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SHORT COMMUNICATION

First Detection of Tacheng Tick Virus 2 in Hard Ticks from Southeastern Kazakhstan

Yuqing JIA [1,2,#,a] Shiyi WANG [1,2,#,b] Meihua YANG [3,c] Nuralieva ULZHAN [4,d]
Karlygash OMAROVA [5,e] Zhiqiang LIU [6,f] Oralhazi KAZKHAN [7,g (*)] Yuanzhi WANG [1,2,h (*)]

[#] *These authors contributed equally to this work*
[1] School of Medicine, Shihezi University, Shihezi, CHINA
[2] NHC Key Laboratory of Prevention and Treatment of Central Asia High Incidence Diseases, First Affiliated Hospital, School of Medicine, Shihezi University, Shihezi, CHINA
[3] College of Agriculture, Shihezi University, Shihezi, CHINA
[4] Department of "Beekeeping, Poultry and Fisheries" Non-profit Joint Stock Company, Kazakh National Agrarian University, Almaty, KAZAKHSTAN
[5] Department of Production and Processing Technology of Animal Husbandry Products, S. Seifullin Kazakh Agro Technical University, Nur-Sultan, KAZAKHSTAN
[6] Institute of Veterinary Medicine, Xinjiang Academy of Animal Science, Urumqi, CHINA
[7] College of Animal Science and Technology, Shihezi University, Shihezi, CHINA
ORCIDs: [a] 0000-0001-6904-1124; [b] 0000-0001-8581-8060; [c] 0000-0002-2238-3781; [d] 0000-0002-7008-7303; [e] 0000-0001-7551-5787
[f] 0000-0002-6382-1135; [g] 0000-0002-1336-9312; [h] 0000-0002-7500-022X

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Abstract

We aim to detect the presence of Tacheng tick virus 2 (TcTV-2) in ticks of southeastern Kazakhstan. A total of 205 ticks were collected and separated into four species, namely *Hyalomma scupense, Dermacentor marginatus, Hyalomma asiaticum* and *Hyalomma anatolicum*. The partial S segment of TcTV-2 was detected in individual RNA of separated ticks by reverse transcriptase polymerase chain reaction. 11.22% (23/205) of ticks were positive to the viral S segment. This is first report of presence of the TcTV-2 in *Hy. scupense* and *Hy. anatolicum* from Kazakhstan. This finding extends tick species and the geographic distribution of TcTV-2.

Keywords: *Hyalomma scupense, Hyalomma anatolicum, Kazakhstan, Tacheng tick virus 2*

Güneydoğu Kazakistan'da Sert Kenelerde Tacheng Kene Virüsü 2'nin İlk Tespiti

Öz

Güneydoğu Kazakistan'a ait kenelerde Tacheng kene virüsü 2'nin (TcTV-2) varlığının araştırılmasını amaçladık. Toplam 205 kene toplandı ve keneler *Hyalomma scupense, Dermacentor marginatus, Hyalomma asiaticum* ve *Hyalomma anatolicum* olmak üzere dört türe ayrıldı. TcTV-2'nin kısmi S segmenti, tür ayırımı yapılmış kenelerin bireysel RNA'sında reverse transkriptaz-polimeraz zincir reaksiyonu ile tespit edildi. Kenelerin %11.22'si (23/205) viral S segmenti yönünden pozitifti. Bu, Kazakistan'da *Hy. scupense* ve *Hy. anatolicum*'da TcTV-2'nin varlığına dair ilk bildirimdir. Bu bulgu, kene türleri ve TcTV-2'nin coğrafi dağılımı hakkında bilgi sunmaktadır.

