



Vector-borne bacteria in blood of camels in Iran: New data and literature review



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ABSTRACT

Despite close association between camels and humans, molecular based studies on vector-borne pathogens infecting camels are scarce compared to other animals in Iran. The current study was carried out to investigate the occurrence of vector-borne bacteria in the blood of dromedaries by molecular tools. A total of 200 peripheral blood samples were collected from apparently healthy animals. Microscopic examination was performed on Giemsa-stained blood smears, and drops of blood were spotted on Whatman FTA[®] cards for molecular analyses. Genomic DNA was extracted from the cards, and PCR amplification followed by sequencing of positive samples was carried out for the detection of Anaplasmataceae, spotted fever group (SFG) rickettsiae, *Bartonella* spp. and *Borrelia* spp. Intra-cytic forms of any blood pathogens could not be detected by light microscopy. PCR results revealed 30 animals (15%) to be infected with Anaplasmataceae bacteria. Analyses of sequences revealed a strain of *Anaplasma* sp. identical to *Candidatus* *Anaplasma camelii* isolated from camels, cattle and deer in Asia and Africa. Neither SFG rickettsiae, nor *Borrelia* or *Bartonella* species were found. Further studies for determining epidemiological role of camels and its zoonotic potential are recommended. This paper reviews the current knowledge on camels' tickborne bacteria including microscopy, serology and molecular studies.

1. Introduction

Camels are susceptible to a wide range of pathogenic microorganisms and they may act as carriers or reservoirs for several animal and zoonotic diseases [1]. However, apart from camel brucellosis and the Middle East respiratory syndrome (MERS), reports of camel-to-human transmission of zoonotic agents are either anecdotal or unsubstantiated.

Human and animal infections with members of the Anaplasmataceae family are increasingly recognized as important, in part emerging and potentially fatal arthropod-transmitted diseases for humans and animals. From the genus *Anaplasma* to date *Anaplasma phagocytophilum*, *A. platys*, *A. ovis* and *A. capra* have been recognized to infect humans [2–5]. In camels *Anaplasma* organisms have been reported in blood smears by light microscopy [6,7] and antibodies against them in blood serum [8,9]. However, so far the only *Anaplasma* species

confirmed by DNA sequencing in camels are *Candidatus* *Anaplasma camelii* (genetically close to *A. platys*), *A. phagocytophilum* and *A. ovis* [10–18]. Clinical signs of natural infections are described as fever, pale and icteric conjunctiva suggestive of anaemia, dullness, anorexia, diarrhoea, loss of appetite, emaciation, coughing, lacrimation, rough hair coat, abortion, and/or infertility [7,19]. Intramuscular administration of oxytetracycline at 20 mg/kg as specific therapy and injectable B-complex, iron, folic acid and hydroxycobalamin as supportive therapy is the recommended therapeutic regimen [7,19]. Infection with and antibodies against several species of *Rickettsia* [20,21], as well as infection with *Bartonella* [22] and *Borrelia* [23,24] have been also documented.

According to official estimates, around 183,900 camels live in Iran [25]. Given the growing scientific and public health interest in camels, we investigated the occurrence of selected vector-borne bacteria;

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Anaplasmataceae, spotted fever group (SFG) rickettsiae, *Bartonella* spp. and *Borrelia* spp. in domestic dromedary camels from Iran to get a deeper insight into the spectrum of pathogens circulating in this host population. We also review the current knowledge on camel tick-borne bacteria including microscopical, serological and molecular studies.

2. Materials and methods

2.1. Sampling and microscopy examinations

Totally 200 clinically healthy one-humped dromedaries (*Camelus dromedarius*) of both sexes (36 females and 164 males), aged between one and nine years were sampled from June to July 2014 in central and south-eastern Iran from six different locations. In previous studies, blood of these animals was examined for the presence of filarioid helminths, piroplasms and trypanosomes. *Deraiphoronema evansi* was detected in 16 out of 200 samples and one positive sample each with *Theileria annulata* and *Trypanosoma evansi* using PCR and sequencing were found. For details on the study population and method of sampling see Sazmand et al. [26,27]. Thin blood smears were prepared from each sample, and stained with Giemsa for light microscopic examination.

2.2. DNA extraction, PCRs and DNA sequencing

Genomic DNA was extracted from blood spots on FTA® cards (5 mm²) with the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommended protocol.

