



**Molecular detection of *Babesia microti* in one-humped camel  
(*Camelus dromedarius*) in Halayeb and Shalateen, Halayeb, Egypt**

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**Abstract**

There is paucity in the epidemiological screening of a blood parasite of zoonotic importance, *Babesia microti* (*B. microti*) in camels in Egypt. Therefore, a total of 142 individuals of one humped camel (*Camelus dromedarius*) that were bred in Halayeb and Shalateen were examined for infection and subjected to phylogenetic analysis on blood parasite infection with *B. microti*. PCR assay focusing on the *Babesia microti* small subunit rRNA (*ss-rRNA*) gene was used for detection of its prevalence in camels. Phylogenetic analysis revealed the detection of 17 case from a total of 142 cases with an infection rate of 11.97% using nested PCR assays. Moreover, phylogenetic analyses showed that *B. microti* was genetically related with German strain that is isolated from voles and rats in France. In conclusion, this is the first evidence of *B. microti* infection in camels in Egypt with evidence of the role of one-humped camels in sustaining the babesia transmission in Egypt.

**Keywords:** *Babesia microti*; *Camelus dromedarius*; Egypt

**INTRODUCTION**

In the past, Camels were supposed to resist various pathological diseases (Hirad et

al., 2018). However, some recent literature described the susceptibility of camels to several infectious agents such as viral, fungal, bacterial

and parasitic diseases (Zarrin et al. 2020).

Vector transmitted diseases cause different clinical signs in livestock in Egypt (Elsify et al., 2015). One of the most important tick-borne pathogens which can cause disease in affected humans is **Babesia spp** (Rizk et al., 2020). *Babesia (B.) microti* is a blood parasite that infects rodents and has zoonotic importance (Chen et al., 2017; Rizk et al., 2017).

Camel babesiosis is characterized by high morbidity with a high economic loss due to tick-borne parasitic protozoan (Qablan et al. 2012). Indeed, there is paucity about the infection of camels by *Babesia* spp. that have zoonotic importance. *Babesia microti* is one of the most important species of babesiosis infecting human, which can be transmitted by blood transfusion or organ transplantation (Vannier et al., 2008). In this regard, in the present study, we investigated the presence of *B. microti* in camel breeds in Halayeb and Shalateen at Upper Egypt using molecular diagnostic techniques while in conducting phylogenetic analysis for the identified parasite.

## MATERIALS AND METHODS

### 1. Animals and blood sampling

A hundred and forty-two blood samples were collected from apparently

healthy one-humped camels throughout 2017. The camels used in this experiment were bred in Halayeb and Shalateen region in southern Egyptian land adjacent to Sudan. An approximately 2 ml of whole blood was collected from the jugular veins of each camel into a Vacutainer tube containing EDTA. The blood samples were identified and kept at  $-20^{\circ}\text{C}$  until the DNA extractions were conducted. All collected blood samples were examined thoroughly for infection with *B. microti*. Sample size was calculated depending on the equation mentioned by Charan et al. (2013) which is stated as:

$$\text{Sample size} = (Z\text{-score})^2 \times \text{StD} \times (1 - \text{STD}) / (\text{Confidence interval})^2$$

Where, Z score indicates Zeta-score that depends on confidence level for conducting the current research

### 2. Molecular detection of *B. microti*

DNA extraction from 300  $\mu\text{l}$  of the blood samples was achieved using a commercial kit (Promega, Madison, WI, USA) following the manufacturer's instructions, and then stored at  $-20^{\circ}\text{C}$  for further use. *B. microti* was detected in the DNA samples using previously described diagnostic PCR assays targeting the *B. microti* small subunit rRNA (ss-rRNA) gene (Persing, 1992). For detection of *B. microti*, nested PCR assays were used. Primer sequences and annealing temperatures were represented in

(Table 1). For optimizing the conditions used for *B. microti* detection, the enzyme activation and denaturation were conducted at 95°C for 5 min and 95 °C for 30 sec (Persing et al., 1992). Then, the product was kept at 4 °C. Afterward, gel electrophoresis was done for the PCR product on a 1.5% agarose gel using TBE buffer and was further stained with ethidium bromide. Finally, PCR product was envisaged under UV light. Amplicons that demonstrated high band intensities in PCR products were extracted from agarose gels using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). After that cloning was done into a plasmid vector (PCR 2.1-TOPO, Invitrogen, USA) and sequencing was carried out using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, USA).

