA), from cattle and yaks in three regions (Lhasa (29° 58′ 12″ N, 91° 6′ 36″ E), Lhoka (29° 14′ 24″ N, 91° 46′ 12″ E), and Tianzhu (37° 14′ 24″ N, 102° 50′ 24″ E)) of Tibet Plateau, China in September 2014. Genomic DNA samples were extracted using a genomic DNA extraction kit (Qiagen, Hilden, Germany).

Identification using species-specific primers

Theileria spp. were detected via polymerase chain reaction (PCR) using species-specific primers; information on the PCR primers is shown in Table 1. Six positive *Theileria* genomic DNA samples (from Lanzhou Veterinary Research Institute) were used as the positive controls, while the genomic DNA of healthy bovines was used as the negative control.

PCR amplification and sequence analysis

The PCR products were purified using a DNA gel extraction kit (Axygen, Hangzhou, China), ligated into the pGEM-T Easy vector (Invitrogen, Carlsbad, CA, USA), and subjected to sequencing. All results of sequencing were compared with sequences in the GenBank database (www.ncbi.nlm.nih.gov/genbank/; National Center for Biotechnology Information, NCBI), and the alignments of multiple sequences were executed in Florence Corpet (http://multalin.toulouse.inra.fr/). The final high homology sequences have been deposited in the GenBank database, and phylogenetic trees of major piroplasm surface protein genes and 18S rDNA genes of *Theileria* were constructed using other sequences from NCBI.

Results

In this study, out of 154 blood samples from cattle and yaks in the Tibet Plateau Region (Lhasa, Lhoka and Tianzhu), China, the prevalence of *T. sinensis* in cattle in Lhasa was 27.6 % (8/29) (Table 2) and the appropriate accession numbers are KU518036, KU518037, and KU518038. The prevalence was 6.9 % (2/29) for *T. orientalis* in cattle in Lhasa, and the accession numbers are KU247950 and KU518033. The prevalence was 26.7 % (8/30) for *T. sinensis* in cattle in Lhoka, and the accession numbers are KU247951, KU518034, and KU518035. The prevalence was 30.5 % (29/95) for *T. sinensis* in yaks in Tianzhu (Table 2), and the accession numbers are KU518039, KU518040, and KU518041. Interestingly, *T. luwenshuni*, which is a *Theileria* sp.

Table 1 Sequences of the oligonucleotide primers used in this study

Pathogen	Target gene	Primer oligonucleotide sequences (5′-3′)		Final amplicon size (bp)	References
Theileria spp.	18S rRNA	989 990	AGTTTCTGACCTATCAG TTGCCTTAAACTTCCTTG	1100	Allospp et al. (1993)
T. annulata	Tams1	Ta-N516 F Ta-N517 R	GTAACCTTTAAAAACGT GTTACGAACATGGGTTT	721	d'Oliveira et al. (1995)
T. orientalis	MPSP	Tser F Tser R	CACGCTATGTTGTCCAAGAG TGTGAGACTCAATGCGCCTA	875	Liu et al. (2010)
T. sinensis	MPSP	Tsin F Tsin R	CACTGCTATGTTGTCCAAGAGATATT AATGCGCCTAAAGATAGTAGAAAAC	887	Liu et al. (2010)
T. luwenshuni	18S rRNA	Tluw310 Tluw680	GGTAGGGTATTGGCCTACTGA TCATCCGGATAATACAAG	340	Yin et al. (2008)
T. uilenbergi	18S rRNA	Tuil310 Tuil680	GGTAGGGTATTGGCCTACCGG ACACTCGGAAAATGCAAGCA	340	Yin et al. (2008)
T. ovis	18S rRNA	Forward Reverse	TCGAGACCTTCGGGT TCCGGACATTGTAAAACAAA	520	Altay et al. (2005)

Table 2 Prevalence of Theileria infection of cattle in Tibet Plateau, China in 2014

Collection sites	Host	No. of samples	Prevalence of *Theileria* spp. infection in cattle and yak			
			T. annulata	*T. sinensis*	*T. orientalis*	*T. luwenshuni*
Lhasa	Cattle	29	0 % (0/29)	27.6 % (8/29)	6.9 % (2/29)	0 % (0/29)
Lhoka	Cattle	30	0 % (0/30)	26.7 % (8/30)	0 % (0/30)	10 % (3/30)
Tianzhu	Yak	95	0 % (0/95)	30.5 % (29/95)	0 % (0/95)	1.1 % (1/95)

previously reported to be pathogenic for sheep and goats, was detected from 10 % (3/30) of cattle blood samples in Lhoka (accession numbers KU247948, KU518031, and KU518032) and in 1.1 % (1/95) of yak blood samples in Tianzhu (accession number KU247949) (Table 2).

