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Research Article

Epidemiological Survey on Tick Borne Diseases of Pet Dogs in Korla, Northwestern China

Jianhui CHEN [1] Shen SHI [2 (*)] Nannan CUI [3] Lixin SU [3] Ziqi WANG [3] Fang LI [1] Shang ZHAN [1] Guoyu ZHAO [2] Yuanzhi WANG [3 (*)] Xijiang WANG [2 (*)]

[1] School of Public Health, Xinjiang Medical University, Urmqi, Xinjiang Uygur Autonomous Region 830000, P.R. CHINA

[2] Key Laboratory of Vector-Borne Infectious Diseases, Center for Disease Control and Prevention, Urmqi, Xinjiang Uygur Autonomous Region 830000, P.R. CHINA

[3] Key Laboratory for Prevention and Control of Emerging Infectious Diseases and Public Health Security, the XPCC, School of Medicine, Shihezi University, Shihezi City, Xinjiang Uygur Autonomous Region 832002, P.R. CHINA

Abstract

Pet dogs pose a potential risk to transmitting zoonotic pathogens by ticks. However information about the prevalence status in pet dogs of tick-borne diseases is currently limited. In this study, 196 blood samples and 223 ticks were collected from pet dogs in Korla, northwestern China. Based on morphological and molecular characteristics, all ticks were identified as *Rhipicephalus turanicus* sensu stricto. We used primers targeting the 16S ribosomal *(16S rRNA)* gene for detection of *Anaplasma bovis* species, targeting the small subunit 18S ribosomal RNA(*18S rRNA*) gene for detection of *Hepatozoon canis* species and targeting htpAB-associated repetitive element gene (IS111) for detection of *Coxiella burnetii* species The nested PCR(nPCR)-positive products were sequenced, aligned, and phylogenetically analyzed. three tick-borne pathogens were detected in the samples. *Coxiella burnetii* were detected both in parasitic ticks and in blood samples with a detection rate of 17.93% (40/233) in tick and 79.1% (155/196) in blood samples, followed by 21.52% *H. canis* (48/233) in tick, 2.5% A. bovis (5/196) in blood samples. This study provided molecular evidence for the occurrence of *A. bovis, H. canis* and *C. burnetii* circulating in pet dogs and their ticks in northwestern China. Understanding the prevalence of Tick-borne diseases in pet dog is essential for developing effective strategies for disease control and management.

Keywords: Pet dogs, Ticks, *Anaplasma bovis*, *Coxiella burnetii*, *Hepatozoon canis*, Northwestern China

(*) Corresponding authors:
Xijiang WANG,Yuanzhi WANG & Shen SHI

Phone: +86-0991-8866415(X.W.)
+86-0993-2648349 (Y.W.)
+86-0991-4662488(S.S.)

E-mail: 2500681817@qq.com (X.W.)
wangyuanzhi621@126.com (Y.W.)
406959660@qq.com (S.S.)

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Introduction

Tick-borne diseases (TBDs) are relatively common in pet dogs, and dog-human relationships may facilitate the spread of tick-borne pathogens among people [1]. Many factors can affect the spread of tick-borne diseases in pet dogs, such as the pet dog's living environment, parasite control, and the health awareness of pet dog owners [2]. However, parasite control in pet dogs is often neglected due to the lack of knowledge about parasite hazards by dog owners [3]. Due to the increasing number of pet dogs in China and their close relationship with humans, there is a need to study the epidemiological status of tick-borne zoonotic pathogens [4].

Korla City is listed as an important transportation hub and material distribution center of Xinjiang Uygur Autonomous Region (XUAR, northwestern China) with more than 477.000 residents. It is located in the northeast edge of the Taklamakan Desert, which is the second largest desert in the world. Extreme dryness with an average annual precipitation of 58.6 mm and an annual maximum evaporation of 2788.2 mm is its climatic characteristics [5]. *Rhipicephalus turanicus*, *Dermacentor marginatus* and *Hyalomma asiaticum* were previously reported as dominant tick species in the oasis of Taklamakan desert [6]. TBPs in pet dogs and their ticks, such as *Candidatus Rickettsia barbariae*, *Rickettsia massiliae*, *Rickettsia conorii*,

Rickettsia sibirica, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Anaplasma ovis* and *Brucella* spp., were previously reported in north region of XUAR [7,8]. In present study, we further investigated the blood samples of pet dogs and tick,which will help disease prevention and control in Korla City, .

