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Short communication

Quest for the piroplasms in camels: Identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR

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ABSTRACT

DNA of two species of piroplasmids was detected in dromedaries during a survey of blood protozoans in Jordan between 2007 and 2009. Ten clinically healthy camels (10%) originating from three Jordanian districts were found, using a PCR assay, to harbor *Theileria* or *Babesia* species in their blood and no mix infection was determined. Analysis of the partial 18S rRNA gene sequences of these parasites allowed their unambiguous identification as equine piroplasmids *Babesia caballi* ($n = 6$) and *Theileria equi* ($n = 4$). In case of latter species, a novel genotype was found in horses. This first molecular-based species determination of piroplasmids from camels further contributes to the growing evidence of low host specificity of piroplasmids.

1. Introduction

The one humped camel, *Camelus dromedarius*, is physiologically and anatomically adapted to survive harsh conditions. As such, it is a widely distributed domestic animal in arid and semi-arid regions of Africa, Arabia and Western Asia up to India. The highest numbers have been reported from Somalia and Sudan, with 6 and 3.5 millions animals, respectively (Wernery and Kaaden, 2002). Camels are resistant to several devastating infections threatening other livestock species (e.g. contagious pleuropneumonia and foot and mouth disease caused by a non-enveloped *Aphtovirus* of the family Picornaviridae), however, several other infections play an important role in camel husbandry (Dirie and Abdurahman, 2003).

Piroplasmids belonging to the genera *Babesia* and *Theileria* are suspected of infecting dromedaries (Yakimoff et al., 1917; Rao et al., 1988; Egbe-Nwiyi, 1994), but data published so far are limited or unreliable. These tick-borne apicomplexans were generally considered as highly specific for a given host species (Uilenberg, 2006). However, the specificity of piroplasms is probably lower than expected, which is supported by the detection of canine piroplasms in horses or equine *Theileria equi* and *Babesia caballi* in dogs (Criado-Fornelio et al., 2003; Beck et al., 2009; Fritz, 2010), as well as by the infections of humans by *Babesia microti*, *B. microti*-like and *Babesia divergens*, which usually infect cattle (Wei et al., 2001; Zintl et al., 2003; Hildebrandt et al., 2007).

Despite numerous studies addressing the taxonomy and host specificity of piroplasmids in domestic animals, the

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Table 1
PCR detection of piroplasmids in camels and their further determination by sequencing. Sequences accession number in the GenBank™ included.

Locality	Coordinates	Total number	PCR positive	*B. caballi*	*T. equi*	Accession number
Suwaymah	31°46′N, 35°36′ E	24	2	1	1	JN596980 and JN596981
Baptism	31°50′N, 35°32′E	7	1	–	1	JN596982
Al Karamah	31°57′N, 35°35′E	6	–	–	–	–
Shuna	31°50′N, 35°37′E	19	–	–	–	–
Ghor Al Safi	31°00′N, 35°30′E	15	4	4	–	JN596975–JN596979
Ar Rishah	31°33′N, 35°36′E	1	1	–	1	JN596983
Azraq	31°50′N, 36°49′E	14	1	1	–	JN596979
Hazim	31°34′N, 37°14′E	10	1	–	1	JN596984
Bayir	30°46′N, 36°41′E	1	–	–	–	–
Um Sayhoon	30°20′N, 35°27′E	3	–		–	–
Total		100	10	6	4	

question of the identification of *Theileria* and/or *Babesia* species in dromedaries remains unresolved. Piroplasmids reported so far from dromedaries can be either specific for these hosts, or they may represent species known from other hosts that have been transmitted to camels via shared ticks. In order to distinguish between these scenarios, we have surveyed Jordanian camels and identified the piroplasmid species based on their 18S rRNA gene sequences.

2. Materials and methods

2.1. Study site and sampling

The study was conducted in Jordan in the time period between 2007 and 2009. In total, 100 camels were sampled from several localities within four districts: (i) Shuna Al Janubiya (Suwaymah, Baptism, Al Karamah and Shuna), (ii) Wadi Musa (Um Sayhoon), (iii) Wadi Araba (Ghor Al Safi and Ar Rishah) and (iv) Eastern Desert (Azraq, Hazim and Bayir) (Fig. 1). Anamnestic data for each animal were obtained using bilingual questionnaires. Blood was collected by the puncture of jugular vein using plastic containers (Hemos H-01, Gama Group, Czech Republic) equipped with 18G needles. Two thin blood smears were made from each sample; smears were methanol-fixed in the laboratory within the same day. Blood for DNA analysis was fixed in either absolute ethanol or in a solution containing 10 mM EDTA and 0.5% SDS, and analyzed further upon transportation to the laboratory.

