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First Record of a Spotted Fever Group *Rickettsia* sp. and *Theileria annulata* in *Hyalomma dromedarii* (Acari: Ixodidae) Ticks in the United Arab Emirates

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First record of a spotted fever group *Rickettsia* sp. and *Theileria annulata* in *Hyalomma dromedarii* (Acari: Ixodidae) ticks in the United Arab Emirates

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Abstract

Rickettsiosis and theileriosis can cause mortalities in camel populations. This study was conducted to achieve 2 objectives: (1) to detect the presence of SFG *Rickettsia* sp. and *Theileria* sp. in *Hyalomma dromedarii* Koch, 1844 (Acari: Ixodidae) ticks and (2) to determine their prevalence in the tick population on the sampled camel farms in Al-Ain, United Arab Emirates (UAE). Camel ticks (*H. dromedarii*) were collected from a total of 625 one-humped camels (*Camelus dromedarius*) in 22 sampling locations in Al-Ain, UAE. Tick samples were analyzed by Polymerase Chain Reaction (PCR). An SFG *Rickettsia* sp., which was 99% similar to *Candidatus* 'Rickettsia andeanae' and *Rickettsia* endosymbionts, was detected only in 2011 and its prevalence in the sampled ticks was 1.12%, while *Theileria annulata* was detected in both years with a prevalence of 2.3% and 1.60%, respectively. Additionally, *T. annulata* was present in all of the sampling zones (east, west, north, and south) of the study area, whereas SFG *Rickettsia* sp. was limited to 2 zones only (east and south). The geographic distributions of SFG *Rickettsia* sp. and *T. annulata* showed no overlap throughout the entire study area except in one location in which both of the disease agents were present. This study is the first published record on the presence of SFG *Rickettsia* sp. and *T. annulata* in camel ticks in the UAE. In addition, the current study should serve as a foundation for more studies leading to a better understanding of the reservoir potential of camels and the risk posed by these 2 disease agents to camels and other livestock.

Key Words: Camel; disease; PCR; prevalence

Resumen

La Rickettsiosis y theileriosis pueden causar mortalidad en poblaciones de camellos. Hasta la fecha, ningún estudio ha examinado la presencia de estas dos enfermedades asociadas con garrapatas en camellos en los Emiratos Árabes Unidos (EAU). Por lo tanto, se realizó este estudio para lograr dos objetivos: (1) detectar la presencia de la *Rickettsia* GFM y *Theileria* sp. en las garrapatas *Hyalomma dromedarii* y (2) determinar su prevalencia en la población de garrapatas en granjas de camellos muestreadas en Al-Ain, Emiratos Árabes Unidos. Las garrapatas de los camellos (*H. dromedarii*) fueron recolectadas en un total de 625 camellos de una sola joroba (*Camelus dromedarius*) en 22 sitios de muestreo en Al-Ain, Emiratos Árabes Unidos. Se analizaron las muestras de garrapatas por medio de la Reacción en Cadena de la Polimerasa (RCP) para detectar la presencia del Grupo de Fiebre Manchada (GFM) de *Rickettsia* y el ADN de *Theileria annulata*. El GFM de *Rickettsia* sólo se detectó en el 2011 y su prevalencia en las garrapatas muestradas fue de 1.12%, mientras que *T. annulata* se detectó en ambos años, con una prevalencia de 2.3% y 1.60%, respectivamente. Además, *T. annulata* estaba presente en todas las zonas de muestreo (este, oeste, norte y sur) de la zona de estudio, mientras que el GFM de *Rickettsia* se limitó a dos zonas solamente (este y sur). La distribución geográfica del GFM de *Rickettsia* y *T. annulata* mostraron ningúna superposición en todo el área de estudio, excepto en una área en donde ambas enfermedades estaban presentes. Este estudio es el primer registro publicado de la presencia del GFM de *Rickettsia* y *T. annulata* en las garrapatas de camellos en los Emiratos Árabes Unidos. Sus descubrimientos, en especial la presencia del ADN del GFM de *Rickettsia*, muestran que la población rural de Al-Ain, Emiratos Árabes Unidos, está en riesgo de rickettsiosis transmitida por garrapatas. Además, el estudio actual debería servir de base para más estudios conducentes a una mejor comprensión del potencial del reservorio por los camellos y el riesgo que representan estas dos enfermedades a los camellos y otros animales.