### 3. Phylogenetic analysis

For conduction of phylogenetic analysis, 17 nucleotide sequences were used for conducting phylogenetic analysis. Codon position contained the first, second, third and non-coding region, where all gaps and missing data were excised. A total of 201 positions were finally assigned and phylogenetic analysis was conducted using MEGA6 (Tamura et al., 2013). The evolutionary history was gathered using the Neighbor-Joining method (Saitou and Nei,

1987). It was demonstrated that the optimal tree with the sum of 3 branches length equal 56.08480007. Bootstrap tests were consisted of 1000 replicates and was shown next to every branch, where the associated taxa were grouped together (Felsenstein, 1985). The evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al., 2013).

### 4. Statistical analyses

A phylogenetic analysis was designed for our isolated sequence and others with a high similarity index using the neighbor-joining method for statistical analysis of phylogenetic analysis with 1000 replicates. The upper and lower boundaries of the confidence intervals of the positive rates were calculated for *B. microti* parasite using the Open Epi program (<http://www.openepi.com/v37/Proportion/Proportion.htm>).

## RESULTS

The genomic DNA sequence of *B. microti* in camel's blood was amplified using specific nested primers assigned for Babesia species to amplify small ribosomal subunit of those parasites. Seventeen samples were positive for *B. microti* with infection rates of 11.97 % and 95% confidence interval C.I. 95% (7.61-18.34). The species-specific PCR assay detected the surveyed Babesia species in the camel populations (Fig. 1).

The nucleotide sequences of PCR-amplified ss-rRNA gene of *B. microti* have been registered and assigned the following GenBank accession number: MF737082.1. The phylogenetic analysis of target genes from other species of *Babesia* was compared as in table 2.

The PCR products of the amplification of small ribosomal subunit of *B. microti* produced 154 bp that was further excised, sequences, trimmed and checked for similarity index on Genbank that was required for performing phylogenetic tree to evaluate the genetically closely related strains for *B. microti* to our isolated sequence. The accession number of the isolated sequence was deposited on Genbank with accession number of (MF737082.1). In addition, Phylogenetic analysis revealed the close genetic relationship of our isolated sequence with German strain of *B. microti* in rats (AB366158.1) and voles in France (KX758442.1) (Fig. 2). Moreover, the interspecies distance of our isolated sequence of *B. microti* was showing a close genetic relationship with also (AB366158.1) from Deutschland (Fig. 3).

## DISCUSSION

This study was including the first molecular identification of *B. microti* based

on small ribosomal subunit in camels, which was firstly discovered in human patients in mature erythrocytes (**Skariah et al., 2017**). Generally, *Ixodes* ticks are the vectors of zoonotic *Babesia*, and *Ixodes scapularis* is the most frequent vector in North America (**Eskow et al., 1999**).

PCR monitoring and DNA sequence analyses of field-collected ticks in Asia and Europe have showed that *Ixodes persulcatus* (**Rar et al., 2011**) and *Ixodes ricinus* (**Duh et al., 2001**) in Asia and Europe, respectively, carry the *B. microti* U.S.

In the current study, phylogenetic analysis of small ribosomal subunit of *B. microti* showed a genetically closer relationship of our isolated sequence with similarly related species in Germany and France that were isolated from rodents. Similarly, A higher similarity index was found between isolated species of *B. microti* in Mongolia and other strains in USA regarding small ribosomal subunit (**Tuvshintulga et al., 2015**).

