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Short Communication

Molecular Identification of Hemoprotozoan Parasites in Camels (*Camelus dromedarius*) of Iran

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Abstract

Background: Although camels represent a valuable source of food, wool and hide in many countries, in-depth information about their vector-borne pathogens is scarce compared to other animals. The aim of the current study was to characterize vector-borne protozoa in the blood of dromedaries from Iran by molecular tools.

Methods: From June to July 2014, 200 peripheral blood samples were collected from asymptomatic one-humped camels in two provinces of Kerman and Sistan- va-Baloochestan in central and southeastern Iran. Microscopic examination was performed on Giemsa-stained blood smears, and drops of blood were spotted on Whatman FTA® cards for further analyses. Genomic DNA was extracted from the cards, and PCR was carried out for the detection of piroplasms and trypanosomes, followed by sequence analysis of positive samples.

Results: One sample was positive *Trypanosoma* spp. trypomastigotes in light microscopy. PCR results revealed one positive sample each with *Theileria annulata* and *Trypanosoma evansi*.

Conclusion: Camels were identified as hosts for bovine Mediterranean theileriosis in the investigated area. The presence of *Tr. evansi*, the causative agent of surra disease, was also confirmed in camels of Iran. Further studies are recommended in order to investigate their impact on the health and productivity of camels and other livestock in this region.

Introduction

One-humped and two-humped camels have a global population of over 27 million animals (1). The on-going desertification of the earth emphasizes the socio-economic role of camels as farm animals in the arid parts of the world and the need for optimized management and appropriate disease control.

Few reports have been published concerning tick-borne pathogens such as members of the Piroplasmida in this host. So far, DNA of several species of the genera *Theileria* and *Babesia* have been detected from peripheral blood of apparently healthy camels (2–6). In Iran, the most prominent hard tick species infesting camels is *Hyalomma dromedarii* which is presumed to be a vector for camel piroplasms (7,8). Heavy tick infestations of Iranian camels, as well as the presence of parasites in blood smears of camels at considerable rates (15.79%) in a previous study (9) encouraged us to seek more detailed information regarding piroplasms in this host.

Trypanosoma evansi can affect a wide range of mammals and even some birds (10), and several reports of human disease caused by *Tr. evansi* are published as well (11). *Tr. evansi* is the most pathogenic and economically important protozoan parasite of camels with up to 43% morbidity and around 3% mortality. The acute form of the disease is almost always fatal within a few weeks, while the more common chronic form is manifested by anemia, emaciation, recurrent fever, edema, conjunctivitis, lacrimation, enlargement of the lymph nodes and abortions (12). No vaccine is available, and treatment with melarsomine is recommended (13). Genetic variations of *Tr. evansi* in camels are reported from different parts of the world.

According to official estimations, around 162,000 camels live in Iran (14). Given the growing scientific and public health interest in camels, we investigated the prevalence of vector-borne hemoparasites by means of molecular genetic identification in domestic dromedary camels from Iran to get a deeper insight into the spectrum of pathogens in this host population.

Materials and Methods

Study area and sampling

From June to July 2014, 200 clinically healthy one-humped dromedaries (*C. dromedarius*) of both sexes (36 females and 164 males) aged between one and nine years were sampled. All camels were kept by local farmers in two provinces of Kerman and Sistan-va-Baloochestan in central and southeastern Iran. The mentioned provinces, chosen for sampling, host almost half of the camels in Iran (14). In a previous study, blood of these animals was examined for the presence of filarioid helminths. *Dipetalonema evansi* was detected in 16 out of 200 samples using PCR and sequencing (15).

Microscopic examinations

Thin blood smears were prepared from each sample, and stained with Giemsa for light microscopic examination for hemoparasites.