Material and Methods

Ethical Statement

This study was reviewed and approved by the ethics committee of School of Medicine, Shihezi University in accordance with the medical regulations of China (Approval numbers A2020-113-01).

Sample Collection

Two shelters for stray dogs and five pet hospitals close to pastures in Korla City (934 m above sea level; 41°14′N 85°11′E) in Tarim Basin, XUAR were selected between late April to mid-May 2021(coinciding with the peak activities of adult ticks), blood and tick samples were collected from pet dogs based on clinical symptoms that include depression, weight loss, and anorexia. All samples were collected with the permission of the pet owner and sample collection was carried out by a local veterinarian. The blood samples are collected into a vacuum tube containing ethylene diamine tetraacetic acid (EDTA) anticoagulant while ticks were picked from dogs and placed in tubes containing 75% ethanol and 5% glycerol.

Identification of Ticks

Extracted total DNA from 200 µL whole blood samples using a blood DNA extraction kit (Omega Bio-tek, Norcross, USA) and genomic DNA from whole ticks using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) following the manufacturer's instructions. All ticks were identified based on morphology before DNA is extracted, as previously described [9,10]. Subsequently, 30 representative ticks, with 4-6 ticks at each veterinary clinic, were subjected to molecular classification analysis based on partial mitochondrial 16S ribosomal RNA *[16S* rRNA (460bp)] gene sequences to confirm tick species [11].

Detection of Tick-Borne Pathogens

We used a partial *16S rRNA* (450bp) gene to detect *A. bovis* [12]. The molecular detection of *C. burnetii* was per6formed using the IS111 (260bp) [13]. *Hepatozoon canis* were detected targeting 18S ribosomal RNA *(18S rRNA)* [14]. DNA from our laboratory was used as positive controls for *A. bovis*, *C. burnetii* and *H. canis*. Double distilled water was used as a negative control (Dongsheng, Guangzhou, China). Characteristics of the amplified fragments and corresponding primer sequences are provided in *Table 1*.

Sequencing and Data Analyses

Sequencing data were subjected to Basic Local Alignment Search Tool (BLAST) searches *(http://www.ncbi.nlm.nih.gov/blast/)* and then aligned and analyzed with reference sequences downloaded from GenBank. Phylogenetic trees

Table 1. *Characteristics of amplified fragments and corresponding primer sequences*

Targeted DNA	Gene	Primer sequence (5'-3)	Fragment	Cycling Conditions of PCR Assays	Reference
Tick	16S rRNA	Forward 1 (CTGCTCAATGATTTTTTAAATTGCTGTGG)	460bp	94°C for 5 min, followed by 37 cycles at 92°C for 30 s, 54°C for 30s, and 72°C for 30s, with a final extension at 72°C for 8 min	[11]
		Reverse 1 (CCGGTCTGAACTCAGATCAAGT)			
Hepatozoon canis	*18S rRNA*	Forward 2 (ATACATGAGCAAAATCTCAAC)	666bp	95°C for 5 min, followed by 35 cycles at 95°C for 60s, 58°C for 60 s, and 72°C for 60 sec, with a final extension at 72°C for 5 min	[14]
		Reverse 2 (CTTATTATTCCATGCTGCAG)			
Anaplasma bovis	16S rRNA	Forward 1 (TTGAGAGTTTGATCCTGGCTCAGAACG)	450bp	94°C for 5 min, followed by 40 cycles at 94°C for 45s, 55°C for 50 s, and 72°C for 1 min, with a final extension at 72°C for 5 min	[12]
		Reverse 1 (CACCTCTACACTAGGAATTCCGCTATC)			
		Forward 2 (TTGAGAGTTTGATCCTGGCTCAGAACG)			
		Reverse 2 (GTACCGTCATTATCTTCCCTA)			
Coxiella burnetii	IS111	Forward 1 (TACTGGGTGTTGATATTGC)	260bp	95°C for 8 min; followed by 35 cycles at 95°C for 15 s, 52°C for 5s, and 72°C for 1 min; and extension at 68°C for 10 min.	[13]
		Reverse 1 (CCGTT TCATCCGCGGTG)			
		Forward 2 (GTAAAGTGATCTACACGA)			
		Reverse 2 (TTAACAGCGCTTGAACGT)			

were constructed based on the sequence distance method using the neighbor-joining algorithms implemented in the Molecular Evolutionary Genetics Analysis MEGA 7.0 *(http://www.megasoftware.net)* software.