We performed PCRs targeting a 345 bp fragment of the 16S rDNA using the primers EHR16SD (5'-GGTACCACAGAAAGTCC-3') and EHR16SR (5'-TAGCACTCATCGTTTACAGC-3') [28]. For the phylogenetic network analyses, we sequenced a 1012 bp fragment of the 16S rDNA using the newly designed primers Ana16SF (5'-GCAGACGGGTGAGTAATGCATAG-3') and Ana16SR (5'-CTTGACATCATCCCCACCTT CCT-3'). Annealing temperatures were 53 °C and 56 °C in the latter two PCRs, respectively. Screening for *Rickettsia* spp. was carried out with the primers ITS-F (5'-GATAGGTCGGGTGTGGAAG-3') and ITS-R (5'-TCGGGATGG GATCGTGTG-3'), amplifying the ca. 360 bp long intergenic spacer region between the 23S and 5S rDNAs, at 52 °C annealing temperature [29]. The samples were also screened for *Borrelia burgdorferi* s.l. using the primers P1 (5'-ACGCTGGCAGTGGCTCT TAA-3') and P2 (5'-CTGATATCAACAGATTCCACCC-3') targeting ab 650 bp section of the 16S rDNA, at 64 °C annealing temperature [30]. For the detection of *Bartonella* spp. a hypervariable intergenic transcribed spacer 16S-23S rRNA (ITS) was targeted using the primers Bart/16-23 F (5'-TTGATAAGCGTGAGGTGGAGG-3') and Bart/16-23R (5'-CAAAGCAGGTGCTCTCCAG-3'), at 64 °C annealing temperature [31].

All PCRs were performed with the GoTaq® G2 Polymerase (Promega, Madison, Wisconsin, USA) and started with an initial denaturation for 2 min at 94 °C, followed by 35 cycles with 30 s at 94 °C, 30 s at the respective annealing temperatures (see above), 1 min at 72 °C, and a final extension for 10 min at 72 °C. PCR-products were sent to Microsynth Austria GmbH (Vienna, Austria) for purification and sequencing in both directions using the PCR primers.

2.3. Sequence analyses

The raw forward and reverse sequences (and electropherograms) were carefully analyzed in Bioedit 7.0.8.0 [32]. In order to visualize the relation between *Anaplasma* lineages, we calculated a Median Joining network with the 16S rDNA sequences (1012 bp) of *Anaplasma*-positive samples and data published on NCBI GenBank®. Calculations were performed with Network v.4.6.0.0 (Fluxus Technology Ltd., Suffolk, UK) applying the default settings. The network was post-processed with the MP (Maximum parsimony) option in order to reduce unnecessary

median vectors.

2.4. Statistical analyses

Pearsons' X² and Kruskal–Wallis tests were used for the determination of relations between infections and sex or age of the camels with IBM SPSS Statistics 20.0 software. *P* values < 0.05 were considered significant.

2.5. Ethical considerations

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

3. Results

Intracellular forms of blood pathogens could not be detected by light microscopy. However, 30 samples (15%; CI₉₅ = 10–20%) were positive in the PCR assays specifically targeting the 16S rDNAs of Anaplasmataceae. The longer 16S fragment (1012 bp) was sequenced from 21 of the *Anaplasma*-positive samples, whereby all featured identical sequences. By performing a BLAST search in NCBI GenBank® database, we retrieved another 68 *Anaplasma* sequences, which covered the complete 16S rDNA sequence and showed more than 99.5% sequence similarity. Median Joining networks were calculated based on the alignment containing all these 68 sequences and the 21 sequences of the present study (Fig. 1).

The lineage detected in the present study is identical to *Candidatus Anaplasma camelii* isolated from blood and spleen, respectively, of dromedary camels in Saudi Arabia (KF843823–28, [11]), Iran (KX765882 [15]), and Tunisia (KM401906–07 [12]). Moreover, the same lineage was found also in blood of Javanese rusa which is a deer native to the islands of Indonesia and East Timor (*Rusa timorensis*) (MG910989) and cattle (MG910990) in Malaysia [33], and in cattle in Bangladesh (MF576175 [34]). The 1012 bp section of the 16S differs only in one position from sequences of *Anaplasma platys* isolated from dogs worldwide. Moreover, several other genotypes, differing in one or a few positions only, were isolated from dromedaries in Tunisia, and in Bactrian camels, Mongolian gazelles, goats, cattle, and blood fed mosquitoes in China. Several related genotypes, differing in one or several positions, were isolated from blood of goats, Mongolian gazelle, cattle, and blood fed mosquitoes in China, and dogs from the Philippines.