Anahtar sözcükler: *Hyalomma scupense, Hyalomma anatolicum, Kazakistan, Tacheng kene virüsü 2*

INTRODUCTION

Tacheng tick virus 2 (TcTV-2) is an emerging tick-borne virus which is a member of the genus *Uukuvirus* in the family *Phenuiviridae* order to *Bunyaviruses*. In 2019, TcTV-2 was detected in human and caused fever, headache and multiple clinical symptoms in the Xinjiang Uygur Autonomous Region (XUAR, northwestern China), and had

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(*) Corresponding Author

Tel: +86-1890-9936353 (K. Oralhazi); +86-1588-69312178 (Y-Z Wang)
E-mail: 1328251980@qq.com (K. Oralhazi); wangyuanzhi621@126.com (Y-Z Wang)

potential risk of person-to-person transmission. Presence of ticks in China for *Dermacentor nuttalli*, *Dermacentor silvarum*, *Dermacentor marginatus* and *Hyalomma asiaticum* were 5.46% (10/183), 16.67% (2/12), 14.81% (16/108) and 11.90% (5/42), respectively [1]. TcTV-2 was detected in *Rhipicephalus sanguineus* in Turkey and in *Dermacentor reticulatus* in Romania by high-throughput transcriptome sequencing [2,3].

Kazakhstan is located in Central Asia and borders five countries (China, the Russian Federation, Kyrgyzstan, Uzbekistan and Turkmenistan). To date, at least 12 species of ticks have been detected in southeastern Kazakhstan [4]. However, it is still unclear whether TcTV-2 can be detected in hard ticks in this region, which is adjacent to XUAR, China.

Our study aims to confirm whether TcTV-2 can be detected in hard ticks in Kazakhstan. In the present study, TcTV-2 was detected in hard ticks in three oblasts of southeastern Kazakhstan.

Material and Methods

Ethical Statement

The sampled ticks were treated and imported into Chinese lab according to the requirement of the Administration of Animal and Plant Quarantine of the People's Republic of China.

Tick Collecting and RNA Extraction

From March to May during 2018-2019, contemporaneously with the peak activities of adult ticks in Kazakhstan, a total of 6107 hard ticks were collected from Eastern and Southern Kazakhstan. According to our previous work, the morphological characteristics of ticks were taxonomically identified using a stereoscopic dissecting microscope [4,5]. Here, 205 adult ticks were sampled from their natural hosts in four oblasts including East Kazakhstan, Almaty, Jambyl and South Kazakhstan. Parasitizing ticks were collected from the entire body of each animal including cattle, horses and sheep. The geographical information was shown in *Table 1*. The total RNA of each tick was extracted by RNAprep Pure Tissue Kit (Tiangen, Biotech Co., Ltd., Beijing, China).

Nested Reverse Transcriptase Polymerase Chain Reaction (nRT-PCR) Conditions and Phylogenetic Analysis

The partial S segment of TcTV-2 was detected in individual RNA by nRT-PCR. The used primers were referred previous literature [1]. The first round PCR: the cycling conditions consisted of an initial 5 min denaturation at 94°C, followed by 35 cycles at 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 8 min. The second round PCR: the cycling conditions consisted of an initial 5 min denaturation at 94°C, followed by 35 cycles at 94°C for 30 sec, 54°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 8 min. A total of 205 samples were amplified in a 10 μL reaction containing 2×SYBR Premix Ex Taq II (Tiangen, Biotech Co., Ltd., Beijing, China). Each PCR assay included a negative control (distilled water instead of tick DNA template) and a positive control (with cDNA from TcTV-2-postive ticks from China). Amplicons were visualized by electrophoresis in a 1.5% agarose gel (1×TAE, pH 8.0) stained with Goldview Nucleic Acid Gel Stain (Equitech-Bio, Shanghai,China). The PCR products were sequenced and analyzed by BLASTn and Mega7 (Maxmum likelihood, Bootstrap 1000) [6].

Results

After the nRT-PCR, the S segment produces a single 252 bp fragment *(Fig. 1)*. The viral S segment was detected in 11.22% (23/205) of ticks including 10 *Hyalomma scupense*, 10 *D. marginatus*, 2 *Hy. asiaticum*, 1 *Hyalomma anatolicum*, shown in *Table 1*. Nucleotide sequences were deposited in the GenBank database (GenBank accession number were MT302558, MK286259, MK282660 and MK282666).

BLASTn analysis indicated that TcTV-2 S segment from Kazakhstan shared 99.5% (190/191) identity with those in China, and shared 96.9% (185/191) identity with those in Romania. Furthermore, phylogenetic analysis showed that TcTV-2 in Kazakhstan was closer to the Chinese virus strains in patient, Asian badger, cattle, sheep and hard ticks, and clustered with Yongjia tick virus 1 *(Fig. 2)*.