In Egypt, El Bahnasawy and Morsy discovered the first incidence of human babesiosis, where all serological, hematology and symptoms didn't give an ultimate diagnosis for babesiosis infection (**El-Bahnasawy and Morsy, 2008**). Surprisingly, Patients were showing a negative result for malaria infection and for proper diagnosis of babesiosis a typical

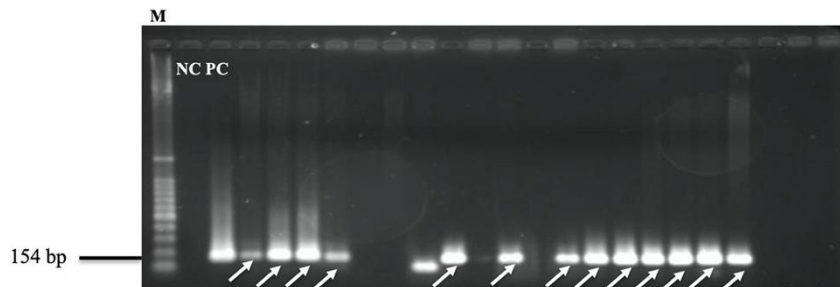
**Table 1:** Primary and nested PCR primers used for PCR amplifications

Species	Assay	Primer sequence (5' → 3')	Annealing	Amplification cycles (No.)	Product size	Target gene	References
<i>B. microti</i>	PCR	F-CTTAGTATAAGCTTTTATACAGC R-ATAGGTCAGAACTTGAATGATACA	55°C	35	238 bp	SS- <i>rRNA</i>	(Persing et al., 1992)
	nPCR	F-GTTATAGTTTATTTGATGTTTCGTTT R-AAGCCATGCGATTCGCTAAT	55°C	50	154 bp		

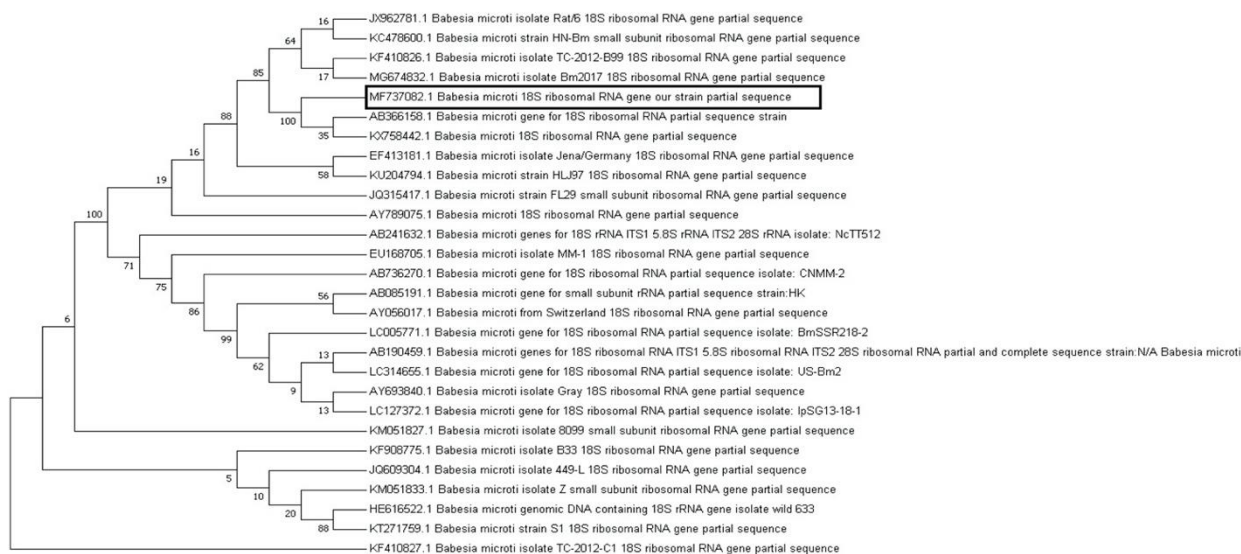
**Table 2.** Origin and accession number of *B. microti* used for genetic analysis in this study