Palabras Clave: Camello; enfermedad; RCP; prevalencia

Ticks are vectors to many pathogens and parasites that are of significance to human, domestic animal and wildlife health (Al Khalifa et al. 2006; Diab et al. 2006). *Hyalomma* (Acari: Ixodidae) ticks are widespread in North Africa, Southern Europe, Middle East, Central Asia and China and may serve as vectors of Crimean-Congo hemorrhagic fever (CCHF), rickettsiae and tropical theileriosis (Wernery & Kaaden 2002;

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Atkas et al. 2004). Few studies document tick-borne diseases in the Middle East region (Wernery & Kaaden 2002). *Rickettsia*-like microorganisms were first described from the tick *Ornithodoros savignyi* collected from camels in Egypt (Roshdy 1968). Several rickettsiae including *Anaplasma marginale*, *Coxiella burnetii*, *Rickettsia aeschlim* were reported from Egypt, Somalia and Nigeria from several *Hyalomma* spp. including *H. dromedarii* Koch, 1844 (Wernery & Kaaden 2002; Loftis et al. 2006). Seventeen species of the genus *Rickettsia* are categorized within the Spotted Fever Group (SFG) rickettsiae (Hackstadt 1996) and several have been documented in the Middle East, although some reports are considered questionable (Wernery & Kaaden 2002). SFG rickettsiae are intracellular, gram-negative bacteria that are transmitted by *Hyalomma* ticks, and generally they are host-specific and have the ability to infect a variety of atypical hosts, including humans. *Theileria annulata* (an apicomplexan) is a parasite of potentially important domestic animal health concern in the region because it infects cattle and is transmitted by *Hyalomma* spp. (Hussein et al. 1991; De Kok et al. 1993; Atkas et al. 2004). The parasite causes acute, often fatal, disease (tropical theileriosis) and occurs from North Africa and Southern Europe, through the Middle East and across Southern Asia. Both diseases could cause great losses in livestock production in the region (Wernery & Kaaden 2002).

The importance of this study is that it explores the presence of 2 pathogens that can infect the one-humped camel (*C. dromedarius*), which is the most important animal in the UAE. Historically and to date, camels in the UAE produce milk and meat plus they have been used in racing, which is a traditional sport, practiced by many Emiratis. *Hyalomma* ticks are abundant in the UAE, with heavy infestation of primarily *H. dromedarii* occurring widely in camels (Wernery & Kaaden 2002; Diab et al. 2006). There are other ticks in the Middle East and North African region including *H. anatolicum*, *H. excavatum*, *Boophilus microplus* and *O. savignyi* reported from camels, cattle and other livestock (Diab et al. 2006). It was reported that the prevalence of *H. excavatum* infesting cattle to be 92.35% in Iran, where *T. annulata* is endemic (Ramzi et al. 2003). In Saudi Arabia, *H. anatolicum* had a prevalence of 77.3%, with considerable geographic variation in the prevalence of different species of ticks (Diab et al. 2006). Thus, multiple species of ticks could be involved in maintaining a number of pathogens and parasites in the Middle East. This study was conducted to achieve 2 objectives: : (1) to detect the presence of SFG *Rickettsia* sp. and *Theileria* sp. in *Hyalomma dromedarii* ticks and (2) to determine their prevalence in the tick population on the sampled camel farms in Al-Ain, UAE.

Materials and Methods

STUDY DESIGN

This study is a cross-sectional prospective exploring the presence of 2 pathogens in camels' ticks that were collected from Al-Ain farms. The inclusion criterion is the approval of the farm owner to enter the farm and collect the ticks. The number of camels that were sampled from each farm represented a percentage of the total number of camels reared at each farm.

STUDY LOCATION AND TICK COLLECTION

Al-Ain area was theoretically divided into 4 sampling zones (east, west, north, south). Camel farms were chosen from each zone randomly. The selected farms represented all of the major camel breeding locations in the study area. Al-Ain is located about 120 km south-southeast of Abu Dhabi (N 24° 11' E 55° 45'). The inland location of Al-Ain makes its environment drier and warmer compared to Abu Dhabi or Dubai. In 2010, ticks were collected from individual camels (n = 133) of 14 farms. In 2011, a larger number of camels (n = 492) was sampled for ticks from 20 farms. In both years, collection was done during spring and summer (Mar-Aug). In each farm, 15-20 ticks were removed manually from each one of the randomly selected camels. Ticks were placed in 50 mL blue cap plastic tubes (Sterilin, UK). The tubes containing ticks were put on ice inside a cooler and were taken to the Entomology Laboratory at UAE University where they were frozen at –20 °C until further processing. All ticks were morphologically identified to the species level by using a tick taxonomic key (Walker et al. 2003). The morphological identification was confirmed by molecular methods.