Table 1. *Geographical distribution, positive rate and tick species of ticks in southeastern Kazakhstan*

Neighboring Country	States	Districts	Number	Tick Species	Origin	Positive Rate
China-Russia	East Kazakhstan	Zaysan County	68	*Hy. scupense*	cattle	14.70% (10/68)
			26	*D. marginatus*	cattle	11.54% (3/26)
China-Kyrgyzstan	Almaty	Karablak County	14	*D. marginatus*	cattle	7.14% (1/14)
			27	*D. marginatus*	horse	11.11% (3/27)
		Ussalle County	14	*Hy. scupense*	cattle	0.00% (0/14)
		Kuksu County	14	*D. marginatus*	cattle	21.42% (3/14)
		Ustobe County	22	*Hy. asiaticum*	horse	9.09% (2/22)
Kyrgyzstan-Uzbekistan	Jambyl	Lugovoy	20	*Hy. anatolicum*	sheep	5.00% (1/20)

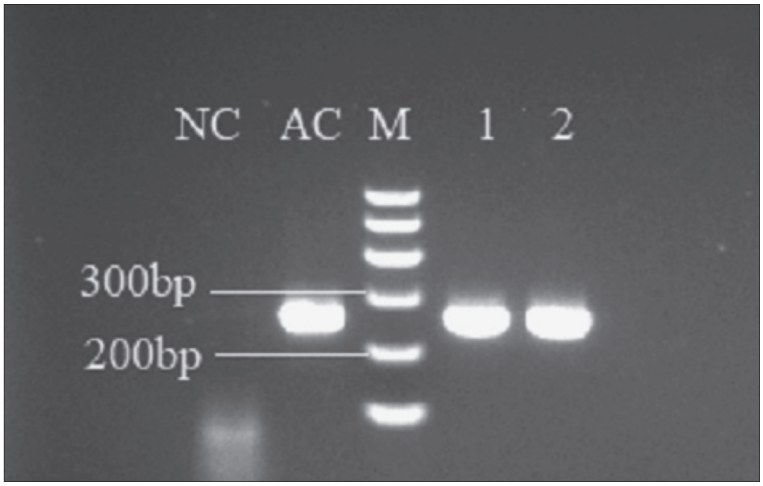

Fig 1. PCR product of TcTV-2 S segment amplification. Lane 1: Blank control; Lane 2: Positive control; Lane 3: 100 bp DNA Ladder Marker; Lane 4 and Lane 5: positive result on 252bp