The phylogenetic tree based on the major piroplasm surface protein showed that a *Theileria* sp. obtained from Lhasa was *T. orientalis* (group 1) and that the *Theileria* sp. from Lhasa, Lhoka, and Tianzhu was *T. sinensis* (group 2) (Fig. 1). Another phylogenetic tree constructed in this study

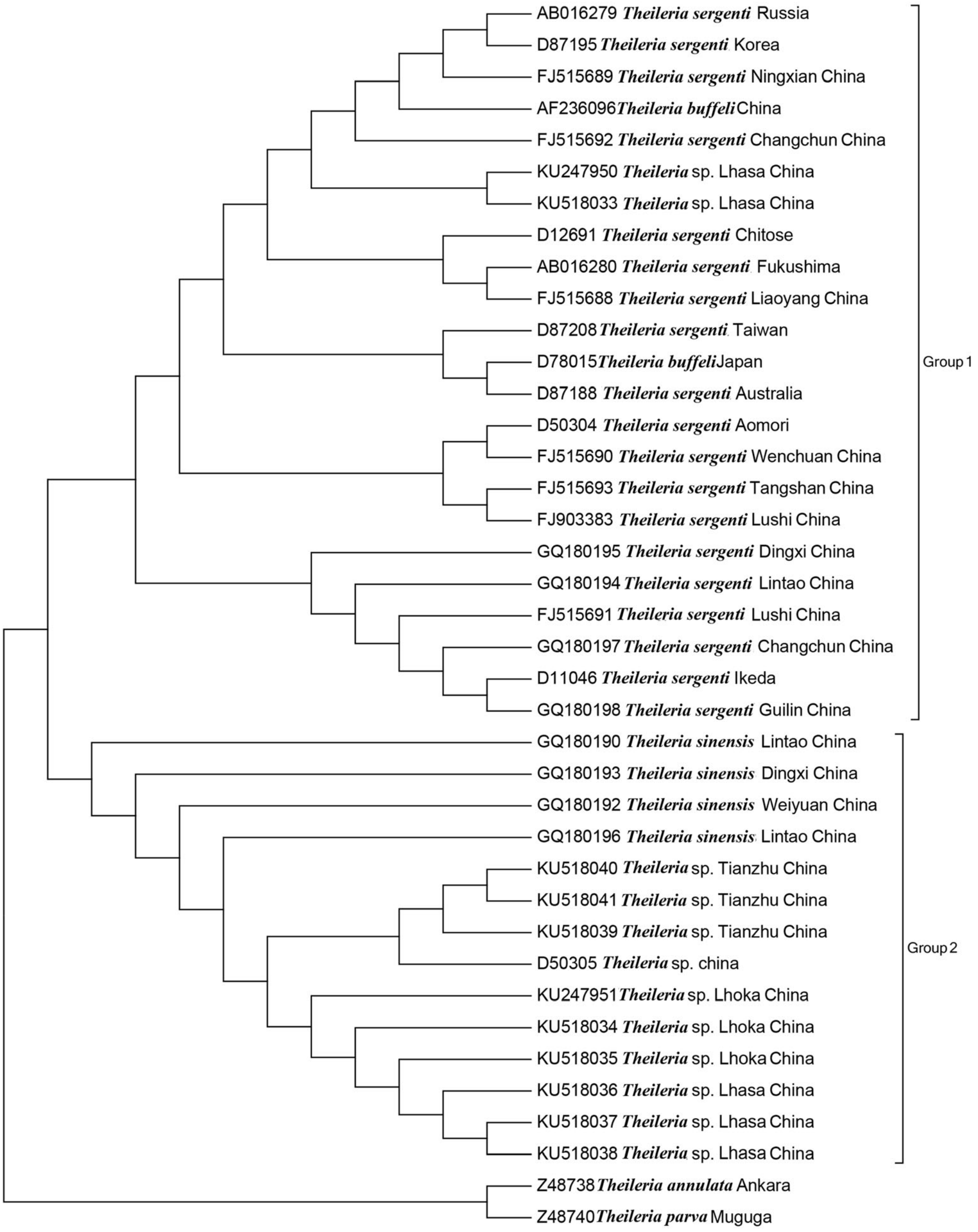


