Genetic diversity of hard ticks (Acari: Ixodidae) in the south and east regions of Kazakhstan and northwestern China

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Short report

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Abstract

Background

The Republic of Kazakhstan, located in Central Asia, ranks as the world’s largest landlocked country, and borders five countries including China. There is a 1783 km long borderline between the South and East regions of Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR, northwestern China). To date, there is no report on the genetic diversity of ticks in these regions.

Methods

During 2015–2019, a total of 4392 hard ticks (representatives of ten species) were collected from 605 animals (sheep, cattle, camels, dogs and hedgehogs) at 24 sampling sites belonging to 15 districts in southeastern Kazakhstan. After morphological identification, 213 specimens of these ticks were selected for molecular analyses. In addition, 157 hard ticks collected from sheep and camels between 2015 and 2018 in seven districts of XUAR were used for comparison. Following DNA extraction, a fragment of the mitochondrial cytochrome c oxidase subunit I (cox1) gene, ranging from 631 bp to 889 bp, was used to analyze genetic diversity among these ticks.

Findings:

Phylogenetic analyses indicated that i) five tick species including Hyalomma detritum, Hyalomma asiaticum, Rhipicephalus turanicus, Dermacentor reticulatus and Haemaphysalis erinacei from Kazakhstan clustered together with conspecific ticks from XUAR; ii) the phylogenetic separation of Dermacentor marginatus from Kazakhstan and XUAR was highly supported; and iii) Rhipicephalus sanguineus sensu lato from Alamaty Oblast was more closely related to a specimen from Iran than to that from XUAR. The network diagram of haplotypes showed that iv) Hy. asiaticum from Almaty and Kyzylorda (Kazakhstan) together with that from Yuli County of XUAR constituted an ancestral haplogroup; and v) three lineages of Rh. turanicus (from Israel, Almaty and South Kazakhstan, as well as from Usu city, Ulugqat and Baicheng Counties of XUAR) might have originated from an ancestral lineage in Alataw city, XUAR.

Conclusions

These findings indicate that: (i) mitochondrial lineages of some tick species are shared between southern, eastern regions of Kazakhstan and northwestern China; (ii) common evolutionary origin of Hy. asiaticum and Rh. turanicus in these regions might be attributed to historical international trade and movements of wildlife; and (iii) certain tick species show clear differences between Kazakhstan and XUAR, either in
terms of abundance (*e.g.* *Hy. scupense, Hy. marginatum*) or exhibiting a phylogenetic split between these regions (relevant to *D. marginatus*).

**Background**

The Republic of Kazakhstan is located in Central Asia between 39°49′ – 55°49′ N and 46°28′–87°18′ E, with its western part extending into Eastern Europe. Kazakhstan is the ninth largest country in the world with a total area of 2,727,300 km², also ranking as the world's largest landlocked country [1, 2]. It borders Russia, Kyrgyzstan, Turkmenistan, Uzbekistan and China [3]. Xinjiang Uygur Autonomous Region (XUAR), occupies one-sixth of China and borders eight countries, covering an area of approximately 1,660,000 km² [4]. The borderline between XUAR and the South and East regions of Kazakhstan is 1,783 km long [5]. The ecological environment, topography, climate and natural landscape are similar both in the XUAR and southeastern Kazakhstan [6].

Recently, fourteen tick species have been molecularly characterized in the border regions of XUAR [7]. At the same time, while at least thirteen tick species have been identified in Kazakhstan [8–11], their molecular characteristics are unknown. Here we report the genetic diversity of ticks in the South and East regions of Kazakhstan, in comparison with those from northwestern China.

**Methods**

**Tick collection and morphological identification**

During 2015–2019, a total of 4392 hard ticks were collected from 605 domestic animals (287 sheep, 210 cattle, 101 camels, 7 dogs) and 22 hedgehogs at 24 sampling sites belonging to 15 districts of five oblasts (East Kazakhstan, Almaty, Jambyl, South Kazakhstan and Kyzylorda) in the East and South regions of Kazakhstan (Fig. 1). After morphological identification, 213 of these ticks (representing all species) were selected for molecular analysis. In addition, 157 hard ticks collected from sheep and camels between 2015 and 2018 in seven districts of XUAR were used for comparison. The information on tick species, their host of origin and collection data (year and coordinates of location) are shown in Appendix Table S1.