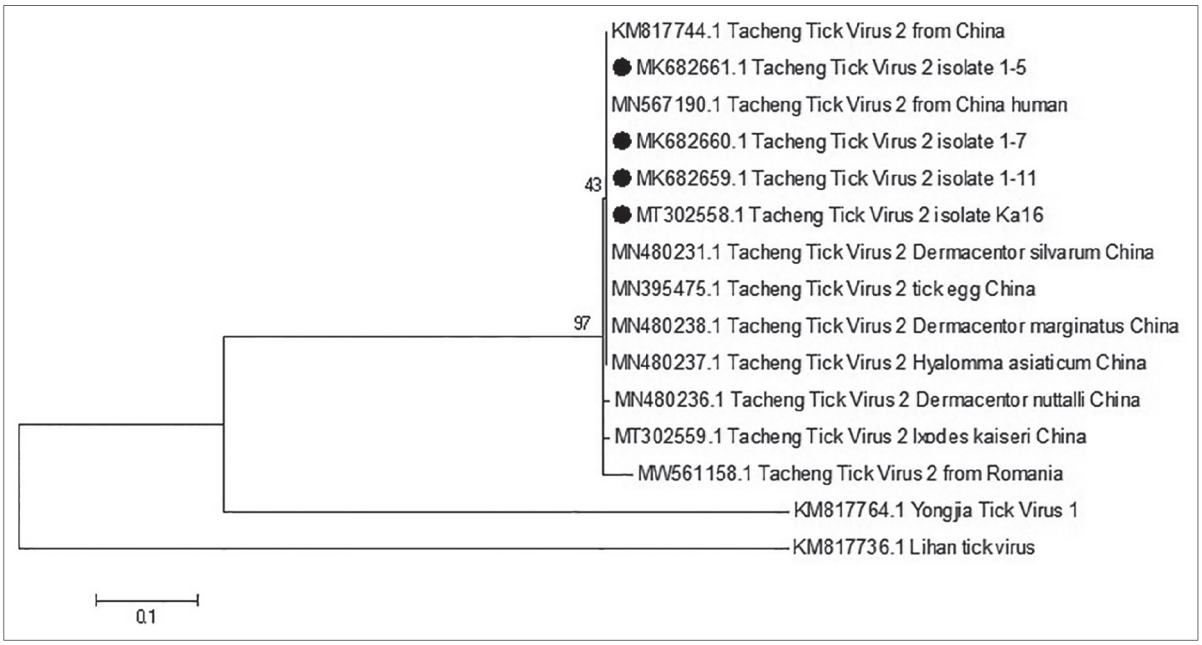

Fig 2. Phylogentic analysis of TcTV-2 partial S segment in Kazakhstan. Phylogenetic analysis was analyzed by Mega7 (Maxmum likehood, Bootstrap 1000), reference sequences were marked with black circle

Discussion

Tick-borne viral diseases have attracted much attention in recent years because of their increasing effects on human and animal health. Previously, a variety of tick-borne emerging viruses were detected in Kazakhstan, including Chim virus *(Orthonairovirus, Bunyavirales)* [7], Tacheng tick virus 1 (MK639367, *Orthonairovirus*, *Bunyavirales*), Tacheng tick virus 5 (MK656451, unclassified ssRNA negative-strand viruses) and Kemerovo virus (*Orbivirus*, *Reovirales*) [8]. In this study, TcTV-2 was detected in four tick species in Kazakhstan, and firstly documented in *Hy. scupense* and *Hy. anatolicum* ticks. To date, more than 30 species of human-biting ixodid ticks in Kazakhstan [9]. In our study, 6107 hard ticks were detected, and shown that 2935 (48.06%) were *Dermacentor*, 1592 (26.07%) were *Hyalomma* and 122 (2%) were *Rhipicephalus* [4]. This suggests *Dermacentor* and *Hyalomma* were dominant tick species in Kazakhstan. In the future, TcTV-2 should be further investigated in more tick species (especially in genera *Hyalomma*, *Dermacentor* and *Rhipicephalus*) sampled from more loci in Kazakhstan.

Previously, TcTV-2 was found in Turkey (36°N-40°N), Romania (Iasi City, 47°N) and China (Qinghe County, Wenquan County, Wusu City, Fuyun County, Gongliu County, Xinyuan County, Shawan City and Fuhai County, 43°N-47°N) [1-3]. Here, TcTV-2 was also detected in hard ticks in Almaty,

Jambyl and East Kazakhstan oblasts (25°N-47°N). This finding suggests that TcTV-2 may be detected in more regions (at least ranging from 25°N to 47°N), although it should be confirmed by more investigations.

TcTV-2, being a marked tick-borne pathogen, was previously detected in Asian badgers, cattle and sheep in China. The data came from GenBank, and their accession numbers were MW725300, MW725298 and MW725299, respectively. Here TcTV-2 was also found in 10.20% (5/49) of horse ticks (*D. marginatus* and *Hy. asiaticum*), which suggested that further investigation on TcTV-2 infection in horses should be carried out in Kazakhstan.

In summary, TcTV-2 was found first time in *Hy. scupense* and *Hy. anatolicum* ticks, and its mean positivity was 11.22% in hard ticks from southeastern Kazakhstan. This finding extends tick species and the geographic distribution of TcTV-2.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author and can be provided on your request.

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Competing Interests

The authors declared that there is no conflict of interest.

Authors' Contributions

YJ, SW and YW conceived and designed the study, and critically revised the manuscript. KG, KO and NU collected the ticks. YJ, SW, MY and ZL performed the experiments and analyzed the data. ZL and YW provided funds and contributed to writing the manuscript. All authors read and approved the final manuscript.

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