2.2. Blood smears, processing of DNA, and PCR assay

Blood smears were stained with Giemsa solution (Merck, Germany) and examined using light microscopy (Olympus AX 70); each slide was examined for 30 min. Total DNA was extracted from blood using DNAesay blood and tissue Kit (Qiagen, Germany) and used for PCR amplification. The primers TB-F (5′-CTTCAGCACCTTGAGAGAAAT-3′) and TB-R (5′-TCDATCCCCRWCACGATGCRBAC-3′), which amplify ~500 bp-long region of the 18S rRNA gene of apicomplexans such as *Theileria*, *Babesia* and *Hepatozoon* spp. were used in a PCR program described elsewhere (Sloboda et al., 2011). PCRs were conducted in a total volume of 25 μl, composed of 12.5 μl of commercial Master Mix (Top-Bio, Slovakia), 10 μM of each primer, ~25 ng of genomic DNA and sterile water. Genomic DNAs isolated from the blood of horses infected with *T. equi* and/or *B. caballi* were used as positive control. DNA isolated from the blood of a piroplasmids-free horse maintained at the clinic was used as a negative control. The amplified PCR products were subjected to electrophoresis stained with Gold-View agarose gel (1.5%), and visualized and documented using Vilber Lourmat system (France).

2.3. Comparative material from horses

Blood samples from 218 horses (data will be published elsewhere) were obtained and processed as described above. Out of these, only five samples of horses originating from Shuna Al-Janubiya tested PCR positive for *T. equi* and were used herein for comparison.

2.4. Sequencing and phylogenetic analysis

PCR products were purified using Qiaquick gel extraction kit (Qiagen, Germany) and commercially sequenced by Macrogen (South Korea). Partial 454 bp-long 18S rRNA sequences from 16 PCR-positive hosts were deposited in the GenBank™ under the accession numbers JN596975–JN596990. Sequences were aligned separately for each species with homologues available in the GenBank™ using Kalign (Lassmann and Sonnhammer, 2005) and consequently manually edited in Bioedit (Hall, 1999). Phylogenetic trees were constructed by maximum likelihood (ML) with GTR model for nucleotide substitutions and discrete gamma distribution in four categories (PhyML; Guindon and Gascuel, 2003) and maximum parsimony (MP) (as implemented in PAUP* 4b 10 (Swofford, 2002)).

3. Results and discussion

Clinical examination of camels revealed various health disorders (mange, mastitis and hair-loss); however, no clinical signs usually associated with piroplasmosis were observed. Neither *Babesia* nor *Theileria* stages were detected in the blood cells of camels during the microscopic examination, blood smears from horses chosen for comparison were also negative for any developmental stages of these parasites.

The PCR with the primers TB-F and TB-R amplified target gene of piroplasmids from the blood of 10 camels from six localities (Table 1). All PCR products of expected size