DNA isolation and PCR assay

Genomic DNA was extracted as described previously (15). For screening of piroplasms, the primer pair BTH targeting 18S rRNA gene of *Babesia*, *Theileria* and *Hepatozoon* spp. (16) was used. In the case of electropherograms' superimposed signals, additional PCR reactions with nested BAB G primers (17) were performed. The approximate size and range of product size of *Theileria* in the case of BTH primers was 700 bp (698-703 bp) and for BAB G was 570 bp (559-584 bp). The group-specific primers for detection of *Trypanosoma* were designed based on complete 18S rRNA sequences and target species of the genus

Trypanosoma in general. Primer pair TrypUni18SF (5'- GCG AAA CGC CAA GCT AAT AC -3') and TrypEva18SR (5'- ACG GCA CAA AAC TAC GTG -3') could amplify a 540-545 bp fragment of the 18S rRNA gene. Primers were customized and tested for primer dimers using AmplifX® v.1.7.0 (http://crn2m.univ-mrs.fr/pub/amplifx-dist). Primer concentrations were 10 pmol/µl in a final volume of 25 µl. Amplifications were performed in 40 cycles with annealing temperature of 57 °C. All PCR reactions were performed using the GoTaq G2® Polymerase (Promega, Wisconsin, USA) using an Eppendorf Mastercycler Pro® (Eppendorf, Hamburg, Germany). The amplified products were visualized by electrophoresis on 1.8% agarose gels stained with Midori-Green Advance® (Biozym, Hessisch Oldendorf, Germany).

DNA sequencing and sequence analysis

Purification and sequencing of PCR products (both directions) were performed at LGC Genomics (Berlin, Germany). Sequence reads were analyzed using BioEdit® Sequence Alignment Editor (18) and curated manually; all primer sequences were removed from the alignments. BLAST searches were performed in the NCBI database (http://www.ncbi.nlm.nih.gov/) for similarity of the sequences obtained in the present study.

Ethical considerations

Samples from Kerman Province were obtained from slaughtered camels, and samples from Sistan-va-Baloochestan Province were taken from live animals with official permission and under supervision of the Provincial Veterinary Organization in accordance with the veterinary laws of .Iran.

Results

Microscopical examination

Tr. evansi was detected in one sample by light microscopy.

Piroplasms

One positive PCR sample was identified as *Th. annulata* (GenBank® accession number: KR184819) with 100% identity to *Th. annulata* isolates from cattle in Iran (HM628581, HM535613, KF429793 – KF429795, KF429799 – KF429800) and cattle and sheep in Iraq (HM628582, KC778785 – KC778786), as well as to isolates from other origins in Asia.

Trypanosoma *spp.*

The camel positive for *Tr. evansi* by microscopy was infected with a different genotype (accession no. KR184820) than previously reported from Iran (JN896754 – JN896755).

No mixed infections were detected in our study. *Theileria*- and *Trypanosoma*-positive samples were collected only from Kerman City (Fig. 1).

Discussion

In the present study, microscopy and molecular techniques were used for examination of healthy Iranian dromedary camels' blood for protozoan parasites (piroplasms and trypanosomes).

One animal was infected with *Th. annulata*. Two *Theileria* species, *Theileria camelensis* and *Theileria dromedarii,* have been reported from camel-breeding areas in the last decades (19,20). Although there are observations of macro- and microschizonts in blood smears of camels (9, 21) and developmental stages of *Theileria* in lymph nodes (22), the taxonomic status of these agents remains unclear due to lack of experimental infections and molecular characterization. The recent reports of theileriosis outbreaks (23) with the typical clinical pictures are in sharp contrast to the common belief that infections with piroplasms in camels are subclinical and thus have only minor economic importance.