Statistically, there were no significant correlations between PCR positive results and age or sex of the animals. *Candidatus Anaplasma camelii* was present in five out of six study sites with prevalence ranging 5–43.3% in different regions (Table 1). One camel positive for *Anaplasma* spp. in Shahr-e-Babak was co-infected with filaroid *Deraiphoronema evansi* detected in a previous study [27]. The PCR screenings for *Borrelia burgdorferi*, *Bartonella* spp. and *Rickettsia* spp. were all negative. The 16S sequences of *Candidatus Anaplasma camelii* obtained from 21 camels in the present study were deposited in Genbank® (www.ncbi.nlm.nih.gov) under the accession numbers MK726038–MK726058.

4. Discussion

In the present study, microscopy and molecular techniques were employed for examination of clinically healthy Iranian dromedaries' blood for Anaplasmataceae, SFG rickettsiae, *Bartonella* spp. and *Borrelia* spp. We detected a strain of *Anaplasma* sp. identical to *Candidatus Anaplasma camelii* isolated from camels, cattle and deer in Asia and Africa [11,12,15,33,34].

In this study, *Anaplasma* bacteria were not detected in Giemsa-

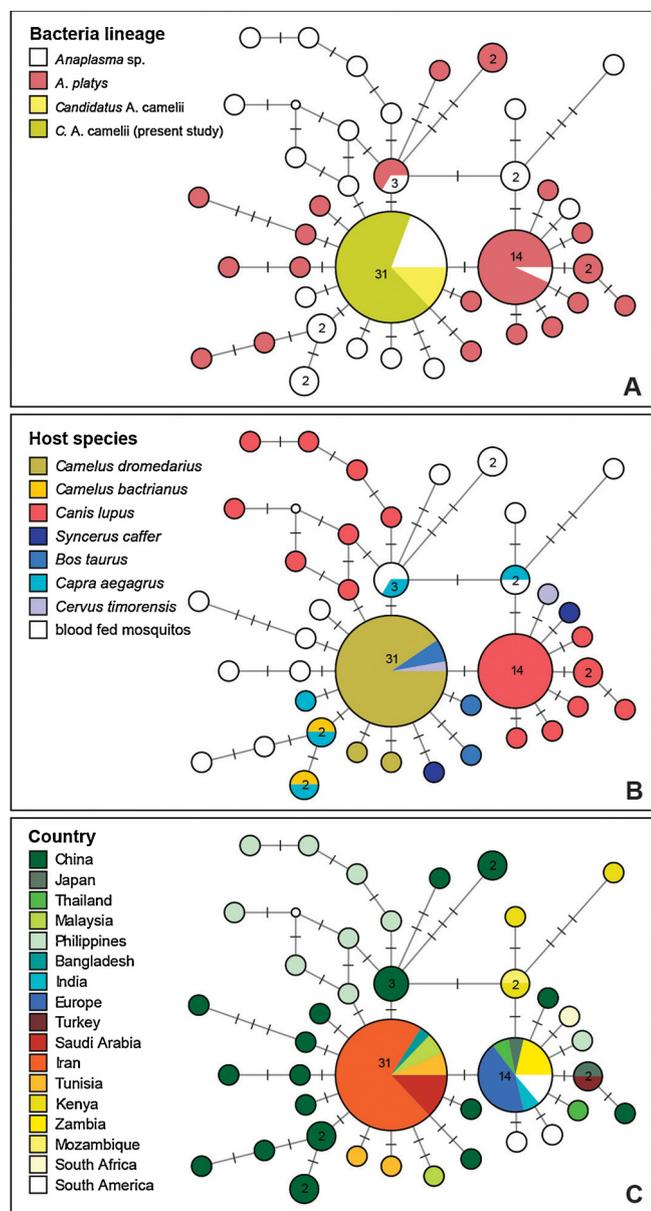


Fig. 1. Median Joining network with 16S sequences (1012 bp) of *Candidatus Anaplasma camelii*, *Anaplasma platys*, and related lineages. The size of the circles corresponds to the number of sequences featuring the same genotype. Bars on branches indicate the number of substitutions between genotypes. In the three figures we indicate (A) the parasite species identified in the original publication, (B) the host species, and (C) the geographic origin of samples.