Results

All ticks (72 male and 151 female) were collected and morphologically identified as *Rhipicephalus turanicus* sensu stricto (s.s.) *(Fig. 1)*. The obtained sequences of *Rh. turanicus* s.s. have been deposited in the GenBank database. Phylogenetic trees analysis further confirmed these results *(Fig. 2)*.

Three tick-borne pathogens were detected in this test, among which the highest detection rate was *C. burnetii,* which was detected in both ticks and blood samples, with a detection rate of 17.94% (40/223) in ticks and 79.1% (155/196) in blood samples, followed by *H. canis* 21.52% (48/223) in ticks and *A. bovis* 2.5% (5/196) in blood samples. Among them, co-detection with *H. canis* and *C. burnetii* was detected in 12 blood samples. All ticks and blood samples were also tested for *Borrelia burgdorferi* and *Leptospira sp.*, However, DNA from these pathogens was not detected in any of the samples.

Among all the positive ticks and blood samples, *A. bovis* and *C. burnetii* showed 99.78% and 99.63 identity to the corresponding sequences of *A. bovis* (MH255939) from Shaanxi Province and *C. burnetii* (KX852471) from

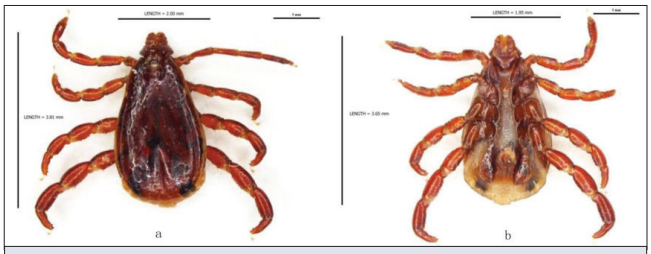

Fig 1. Morphological analysis of *Rhipicephalus turanicus* sensu stricto collected from pet dogs. a Male, dorsal; b male, ventral

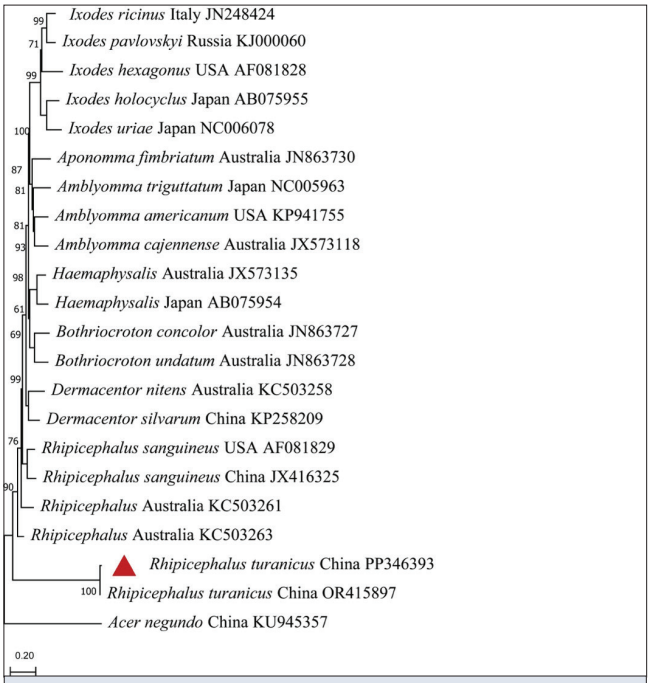

Fig 2. Phylogenetic tree based on partial gene of the 16S rRNA of *Rhipicephalus turanicus* sensu stricto (▲) collected from pet dogs obtained in this study in northwestern China. The evolutionary history was inferred using the neighbor-joining method (bootstrap replicates: 1000) with MEGA 7.0