DNA EXTRACTION FROM TICKS

As previously mentioned ticks were collected from 625 camels and were placed in tubes. From each tube, one unsexed engorged adult tick was picked for DNA extraction. Each tick was washed individually in 500 µL 70% ethanol followed by 500 µL sterile double distilled H_2O to remove any contamination attached to its body. To extract genomic DNA, each tick separately was manually crushed with a sterile ceramic mortar and was placed in a sterile 1.5 mL Eppendorf tube. DNA was extracted from the tick homogenate using the QIAamp Tissue Kit (Qiagen, Hilden, Germany), following the protocol of the manufacturer. To save time and labor, a second extraction method was applied using a Maxwell 16 automated DNA extractor (Promega, USA) following the manufacturer's protocol. The 625 extracted DNA samples were stored in a -80 °C freezer.

POLYMERASE CHAIN REACTION AMPLIFICATION

The template DNA was amplified in a 25 µL reaction mixture containing about 80 ng of tick DNA, 20 pmol of each primer pair, and 12.5 µL 2X PCR Master Mix (Promega, USA). PCR amplifications were carried out in a Swift MaxPro thermocycler (ESCO, Singapore).

In order to confirm the morphological identification of the ticks the primer pair Fish1F: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and Fish1R: 5'- TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005) was used to amplify the cytochrome oxidase *c* sub-unit I (COI) of the mitochondrial DNA. Amplification started with an initial denaturation for 2 min at 95 °C followed by 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 54 °C, and extension for 90 s at 72 °C. A final extension step was performed for 10 min at 72 °C.

Rickettsia pathogen was detected according to a published method (Blair et al. 2004). Briefly, the detection involved a nested PCR of the outer membrane protein (*ompA*) gene. In the first PCR, the amplification of a 590 bp fragment was obtained by using the forward primer 5'- ATGGCGAATATTTCTCCAAAA-3' and the reverse primer 5'- GTTCCGTTAATGGCAGCATCT-3'. In the second PCR, the amplification of a 540 bp fragment was obtained by using the forward primer 5'- AAGCAATACAACAAGGTC -3' and the reverse primer 5'- TGACAGTTATTATACCTC -3'. Following initial denaturation for 1 min at 94 °C, 35 cycles of denaturation for 30 s at 94 °C, annealing for 1 min at 50 °C, and extension for 4 min at 68 °C were performed. A final extension step was done for 20 min at 72 °C. Every PCR included a negative control (no-template DNA) to make sure there was no contamination. Also, a positive control (*Rickettsia* endosymbiont of *Amblyomma maculatum ompA*, GenBank# JX134638) was used to insure that the conditions were optimum and the primers were properly annealing to the target region on the template DNA. *Theileira* genus-specific primers were used in the PCR to produce a specific band size in the positive samples. A 560 bp fragment of the ssrRNA gene was amplified from DNA by using the forward primer 5'-GTCTTGTAATTGGAATGATGG-3' and the reverse primer

5'-CCAAAGACTTTGATTTCTCTC-3'. An initial denaturation step at 94 °C for 2 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 60 s. A final extension was done at 72 °C for 7 min, followed by a hold step at 4 °C. Every PCR included a negative control (no-template DNA). The PCR products were resolved by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and photographed by UVDI gel documentation system (Major Science, Taiwan). By the end of each PCR amplification process the amplicons of the positive samples (i.e. producing the expected band size on electrophoresis gel) were saved for DNA sequencing. The amplicons were purified by a Qiagen PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

DNA SEQUENCING AND SEQUENCE ANALYSIS

The identification of the SFG *Rickettsia* (group level) and *Theileira* (species level) was done based on the similarity between the DNA sequences of the bands produced by the specific primers used in the present study and GenBank sequences. The PCR purified amplicons were sequenced by Source Bioscience (Cambridge, UK) and Macrogen (Seoul, South Korea). Sequences were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) sequence analysis tool in the GenBank database. Sequences were submitted in the GenBank and received accession numbers. MEGA5 (Tamura et al. 2011) was used to draw a phylogenetic tree for the *Theileira* species by using the Neighbor-Joining method (Saitou & Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Felsenstein 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequence and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences (the *Theileria* DNA sequence reported in the present study plus 11 sequences taken from its BLAST search results). All positions containing gaps and missing data were eliminated. There were a total of 510 positions in the final dataset.