Table 1
Distribution of *Anaplasma* infection in camels according to sampling sites.

Province	Sampling site	Number of collected samples	Number of infected camels (%)
Kerman	Shahr-e-Bababk	20	3 (15)
	Kerman	60	5 (8.3)
	Kahnooj	20	1 (5)
Sistan-va-Baloochestan	Zabol	30	13 (43.3)
	Zahedan	10	0 (0)
	Mirjaveh	60	8 (13.3)
Total		200	30 (15)

Table 2
Prevalence rate of *Anaplasma* infection in dromedary camels by light microscopical examination.

Country	Region	No. tested	No. positive	% Positive	Reference
Egypt	Kom Hamada	135	15	11.1	[38]
	Matruh	53	8	15.1	[39]
	Matrouh	331	157	47.4	[40]
Iran	Golestan	100	2	2	[17]
	Kerman	200	0	0	This study
	Sistan-va-Baloochestan				
	Khorasan	35	6	17.1	[41]
	Razavi				
	Mashhad	262	0	0	[42]
Iraq	Yazd	114	0	0	[43]
	Najaf	160	44	27.5	[44]
Nigeria	Ninava	62	52	83.9	[6]
	Maiduguri	105	4	3.8	[45]
Saudi Arabia	Maiduguri	202	41	20.3	[46]
	Zaria	85	2	2.3	[47]
	Riyadh	138	32	23.2	[48]
	Riyadh, Makkah	237	72	30.4	[19]

stained blood smears. As presented in Table 2, *Anaplasma* spp. have been detected in erythrocytes of camels by light microscopy examination in Iran, Iraq, Saudi Arabia, India, Nigeria and Egypt with prevalence rates of up to 83.9%. One report also described *Anaplasma* organisms in cytoplasm of monocytes in Omani dromedaries [35]. It is worth noting that haemotropic *Mycoplasma* spp. can also infect dromedaries [36,37], so due to a high possibility of misdiagnosis of *Anaplasma* spp. morulae with other pathogens, application of PCR is highly recommended, as confirmed in the current study.

Camel anaplasmosis with species that have tropism to erythrocytes, monocytes or granulocytes have been studied serologically in the past decades. As summarized in Table 3 seroprevalences of 0–53.85% have been reported. However, since correct serological testing for *Anaplasma/Ehrlichia* infections is hampered by cross-reactivity [49] and the phase of bacteraemia may be shorter than the period of seropositivity, no DNA of *Anaplasma* was detected in serum-positive dromedaries [21].

We detected a strain of *Anaplasma* sp. in 15% of the tested camels which featured 16S sequences identical to *Candidatus Anaplasma camelii* isolated from camels in Saudi Arabia, Tunisia and Iran [11,12,15]. Phylogenetic analyses based on DNA sequencing in our study supports the assumption that the *Candidatus Anaplasma camelii* lineage is genetically divergent from *A. platys* and may present a novel species.

With recent advances in diagnosis using molecular methods, reports of infection with *Anaplasma* spp. with tropism for platelets are increasing in one- and two-humped camels (Table 4). In six out of eight studies *Anaplasma* genotypes confirmed by nucleotide sequencing were genetically related to *A. platys* and Bastos et al. [11] proposed to name this genotype “*Candidatus Anaplasma camelii*”. Phylogenetic analyses in our study supports that *Candidatus Anaplasma camelii* lineage is genetically divergent from *A. platys* and may present a novel species.

In two studies in Iran in 2018, *A. ovis* was confirmed in two dromedaries, which were positive in microscopy [17], and *A. phagocytophilum* was detected in the blood of 34.2% of 207 tested dromedaries in five areas of the country [18]. In a previous study, *Anaplasma* strains from dromedary camels in Iran and Tunisia were consistently placed on a divergent cluster from those found in Bacterian camel in China suggesting that (i) the causative agents of anaplasmosis in two species of *Camelus dromedarius* and *Camelus bactrianus* are independent from each other, (ii) genetic diversity of *Candidatus Anaplasma camelii* is not dependent on the geographical area, and (iii) it is related to host species [15]. Our finding of identical lineages in deer and cattle in Malaysia [33] and cattle in Bangladesh [34], suggest that *Candidatus Anaplasma camelii* is not geographically restricted to the Middle East and North

Table 3
Seroprevalence of *Anaplasma* in camels (*Camelus dromedarius* and *Camelus bactrianus*).