Fig. 1 Phylogenetic tree of *Theileria* based on MPSP protein gene sequences

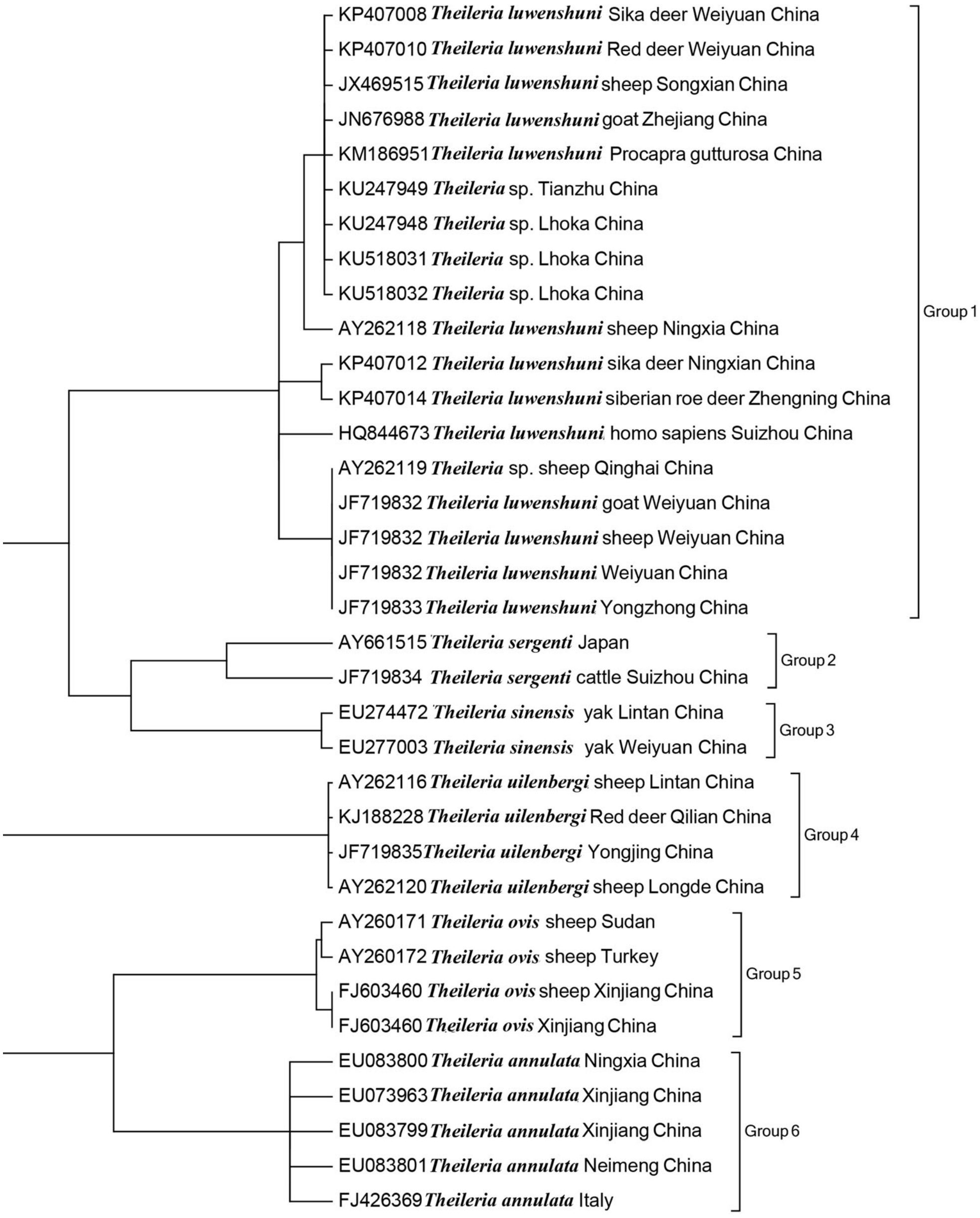
showed that the newly isolated *Theileria* sp. from the Tibet Plateau was in the same clade as *T. luwenshuni* (group 1) (Fig. 2). No other *Theileria* sp. was detected in these samples.