STATISTICAL ANALYSIS

Pearson's chi-squared test (χ^2) was performed to test if there is any difference in the prevalence of SFG *Rickettsia* sp. and *T. annulata* in ticks using an interactive calculation tool [Computer software available from http://quantpsy.org.] (Preacher 2001).

Results

The morphological identification of the ticks reported in this study as *H. dromedarii* was confirmed based on a DNA identification of the sequences of the cytochrome oxidase subunit I (COI). The sequence was submitted in the GenBank and received an accession number (KJ022631).

The 540 bp DNA fragment specific for the SFG *Rickettsia* sp. (Fig. 1) was not detected in ticks in 2010. However, it was detected in 2011 in 7/492 (1.42%) of the sampled tick population (Table 1). This detection occurred in 3 sampling locations: Al-Arad, Malaket, and Al-Wagan. The sequence of the *ompA* gene of the SFG *Rickettsia* sp. reported in this study was submitted in the GenBank and has been assigned an accession number (KF156874). The molecular identification of SFG *Rickettsia* sp. based on a BLAST analysis (Table 1) showed 99% sequence identity and 100% coverage with *Candidatus* 'Rickettsia andeanae' and

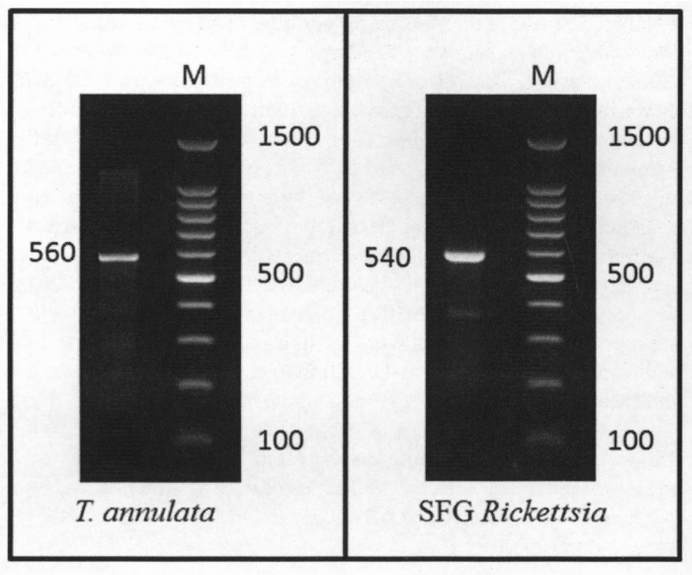

Fig. 1. Gel electrophoresis stained with ethidium bromide showing 2 DNA positive samples of *Theileria annulata* and spotted fever group (SFG) *Rickettsia* sp. amplified by PCR. Lane M: represents 100-bp DNA ladder (Promega, USA).

several *Rickettsia* endosymbionts, all of which belong to the spotted fever group.

The molecular identification of *Theileria* sp. based on a BLAST analysis revealed that it belonged to the species *T. annulata.* This

Table 1. Positive samples of SFG *Rickettsia* sp. and *T. annulata* in *H. dromedarii* ticks according to farm locations in Al-Ain, UAE.

	SFG *Rickettsia* sp.		*T. annulata*	
Farm Location	2010	2011	2010	2011
Al-Ajban	—	10	—	10
Al-Arad	7	23 (4)	7	23 (1)
Bede'Bent Suod	—	17	—	17 (1)
Bede' Fares	—	20	—	20 (1)
Dubai Road	—	60	—	60
Dwar Al-Shahenat	7	26	7	26 (1)
Al-Hama	20	10	20	10
Malaket	15	24 (1)	15	24
Maragh	20	19	20 (2)	19
Mezyad	10	23	10	23 (1)
Al-Nesoreya	—	4	—	4
Remah	—	20	—	20
Al-Sad	7	15	7	15 (1)
She'ab Al-Ghaf	7	28	7	28 (1)
Al-Selemat	—	25	—	25
Swehan	9	75	9 (1)	75 (1)
Al-Dhahera	7	23	7	23
Omghafa	5	23	5	23
Al-Wagan	—	32 (2)	—	32
Al-Yahar	6	15	6	15
Al-Eyada	5	—	5	—
Al-Matla'	8	—	8	—
Total	133 (0)	492 (7)	133 (3)	492 (8)
Percent Positive %	0	1.42	2.25	1.62