Host	Country	Region	No. tested	No. positive	% Positive	Assay	Target organism	Reference
<i>Camelus dromedarius</i>	Iraq	Najaf, Wasit	120	13	10.8	ELISA ^d	<i>A. marginale</i>	[50]
	Kenya	Ol Jogi	NS ^e	0	0	CF ^f	<i>Anaplasma</i> spp.	[51]
	Nigeria	Kano, Maiduguri, Jos	28	3	10.7	IFAT ^a , CTAT ^b , RCAT ^c	<i>A. marginale</i>	[39]
	Spain	Canary Islands	100	3	3	ELISA ^d	<i>A. marginale</i> , <i>A. ovis</i> , <i>A. centrale</i>	[21]
	Tunisia	Sidi Bouzid, Bouficha, Douz	226	66	29.2	IFAT ^a	<i>A. phagocytophilum</i>	[9]
	United Arab Emirates	Dubai	1119	5	0.4	ELISA ^d	<i>A. marginale</i> , <i>A. ovis</i> , <i>A. centrale</i>	[8]
<i>Camelus bactrianus</i>	Mongolia	Terelj, Dalanjargalan, Sainshand	104	56	53.8	IFAT ^a	<i>A. phagocytophilum</i>	[52]

^a IFAT: indirect fluorescent antibody test.

^b CTAT: capillary-tube agglutination test.

^c RCAT: rapid card agglutination test.

^d ELISA: enzyme-linked immunosorbent assay.

^e NS: not stated.

^f CFT: complement fixation test.

Africa as well as lack of host specificity.

Interestingly most genotypes differing in just one nucleotide were found in cattle, goat or blood-fed mosquitoes in China, but the *Candidatus Anaplasma camelii* genotype was not found in China. However, the largest diversity of similar genotypes was found in Asia (China, Philippines, Malaysia), and the same is the case with *A. platys* which differs only by one nucleotide [10,33,34,53,54]. The occurrence of identical genotypes in Iran, Bangladesh and Malaysia might suggest that *Candidatus Anaplasma camelii* did not originate in camels in the Middle East but in Eastern Asia.

All of the Bactrian and dromedary camels tested for *Anaplasma* spp. by means of PCR were apparently healthy with no obvious signs of anaplasmosis, except for camels investigated by Ait Lbacha et al. in Morocco [14]. However, in an outbreak of disease in dromedaries in Morocco clinical signs of oedema, anorexia, respiratory distress, and sudden death similar to the clinical signs observed in cattle acutely infected with *A. phagocytophilum* were observed [14]. As *Anaplasma* infection might not be the sole cause of the observed clinical signs in camels of the latter study, experimental infections of will clarify the outcome of infection in this species.

The vectors of *Candidatus Anaplasma camelii* are still not known. Camels of Iran are mainly infested with hard ticks of the genus *Hyalomma* [1]. Similarly in Saudi Arabia, Tunisia and Morocco, infection of dromedaries with *Anaplasma platys*-like organisms occurs in dromedaries primarily infested with *Hyalomma* spp. ticks [11,12,14], suggesting their potential role as vectors of *Candidatus Anaplasma camelii*. Furthermore, genotypes similar to *Candidatus Anaplasma camelii* were found in blood-fed mosquitoes in China indicating that mosquitoes might play a role in the transmission and evolution of *Anaplasma* species [53]. Researchers have identified *Anaplasma*, *Ehrlichia*, *Candidatus Neohrlichia*, and *Rickettsia* bacteria in multiple mosquitoes and different life stages (egg, larvae, pupae, and adult) showing that mosquitoes may have played an important role in the transmission and evolution of Rickettsiales [53]. Hence, further studies are needed to investigate the actual vector competences for *Candidatus Anaplasma camelii*.