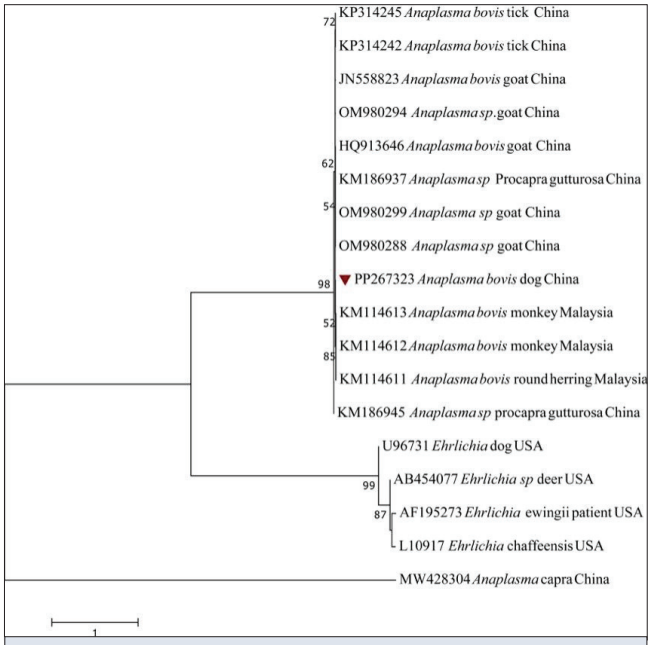

Fig 3. Phylogenetic tree based on partial gene of the 16S rRNA of *Anaplasma bovis* (▼) from blood samples obtained in this study in northwestern China. The evolutionary history was inferred using the neighbor-joining method (bootstrap replicates: 1000) with MEGA 7.0

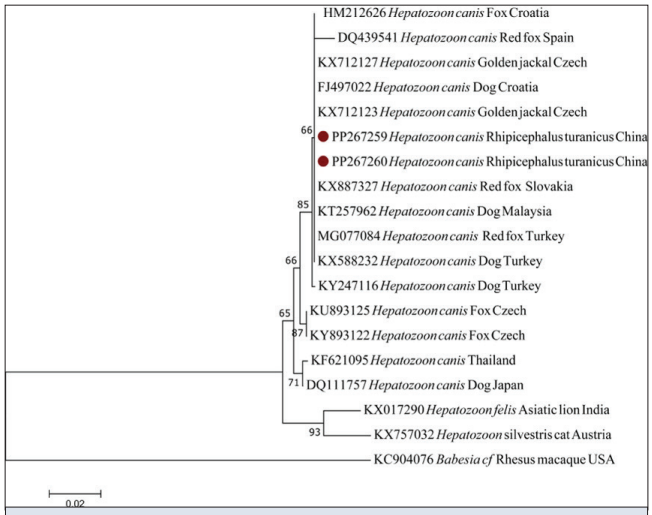

Fig 4. Phylogenetic tree based on partial gene of the 18S rRNA sequence of *Hepatozoon canis* (●) from *Rhipicephalus turanicus* sensu stricto obtained in this study in northwestern China. The evolutionary history was inferred using the neighbor-joining method (bootstrap replicates: 1000) with MEGA 7.0