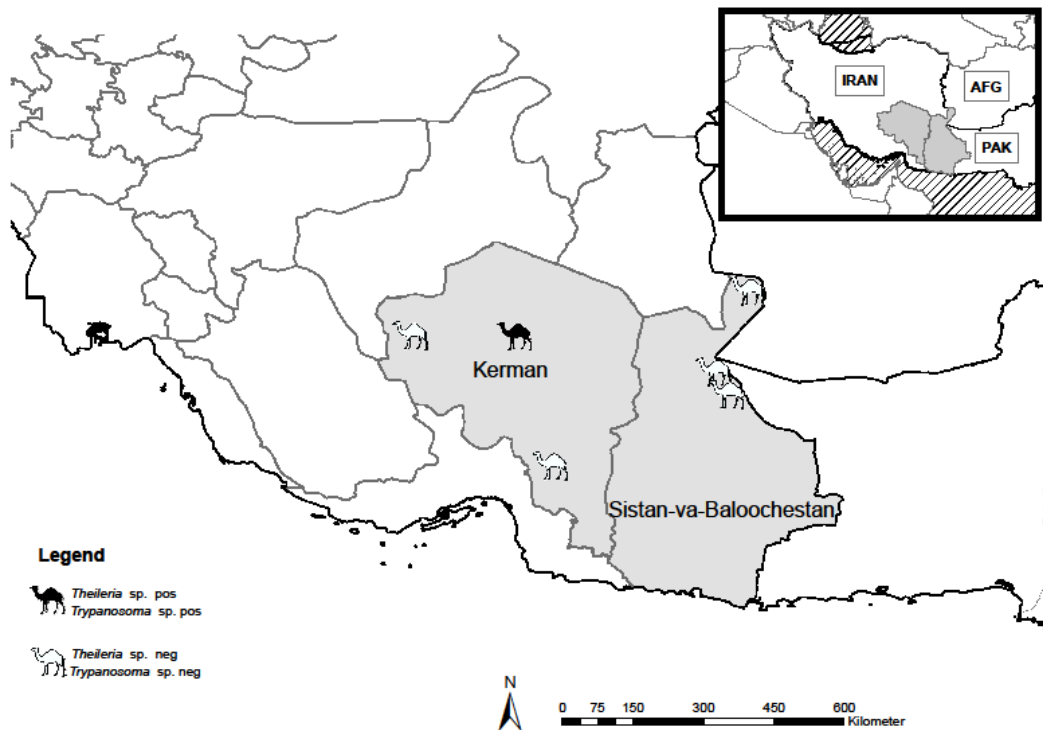

Fig.1: Map of Iran showing sampling sites in Kerman (Shahr-e-Babak, Kerman, and Kahnoodj cities) and Sistan-va-Baloochestan provinces (Zabol, Zahedan, and Mirdjaveh cities). The map was drawn by using Arcinfo (ESRI®Arcmap™10.0, Redlands, CA, USA) and DIVA- GIS (http://www.diva-gis.org/ Data)

One report from Iran describes the successful treatment with buparvaquone in camels with *Theileria* organisms in the blood smear and the typical signs of bovine *Th. annulata* infection (24). So far DNA of *Th. equi*, *Th. mutans*, *Th. annulata*, and *Theileria* spp. have been detected from peripheral blood of apparently healthy camels (2–4). In the only PCR-sequencing based study from Iran *Th. equi* was confirmed in the blood of 7 out of 161 (4.3%) randomly tested camels (6). Our finding of *Th. annulata* (of bovine origin) in south-eastern Iran where camels commonly share pastures with ruminants is similar to the finding of *Th. equi* (of equine origin) in Jordan, where camels live in direct or indirect contact with horses (2). Therefore, it seems that the pathogens have been transmitted to camels via shared ticks. Further studies are necessary to expand the current understanding of the capability of camels to harbor different piroplasms.

Tr. evansi was detected in one animal by both microscopy and PCR (0.5%). Prevalence rates between 0 and 19.47% for *Trypanosoma* infections have been reported for camels in Iran (25,26). These findings may be attributed to differences in the study population, such as host age, length of seasonal migration and season of sampling (27). Since Iran does not lie within the tsetse belt, trypomastigotes in camels have usually been assigned to *Tr. evansi* according to their morphological and morphometrical features upon microscopic examinations (28,29). There is only one sequence-confirmed study on camel trypanosomosis from Iran. PCR and phylogenetic analysis of a limited number of microscopically positive samples from camels in Iran showed that the detected trypanosomes had a close homology to cattle isolates from Thailand (30).

Molecular detection of hemoparasites using filter papers was more sensitive than light mi-

croscopy, as PCR could detect single positive samples for *Tr. evansi* and *Th. annulata*, while only one sample was positive upon microscopic examination (*Tr. evansi*) which was also positive in PCR.

Conclusion

In the present study, Iranian one-humped camels could be described as hosts for *Th. annulata*, and they were confirmed as hosts for *Tr. evansi* by molecular analysis. This adds to our current knowledge on vector-borne diseases of camels in the Middle East. Further studies on the distribution and the clinical importance of piroplasmosis in camels will shed light on the disease burden and reservoir role of this domestic animal species.

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