**Molecular Analysis Of Ticks**

The genomic DNA was extracted from the selected 370 ticks individually using a tissue kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit I (*coxi*) gene, ranging from 631 bp to 889 bp, was used to analyze inter- and intraspecific genetic diversity of ticks. PCR primers and cycling conditions are shown in Appendix Table S2.
Phylogenetic Analysis Of Ticks

Sequences of the \textit{cox1} gene were compared with GenBank data using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) after manual editing. This dataset was resampled 1000 times to generate bootstrap values. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method. The best-fitting substitution model was determined with the Akaike Information Criterion using the ML model test implemented in MEGA 7.0 software [12, 13]. Genetic diversity was estimated as haplotype (h) and nucleotide diversity (\(\pi\)) indices with the programme DNAsp v5.10.1 [14]. True diversity (DIV\textsubscript{mtDNA}) was assessed as the effective number of haplotypes on the sequence level [15]. Median-joining (MJ) networks were generated with the software Network v5.10.1 [16] to display the configuration of haplotypes and geographically localised phylogenetic haplogroups [17].

Results

Morphological and molecular identification revealed the presence of ten tick species in the five evaluated southeastern border oblasts of Kazakhstan, including \textit{Hyalomma asiaticum}, \textit{Hyalomma marginatum}, \textit{Hyalomma scupense}, \textit{Hyalomma detritum}, \textit{Hyalomma anatolicum}, \textit{Dermacentor marginatus}, \textit{Dermacentor reticulatus}, \textit{Rhipicephalus turanicus}, \textit{Rhipicephalus sanguineus} and \textit{Haemaphysalis erinacea}. Seven of these tick species (i.e., \textit{Hy}. \textit{asiaticum}, \textit{Hy}. \textit{detritum}, \textit{Hy}. \textit{anatolicum}, \textit{D}. \textit{marginatus}, \textit{Rh}. \textit{turanicus}, \textit{Rh}. \textit{sanguineus} and \textit{Ha}. \textit{erinacea}) were also represented in the sample group from XUAR, and could thus be used for molecular comparison. According to the latter, intraspecific sequence identities ranged from 96.27–100\% using BLAST analysis of the \textit{cox1} gene (shown in Appendix Table S3).

Phylogenetic analysis based on the \textit{cox1} gene (Fig. 2) revealed that: i) \textit{Hy}. \textit{detritum} from East Kazakhstan (MN841460) clustered together with conspecific ticks from Turpan city of XUAR (KF583581) and Inner Mongolia Province of China (JQ737068); ii) \textit{Hy}. \textit{scupense} from Almaty (MN853164) and East Kazakhstan (MN853163) shared a clade with \textit{Hy}. \textit{scupense} from France and Russia (KX000638 and KU130633); iii) \textit{Ha}. \textit{erinacea} ticks from Almaty Oblast, Kazakhstan (MN841464) and Altaw city of XUAR (KU364301) belonged to a different phylogenetic group than those from European countries; iv) \textit{Hy}. \textit{anatolicum} ticks from Jambyl Oblast (MN853167), Kazakhstan, Karshgar city of XUAR (KF583576) and Iran (KT920180) represented an ancestral clade to those from Iraq, India and Pakistan; v) \textit{D}. \textit{marginatus} from Qapqal County of XUAR (JX051151), East Kazakhstan (MN868592) and South Kazakhstan (MN868560) showed separation from \textit{D}. \textit{marginatus} from Alataw city of XUAR (KU364300) with high (100\%) bootstrap support; and vi) \textit{Rh}. \textit{sanguineus} sensu lato from Alamty (MN862754) was more closely related to a specimen from Iran than to that from Altaw city of XUAR (KU364307).

The network diagram of haplotypes showed that \textit{Hy}. \textit{asiaticum} from Almaty (MN892553) and Kyzylorda (MN961479) together with that from Yuli County of XUAR (KF527400) formed an ancestral haplogroup (H-2, in Appendix File 1). In addition, according to the present data, three lineages of \textit{Rh}. \textit{turanicus}, i.e. from Almaty and South Kazakhstan (MN853166 and MN841462, H-1 haplotype), XUAR (MF002579-
MF002581, H-3 haplotype), and Israel (KF219748, H-4 haplotype), originated from an ancestral lineage from Alataw city of XUAR (KY996841, H-2 haplotype) (Appendix File 2).