No *Rickettsia* spp. were detected in the present study that could be due to very short bacteremic period. Similarly, Erbaş et al. could not detect this bacteria in blood samples from 50 camels in Turkey [57]. However, there are two reports of *R. aeschlimannii* and *Rickettsia* sp. DNA in serum and blood samples from dromedary camels [20,21]. Antibodies against *R. prowazekii*, *R. mooseri*, *R. rickettsia*, *R. conorii* and *Rickettsia* sp. have been detected in camel blood serum with prevalences of up to 83% [21,58] and *R. aeschlimannii*, *R. africae*, *R. sibirica mongolitimonae* and *Rickettsia* sp. have been identified in several tick species

collected from camels [20,59–66]. Despite this, there are no reports on diseases in camels caused by these organisms.

Also, *Bartonella* DNA was not detected in the blood of camels in the present study, although in 2014 a novel species, *B. dromedarii*, was isolated from 18% of apparently healthy domesticated dromedaries in Israel [22]. The potential role of *B. dromedarii* as a zoonotic agent is still unknown. Recently, DNA from *B. bovis* and *B. rochalimae* was confirmed in *Hyalomma dromedarii* ticks collected from a single camel in Palestine. None of the 19 blood samples from camels in the latter study area were positive for *Bartonella* spp. [67].

Borrelia spirochetes DNA could not be found in our study which could be due to the fact that the bacteria do not prevail in the blood for longer time periods after infection. In principle, camels are susceptible to infections with *Borrelia* and in a seroprevalence survey on *Borrelia* in humans and domestic animals from Egypt, 47.8% of the camels had serum antibodies against *Borrelia* sp., which was much higher than in buffaloes, cattle, goats or sheep (10.9–23.8%) [68]. In recent years DNA of *Borrelia burgdorferi* sensu lato has been detected in blood of 1.3% (3/232) of tested dromedary camels in Tunisia [23] and 3.6% (5/138) Bactrian camels in China [24]. These findings suggest that camels could play a role in Lyme disease and/or relapsing fever.

5. Conclusion

In the present article we reviewed literature about infection of camels with vector-borne bacteria, in particular Anaplasmataceae, SFG rickettsiae, *Bartonella* spp. and *Borrelia* spp. We also confirmed infection of Iranian one-humped camels with *Candidatus Anaplasma camelii* by molecular analysis. However, further investigations on vectors, hosts, reservoirs, pathogenicity in camels and zoonotic potential of this pathogen are required. Control of tick infestation is highly recommended to reduce infection pressure with tick-borne pathogens in camels. The risk of transmission of infections of camels with Rickettsiales by mosquitoes must be evaluated further.

Conflict of interests

No conflict of interests is declared.

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Table 4
Overview on molecular detection of *Anaplasma* in dromedary and Bactrian camels.

Host	Country	Region	Type of sample	No. tested	No. positive	% Positive	Sequencing of PCR Products	Accession No.	Reference
<i>Camelus dromedarius</i>	Spain	Canary Island	Serum	3	0	0	-	-	[21]
	Egypt	Matrouh	Blood	331	223	67.4	-	-	[40]
	Iran	Golestan	Blood	100	2	2	+	KT601343, KU321250	[17]
		Fars	Blood	100	6	6	+	KX765882	[15]
		Yazd, Zabol, Kerman, Bandar-Abbas, Hoveyze	Blood	207	71	34.3	Five positive samples	-	[18]
	Morocco	Kerman, Sistan-va-Baloochestan	Blood	200	30	15	+	MH794244, MH794245	This study
		Tiznit, Guelmim, Laâyoune, Es-Semara, Boujdour, Oued Ed Dahab	Blood	106	42	39.6	+	KX074079	[14]
		Sokoto	Blood	36	22	61.1	+	KJ832066, KJ832067	[13]
	Nigeria	Mianwali	Blood	150	20	13.3	+	NS ^a	[16]
	Pakistan	Riyadh	Blood	77	0	0	-	-	[55]
	Saudi Arabia	Unizah	Spleen	100	26	26	+	KF843823–KF843828, KJ814955–KJ814959	[11]
		Sidi Bouzid, Bouficha, Douz	Blood	226	40	17.7	+	KM401905–KM401908	[50]
	Tunisia	Dubai	Blood	55	0	0	-	-	[56]
United Arab Emirates									
<i>Camelus bactrianus</i>	China	Xinjiang	Blood	279	20	7.2	+	KP939254, KP939255, KP939257, KP939258, KP939260, KP939261, KR011925, KR011927	[10]

^a NS: not stated.

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