Values from locations represent the number of tested ticks followed by the number of positive samples in parenthesis. The number of tested ticks also represents the number of sampled camels because one unsexed tick was taken from every camel tick pool (15-20 ticks) for DNA extraction and pathogen detection.

identification was supported by 99% sequence identity with other *T. annulata* GenBank sequences and an E-value = 0 (Table 2). Also, the phylogenetic tree showed a high genetic relatedness with 7 GenBank *T. annulata* DNA sequences and a 95% bootstrap value (Fig. 2). The *T. annulata* DNA sequence reported in this study was submitted in the GenBank and has been assigned an accession number (KC902832.1). The 560 bp DNA fragment specific for the *T. annulata* (Fig. 1), was detected in 8/492 (1.62%) of the ticks (Table 1). The *T. annulata* pathogen was detected in both years in camel ticks (Fig. 1) and its prevalence in the sampled tick population was 2.3% and 1.60% in 2010 and 2011, respectively (Table 1). In 2010, positive samples were discovered in Maragh and Swehan, which were located in the east and west of Al-Ain, respectively. In 2011, the detection occurred in the east (Mezyad and Dwar Al-Shahenat), west (Swehan, Al-Sad and Bede' Fares), north (Bede'Bent Suod), and south (Al-Arad and She'ab Al-Ghaf) of Al-Ain (Table 1). No significant difference was found between the prevalence of SFG *Rickettsia* sp. and *T. annulata* in ticks ($\chi^2 = 0.068$, df = 1, $P = 0.794$) at the significance level ($P < 0.05$).

Discussion

Constant surveillance is imperative to maintain good camel health status and for the early detection of disease emergencies. In this study, a DNA-based detection technique was used to document the presence of SFG *Rickettsia* sp. and *T. annulata* in camel ticks. The presence of the pathogen's DNA in a tick sample was considered as an evidence of the existence of the pathogen itself in the sample. The accuracy of the detection process was confirmed when SFG *Rickettsia*-specific primers (Blair et al. 2004) produced the exact expected band size in the nested PCR and by the BLAST results of the DNA sequence of the amplicon. Because the SFG *Rickettsia* sp. reported in the current study had 99% sequence identity and 100% coverage with 2 organisms in the GenBank namely *Candidatus* 'Rickettsia andeanae' and several *Rickettsia* endosymbionts a species name was not assigned to it. More primers need to be used with the *ompA* primers in future studies to provide an accurate identification at the species level, which was outside the scope of the current study. According to the detection process, the SFG *Rickettsia ompA* gene occurred in 3 locations: Al-Arad, Malaket, and Al-Wagan. Malaket is located in the eastern part of Al-Ain while Al-Arad and Al-Wagan are in the south. This showed that the pathogen was not found in 2 of the sampling zones (west and north). However, this situation may change in the future if infested camels or camel ticks reach these zones possibly through sales or exchange of camels. Wernery & Kaaden (2002) mentioned that disease has not been reported in old world camels as a result of *Rickettsiae* and they are not believed to be an important part in the cycle of *Rickettsiae*. However, the detection of SFG *Rickettsia* sp. in the present study implies that there is a possibility for this pathogen to be present in domestic camels because it was detected in ticks feeding on their blood. Because SFG *Rickettsia* is considered a threat to human as well as cattle health, reporting its presence associated with camel ticks has a great significance to the health authorities in UAE and the neighboring region. Furthermore, this may indicate that the rural population of Al-Ain, UAE, can be at risk for tick-borne rickettsioses if pathogenic species were to be present. Certainly, further studies are needed for better understanding of its epidemiological characteristics. *T. annulata* was found in camel ticks in all of the sampling zones whereas *SFG Rickettsia* sp. was limited to 2 zones only. The geographic distributions of SFG *Rickettsia* sp. and *T. annulata* did not overlap in the entire study area except in one location (Al-Arad) in which both of the pathogens were present. Although *Theileria* spp. are regarded as common in livestock (e.g. *T. camelensis* and *T. dromedarii* in camels, (Wernery & Kaaden 2002)), *T. annulata* is regarded as a parasite of cattle (Beck et al. 2009; Wernery & Kaaden 2002; Atkas et al. 2004). The prevalence of *T. annulata* from cattle in Hofuf, Saudi Arabia was 20.9% (Hussein et al. 1991). In an experiment 5 healthy camels were injected with *T. annulata* but the pathogen could not be detected during the course of the experiment (one month) (Wernery & Kaaden 2002). Thus it was concluded that camels did not play a role in the epidemiology of *T. annulata*. On the contrary, the present study found *T. annulata* in ticks feeding on camels' blood, suggesting that the parasite circulates between camels and ticks albeit in low prevalence in Al-Ain region, UAE. This aspect needs to be examined in future studies. Furthermore, in this study some of the camels' farms had sheep and goats enclosed in separate areas, so it is possible that ticks were serving as mechanical or biological vector between camels, sheep and goats. *T. annulata* poses a greater risk to cattle; hence, a comprehensive study is warranted to explore the possible role of camels' ticks as a vector of *T. annulata* to cattle when the 2 animal species are reared on the same farm. For both of the SFG *Rickettsia* sp. and *T. annulata*, it needs to be mentioned that we were not able to affirm or negate whether the sampled camels showed signs of illness at the time of sampling because ticks were not collected by veterinarians. Still, we can confirm that at the sampling time none of the camels included in this study exhibited major signs of illness warranting isolation from the rest of the camels on the farm.