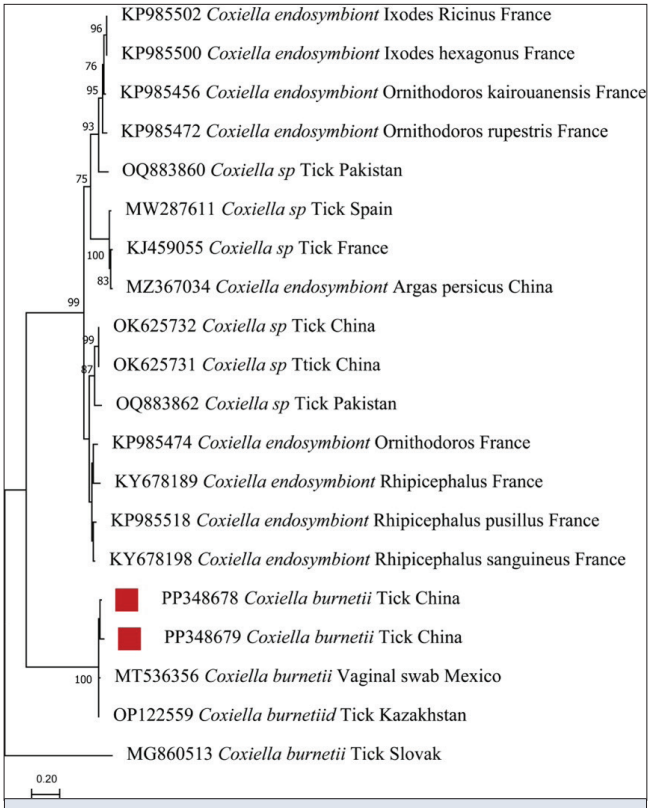

Fig 5. Phylogenetic tree based on partial gene of the htpABgene sequence of *Coxiella burnetii* ( ) from *Rhipicephalus turanicus* sensu stricto and blood samples obtained in this study in northwestern China. The evolutionary history was inferred using the neighbor-joining method (bootstrap replicates: 1000) with MEGA 7.0.

XUAR, respectively. *Hepatozoon canis* showed 99.84% identity to the corresponding sequences from Czech Republic (KX712129). Phylogenetic trees analysis further confirmed these results *(A. bovis - Fig. 3; H. canis - Fig. 4; C. burnetii - Fig. 5).*

All sequences from this study were deposited in the GenBank *(http://www. ncbi.nlm.nih.gov)* database *(Rh. turanicus* s.s PP346393; *A. bovis* PP267323; *C. burnetii* PP348678-PP472476; *H. canis* PP267259-PP267260).

Discussion

There have been increasing numbers of cases of zoonotic tick borne diseases in humans and pet dogs [15]. In this study, *C. burnetii, H. canis* and *A. bovis* were screened out in pet dogs and their parasitic ticks. To the best of our knowledge, this is the first report of *C. burnetii* and *H. canis* in *Rh. turanicus s.s.* in China.

Anaplasma spp. is transmitted by ticks and contains seven proven species. Two of these species, *A. phagocytophilum* and *A. capra*, commonly cause disease in humans [16]. *Anaplasma bovis* was initially thought to be just an animal pathogen until the first patient case was reported in 2019 [17,18]. In this study, *A. bovis* was detected both in dog ticks and blood samples. This finding suggests that it is vital to further survey *A. bovis* among pet dogs, ticks and dog owners especially in oasis of Taklamakan Desert in the future.

Coxiella burnetii can infect a variety of domestic and wild animals, including mammals, birds, and reptiles. Previously, cattle, sheep, and goats were considered the primary hosts [19]. At the same time, dogs and cats are classified as mammals susceptible to *C. burnetii* [20]. Pet animals, especially those in close contact with their owners, play an important role in reservoirs of *C. burnetii*, which causes urban Q fever and sporadic Q fever [21]. In this study, it was not only found in the samples of dog ticks, but also in dog blood samples with 79.1% (155/196) positive rate. This result gives a strong warning to public health security against Q fever.

To date, *Hepatozoon* spp. includes at least 340 species and can infect a wide range of vertebrate hosts, such as mammals, reptiles, birds, fish, and invertebrates. In terms of its primary vectors, *Amblyomma ovale*, *Rhipicephalus microplus*, *Haemaphysalis longicornis* and *Haemaphysalis flava* have been identified as definite hosts for *Hepatozoon* [22-24]. In this study, we found *H. canis* with 21.52% (48/233) positivity in *Rh. turanicus s.s.* Although this study does not confirm *Rh. turanicus s.s* being vectored as *H. canis*, we still believe *H. canis* pose a potential risk to dogs and dog owners in local people.

Co-infection is common in tick-bitten mammals. Previously, some scholars have discovered that co-infections were identified in 16.7% of *Ixodes ricinus* (89/534), which accounted for 64.5% (89/138) of all infected ticks. Co-infection prevalence was 14.3% (11/77) in adults and 17.1% (78/457) in nymphs [25]. Meanwhile, ticks can acquire a variety of pathogenic species (such as parasites, bacteria or viruses) through blood-sucking to different vertebrate hosts or through systemic transmission of common feeding or co-feeding [26]. In this study, *H. canis* and *C. burnetii* were simultaneously detected in 12 dog blood samples. This study extends tick-borne pathogen co-detection in pet dogs.

With the number of pet dogs increasing in China, it is necessary to strengthen the supervision of pet dogs and stray dogs in order to control tick-borne zoonotic diseases in the horizon of "One World One Health".

Declarations

Availability of Data and Materials: The datasets generated during and/or analysed during the current study are available from the corresponding author (XW) on reasonable request.

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