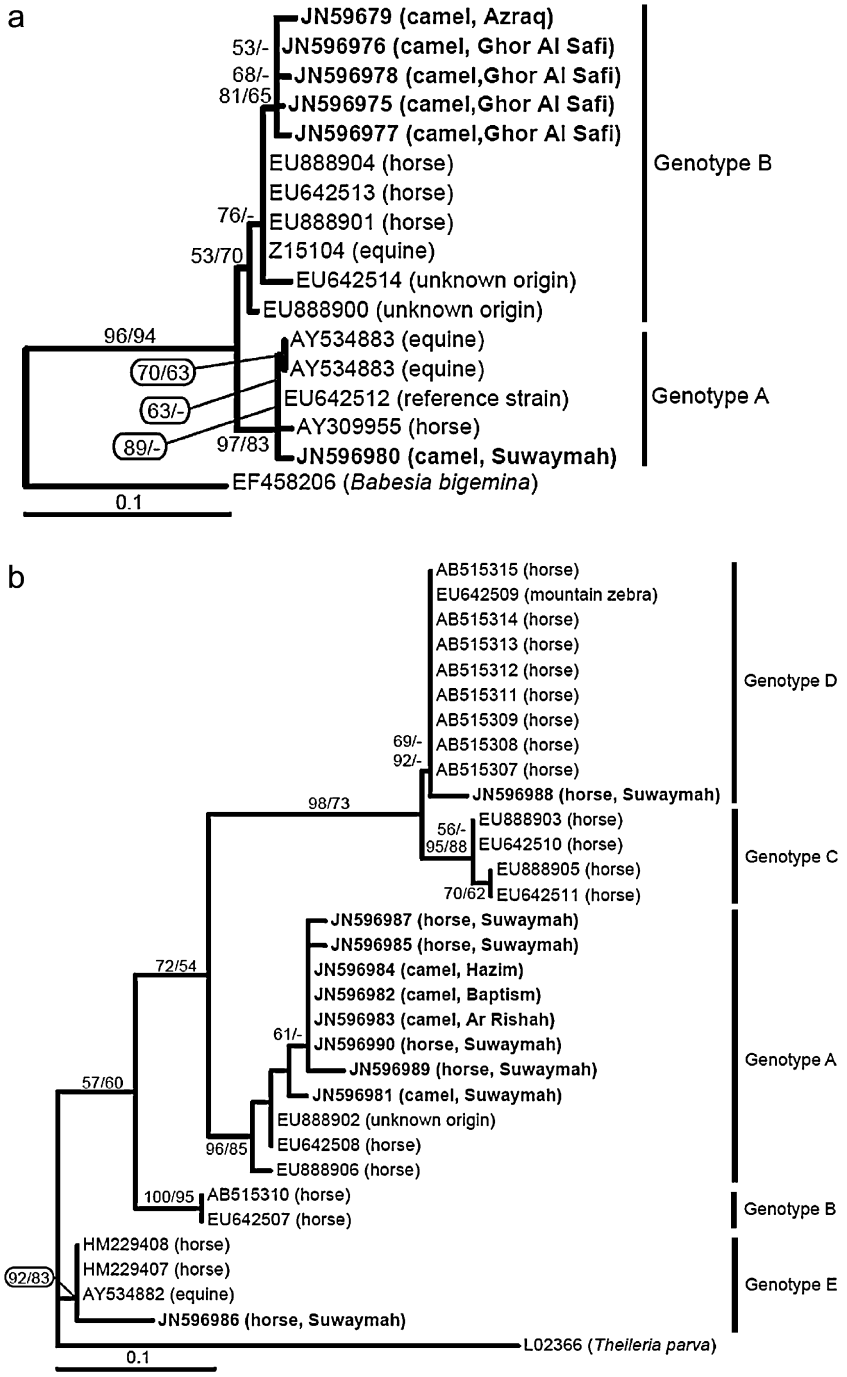

Fig. 1. Phylogenetic tree of (a) *B. caballi* (454 nt) and (b) *T. equi* (449 nt) as inferred from partial sequence of the 18S rDNA gene. The numbers above branches indicates maximum likelihood/maximum parsimony bootstrap supports (500/1000 replicates). Sequences in bold refer to those obtained in our study; host and localities are mentioned in brackets.

were subjected to sequencing. Comparisons of the obtained sequences with the most closely related sequences available in the GenBank™ database are shown in Table 2. In six camels from three localities (Suwaymah, Ghor Al Safi and Azraq) the piroplasmids were identified as *B. caballi*, a further four camels from four localities (Suwaymah, Baptism, Ar Rishah and Hazim) were positive for *T. equi*.

Five *B. caballi* sequences (Fig. 1a) belong to *B. caballi* genotype B, whereas a single isolate was identified as a genotype A. All four *T. equi* sequences from camels, together with four sequences retrieved from horses, belong to *T. equi* group genotype A (Fig. 1b). However, comparative analysis of sequences obtained from horses (accession nos. JN596985–JN596990) indicates even wider diversity in

Table 2
Similarity of sequences detected in the study with sequences of piroplasmids in GenBank™ using Blast searching tool; in all cases, 99% similarity was revealed.

Sample (animal)	Pathogen	Closest hit (accession number/geographic origin)
JN596981–JN596985	*T. equi*	(EU642508/South Africa) and (Z15105/Spain)
JN596986	*T. equi*	(HM229408/South Korea)
JN596987	*T. equi*	(EU642508/South Africa)
JN596988	*T. equi*	(AB515315/Sudan)
JN596989	*T. equi*	(EU888906/South Africa)
JN596990	*T. equi*	(EU642508/South Africa)
JN596975–JN596979	*B. caballi*	(EU642513/South Africa) and (Z15104/Spain)
JN596980	*B. caballi*	(EU642512/South Africa) and (AY534883/Spain)

these hosts. We identified a single sequence from horse clustering within the genotype D and a sequence that clusters together with HM229408, HM229407 and AY534882 from South Korea and Spain, respectively, forming a new separate clade, referred here as a genotype E.