Discussion

In this study, only two bovine *Theileria* spp., *T. orientalis* and *T. sinensis*, were detected in cattle and yaks in the Tibet Plateau Region of China. Interestingly, *T. luwenshuni*, which has been previously reported to be pathogenic to sheep and goats, was detected for the first time in blood samples from cattle and yaks in the Tibet Plateau. No other *Theileria* sp. was detected in this study.

Although the pathogenicity of *T. orientalis* is lower than that of *T. annulata*, it is widely distributed in most provinces in China and also, to some extent, has limited the development of the cattle industry. The prevalence of *T. orientalis* in cattle is up to 72 % in northeast China (Jin et al. 2007). *T. sinensis* differs from all other known *Theileria* spp. It was first found in Gansu province (Bai et al. 2002), transmitted by *Haemaphysalis qinghaiensis* (Yin et al. 2004); the adult *Haemaphysalis japonica* ticks also transmit *T. sinensis* (Yin et al. 2004). A previous report revealed that *T. sinensis* was distributed only in Gansu province (Yin et al. 2004), but Li

Fig. 2 Rooted neighbor-joining phylogenetic tree of 18S rDNA of *Theileria* species



et al. (2015) reported that the prevalence of *T. sinensis* in cattle was 3.3 % in Jiangxi, China. Generally, the distribution of *Theileria* spp. closely matches the distribution of their vectors. *H. qinghaiensis* ticks were first reported in Qinghai province in 1978. They are distributed in Qinghai, Gansu, Ningxia, Sichuan, Yunnan, and Tibet; these regions are 1600–4200 m above sea level (Teng and Jiang 1991). We can conclude that *T. sinensis* may be extensively distributed in these areas of China.

Theileria luwenshuni was first reported in sheep and goats; it is widely distributed in most parts of China (Yin et al. 2007, 2008; Li et al. 2007, 2014b; Chen et al. 2014; Yang et al. 2014) and is transmitted by both *H. qinghaiensis* and *Haemaphysalis longicornis* (Li et al. 2007). There is a lack of relevant information about *T. luwenshuni* infection in other animals. In the past, *T. luwenshuni* was considered to be a parasite of only sheep and goats. However, *T. luwenshuni* has been detected recently in roe, sika, and red deer (Li et al. 2014b), hedgehogs (Chen et al. 2014), and Mongolian gazelle (Li et al. 2014c) in China. Therefore, Li et al. concluded that *T. luwenshuni* is a multi-host parasite and that at least seven mammals, including sheep, goats, roe deer, sika deer, red deer, hedgehogs, and Mongolian gazelle, can be infected by the parasite (Li et al. 2014a, 2014b, 2014c). In this study, *T. luwenshuni* was also detected in blood samples taken from cattle and yaks in the Tibet Plateau Region of China. Given that *T. luwenshuni* is highly pathogenic in sheep and goats and its infection of cattle and yaks was previously unknown, the influence of *T. luwenshuni* on cattle and yaks requires further research. The results of this study are very important to herders in the Tibet Plateau Region, where the main livestock breeding industry involves cattle, yaks, sheep, and goats.

Our results provide important data that extend our understanding of the epidemiology of theileriosis in cattle and yaks and should facilitate the implementation of measures to control the transmission of *Theileria* parasites among ruminants in the Tibet Plateau, China.

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Compliance with ethical standards

Conflict interests The authors declare that they have no competing interests.

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