Discussion

The mitochondrial cox1 gene, as the standard DNA barcode, is one of the most popular markers for population genetic and phylogeographic studies across the animal kingdom [18]. At the same time, it is the best choice for identification of tick species [19]. In the present study, five tick species from the South and East regions of Kazakhstan, including Hy. detritum, Hy. asiaticum, Rh. turanicus, D. reticulatus and Ha. erinacei, clustered together with conspecific ticks from XUAR. Nevertheless, different mitochondrial lineages were also shown to exist within some of these tick species (e.g. for Hy. asiaticum). The most likely reasons why specimens of diverse geographical origin shared common clades in the phylogenetic tree are as follows.

First, international trade of livestock and their products (e.g. skin and fur) may represent a historical link underlying genetic connection between tick populations in China and Kazakhstan. In this study, Hy. asiaticum in Almaty Oblast (MN892553), Kyzylorda (MN961479) and Yuli County of XUAR (KF527400) constituted one, ancestral haplogroup (H-2), while the H-1 haplogroup of Hy. asiaticum in South Kazakhstan (MN865123) originated from the H-3 haplogroup in XUAR (MK610453, MK307807 and MK213071). In addition, the network diagram of haplotypes indicated that Rh. turanicus in Alataw city (the largest economic port of XUAR), represents an ancestral lineage, which gave rise to three lineages. In particular, in the western direction it evolved as the H-1 haplogroup in Almaty (MN841462), Kazakhstan, whereas towards the eastern and southern directions it evolved as the H-3 haplogroup including ticks from Usu city (MF002580), Ulugqat County (MF002581) and Baicheng County (MF002579) in XUAR. Second, movements of wildlife and migratory birds are well-documented in these regions [20, 21], which might contribute to common genetic lineages of tick species. For instance, Ha. erinacei from hedgehog in Almaty, Kazakhstan and from marbled polecats in Altaw city of XUAR belonged to the same clade, which was well-separated from another phylogenetic group including specimens from Turkey and Romania.

However, the present study also has some limitations. First, tick species of the genus Ixodes were not available in Kazakhstan, and therefore could not be compared with those from XUAR. Second, cox1 sequences of Hy. scupense, Hy. marginatum, and D. reticulatus were available from Kazakhstan, unlike corresponding data from XUAR, due to the scarce distribution of these tick species in the latter region. By themselves, these limitations also indicate that there are some differences in the occurrence of tick species and their abundance between XUAR and Kazakhstan. Therefore, it is an important task for the future to investigate further background factors of these differences, as well as to extend the scope of this study to ticks infesting domestic animals and wildlife in further countries neighboring XUAR, i.e. Russia, Kyrgyzstan, Turkmenistan, Uzbekistan, Afghanistan, Pakistan and India.

Abbreviations
XUAR: Xinjiang Uygur Autonomous Region; cox1: cytochrome c oxidase subunit I; Hy. Asiaticum: Hyalomma asiaticum, Hy. marginatum: Hyalomma marginatum; Hy. Scupense: Hyalomma scupense, Hy. Detritum: Hyalomma detritum, Hy. Anatolicum: Hyalomma anatolicum; D. marginatus: Dermacentor marginatus; D. reticulatus: Dermacentor reticulatus, Rh. turanicus: Rhipicephalus turanicus, Rh. Sanguineus: Rhipicephalus sanguineus; Ha. Erinacei: Haemaphysalis erinacei; ML: Maximum Likelihood; MJ: Median-joining.

Declarations

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Authors’ contributions

YY, TJ and YW conceived and designed the study, and wrote the manuscript. YY, TJ, RH, YM, SC, LG, HW, XB, RK and GK performed the experiments, and analyzed the data. SH critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article. The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Figures**
Figure 1

A map of the study area. Left: Distribution of ticks in Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR) in northwestern China. Right: tick species represented with different colors. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
**Figure 2**

Phylogenetic analysis of tick species collected in Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR) in northwestern China. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000) based on cox1 sequences with MEGA7.0. Sequences of the tick species from Kazakhstan and XUAR of China obtained in this study are indicated by solid triangle (▲) and hollow triangle (△), respectively.

**Supplementary Files**

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