Historically, the taxonomy of piroplasms of dromedaries is rather uncertain. Two species, namely *Theileria camelensis* Yakimoff, Schokhor, Kosel-Kine, 1917 and *T. dromedarii* Rao, Mishra, Sharma, Kalicharan, Prasad, 1988 were described (Yakimoff et al., 1917; Rao et al., 1988). However, the validity of both taxa is questionable. The description of *T. camelensis* lacks any information on developmental stages (Boid et al., 1985) and proper taxonomic description of *T. dromedarii* reported in India (Rao et al., 1988) is also missing. In addition, the single and unconvincing report of a *Babesia*-like infection in camels (Egbe-Nwiyi, 1994), again failed to provide any description of life cycle stages of the parasite.

Undoubtedly, the application of molecular diagnostic methods has promptly improved our understanding of the diversity of piroplasms in terms of increased diagnostic sensitivity, determination of new or cryptic species, and understanding the intraspecific genetic diversity (Criado-Fornelio et al., 2003). Our study represents the first detection and consequent identification of piroplasms in dromedaries by PCR and sequencing. The sequences obtained unequivocally confirmed the presence of *B. caballi* and *T. equi* in these hosts which provokes questions regarding the status of piroplasmids taxa previously described from dromedaries. Interestingly, it was already Wenyon (1926) who had suggested the synonymy of *T. camelensis* with *Nuttallia* (nowadays *Theileria*) *equi*.

In our study, we examined camels from 10 localities and found the piroplasmid infection in six of them. To prove or exclude the circulation of piroplasms between camels and horses, we sampled limited number of horses from Suwaymah and sequenced 6 randomly selected amplicons from horses from the same locality. Regardless of the low number of horses involved, we obtained sequences of three different genotypes of *T. equi*. In contrast, *T. equi* reported from the camels in our study invariably belong to the genotype A. Besides the camels, *T. equi* of the genotype A was so far detected in dogs in Croatia (Beck et al., 2009) and, recently, also in dogs in Jordan (Qablan et al., in press), which indicate that dogs might contribute to the circulation of the infection, as well as suggests the potential of this genotype to infect wider spectrum of hosts. One of the *T. equi* sequences retrieved from horses clustered with three sequences from GenBank™ into a distantly separate clade, herein referred to as a new genotype E. Evidently, this clade appeared as a result of splitting of genotype B from previous studies (Bhoora et al., 2009) after the addition of three new sequences (this study and two sequences from South Korea).

Analysis of *B. caballi* sequences placed five out of six of those obtained from camels into genotype B, which shows a high degree of diversity similar to previous studies (Bhoora et al., 2009). The sixth sequence of *B. caballi* from camels belongs to genotype A, which is the same genotype that was identified in two previous reports from canines from Croatia and Jordan, respectively (Beck et al., 2009; Qablan et al., in press).

In three out of four localities where we proved the presence of piroplasmids, the camels live in direct or indirect contact with horses. Detection of piroplasmid infections in dromedaries from the heart of the Eastern desert where the equids are absent can possibly be attributed to the animal migration or to the within-herd circulation (or both). Which tick species are responsible for the transmission of the diseases to and/or between camels' remains to be established. In fact, several tick species in the area studied (*Hyalomma anatolicum*, *Boophilus annulatus*, and *R. sanguineus*) infest both camel and horses (Saliba et al., 1990; El-Rabie et al., 1990; Walker et al., 2000) and probably play a role in the transmission of piroplasmid infections. Furthermore, *Hyalomma dromedarii* is known to infest domestic animals other than camelids (Apanaskevich et al., 2008), yet this species was so far not reported from equids from Jordan.

This study brings novel data about the host specificity and genetic diversity of piroplasmids and proved the presence of equine piroplasmids in camels in Jordan. Further studies from other geographic regions, where the dromedaries are kept, are necessary to address the possibility that dromedaries are capable of hosting other host-specific piroplasms. Moreover, studies focusing at possible clinical impact of the disease are necessary.

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