The selection of farms was not based upon a systematic random sampling, which was due mainly to logistic and financial limitations. Nevertheless, the results of this study remain valid because increasing the number of samples will increase the power of detection. Consequently, more accurate estimations of the prevalence will be made. Systematic random design is likely to result in having greater chance to detect the pathogens in all sampling locations. On the other hand, the above limitations have no effect on the molecular reliability of the identification of the pathogens nor on the identification of the collected ticks.

Table 2. Molecular identification of SFG *Rickettsia* sp. and *T. annulata* based on DNA similarity according to BLAST.

UAE sample accession number	Best match specie	Accession number	Sequence identity, %	Sequence coverage, %	*E*-Value*
KC902832.1	*Theileria annulata*	KF559356.1	99	99	0
	Theileria annulata	KF429800.1	99	99	0
	Theileria annulata	KF429799.1	99	99	0
	Theileria annulata	KF429795.1	99	99	0
	Theileria annulata	KF429794.1	99	99	0
	Ca. Rickettsia andeanae	KF179352.1	99	100	0
KF156874.1	*Ca.* Rickettsia andeanae	KF030932.1	99	100	0
	Rickettsia endosymbiont	EF524203.1	99	100	0
	Rickettsia endosymbiont	EF372578.1	99	100	0

*The typical threshold for a good *E*-Value from a BLAST search is 10^{-5} or lower. *Ca* = *Candidatus*.

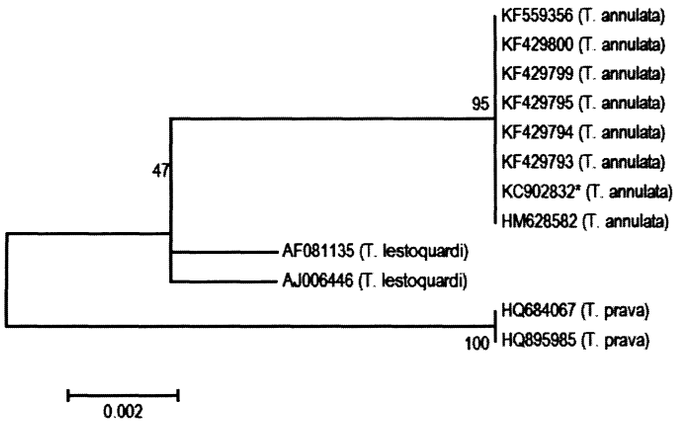

Fig. 2. Kimura 2-parameter distance Neighbor Joining (NJ) tree of 12 nucleotide sequences belonging to the genus *Theileria*. Codes denote the accession numbers of NCBI GenBank database. * Sequence from Al-Ain, UAE. Numbers next to branches are the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein 1985).

In conclusion, the current study reported the presence of SFG *Rickettsia* sp. and *T. annulata* in camel ticks in the UAE. The prevalence of the 2 organisms in the ticks was < 2%, which is important for the public and animal health authorities in the UAE. However, a real risk of tick-borne rickettsioses in Al-Ain rural population becomes conceivable if pathogenic species were to be present. Therefore, a study covering the entire UAE camel breeding range with a complete systematic sampling is warranted. Furthermore, we suggest that SFG *Rickettsia* sp. and *T. annulata* detection in future studies should be coupled with examination of camels for signs of illness at the time of tick collection. To our knowledge, this is the first published record on the presence of SFG *Rickettsia* sp. and *T. annulata* in *H. dromedarii* ticks in the UAE.

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