Origin		Accession number
Geographic	Biological	
Germany	Vole	AB085191.1
Japan	Mouse	AB190459.1
China	Rat	AB241632.1
Germany	Rat	AB366158.1
China	Monkey	AB736270.1
Switzerland	Human	AY056017.1
USA	Human	AY693840.1
Poland	Vole	AY789075.1
Germany	Human	EF413181.1
Holland	Monkey	EU168705.1
Russia	Vole	HE616522.1
USA	Rat	JQ315417.1
China	Human	JQ609304.1
China	Rat	JX962781.1
China	Rat	KC478600.1
China	Human	KF410826.1
China	Human	KF410827.1
China	Human	KF908775.1
Russia	Tick	KM051827.1
Russia	Tick	KM051827.1
Spain	Human	KT271759.1
China	Ticks ( <i>Ixodes persulcatus</i> )	KU204794.1
France	Vole	KX758442.1
Mongolia	Ticks	LC005771.1
Japan	Hamster	LC127372.1
Japan	Hamster	LC314655.1
<b>Egypt</b>	<b>Camel</b>	<b>MF737082.1<sup>a</sup></b>
China	Human	MG674832.1

<sup>a</sup> GenBank accession numbers submitted by this study



**Figure 1.** PCR detection of *B. microti* from a camel in Egypt. PC: positive control; NC: negative control. The expected size of the PCR products was 154 bp. Arrows indicate a positive sample.



**Figure 2. Phylogenetic tree of the *B. microti* ss-rRNA gene.** The nucleotide sequences determined in this study are shown in boldface type letters. Bootstrap values are provided at the beginning of each branch. The sequence identified from Egypt in this study is boxed in black.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 AB085191.1_Babesia_microti_gene_for_small_subunit_rRNA_partial_sequence_strain_HK																											
2 AB190459.1_Babesia_microti_genes_for_18S_ribosomal	0.01																										
3 AB244632.1_Babesia_microti_genes_for_18S_rRNA ITS1_5.8S_rRNA ITS2_28S_rRNA_isolate_NcTT512	0.35	0.36																									
4 AB366158.1_Babesia_microti_gene_for_18S_ribosomal_RNA_partial_sequence_strain	0.75	0.74	0.74																								
5 AB736270.1_Babesia_microti_gene_for_18S_ribosomal_RNA_partial_sequence_isolate_CNMM-2	0.31	0.30	0.35	0.47																							
6 AY056017.1_Babesia_microti_from_Switzerland_18S_ribosomal_RNA_gene_partial_sequence	0.00	0.01	0.35	0.75	0.31																						
7 AY693840.1_Babesia_microti_isolate_Gray_18S_ribosomal_RNA_gene_partial_sequence	0.01	0.00	0.36	0.74	0.30	0.01																					
8 AY789075.1_Babesia_microti_18S_ribosomal_RNA_gene_partial_sequence	0.49	0.50	0.40	0.66	0.44	0.49	0.50																				
9 EF413181.1_Babesia_microti_isolate_Jena/Germany_18S_ribosomal_RNA_gene_partial_sequence	0.66	0.67	0.35	0.48	0.65	0.66	0.67	0.52																			
10 EU168705.1_Babesia_microti_isolate_MM-1_18S_ribosomal_RNA_gene_partial_sequence	0.33	0.32	0.33	0.70	0.26	0.33	0.32	0.20	0.63																		
11 HE616522.1_Babesia_microti_genomic_DNA_containing_18S_rRNA_gene_isolate_wild_633	0.76	0.77	0.71	0.79	0.74	0.76	0.77	0.68	0.71	0.69																	
12 JQ315417.1_Babesia_microti_strain_FL29_small_subunit_ribosomal_RNA_gene_partial_sequence	0.44	0.44	0.13	0.70	0.43	0.44	0.44	0.30	0.22	0.41	0.69																
13 JQ609304.1_Babesia_microti_isolate_449-L_18S_ribosomal_RNA_gene_partial_sequence	0.75	0.76	0.68	0.77	0.74	0.75	0.76	0.71	0.67	0.72	0.15	0.67															
14 JX962781.1_Babesia_microti_isolate_Rat/6_18S_ribosomal_RNA_gene_partial_sequence	0.71	0.72	0.62	0.43	0.67	0.71	0.72	0.23	0.29	0.42	0.71	0.51	0.72														
15 KC478600.1_Babesia_microti_strain_HN-Bm_small_subunit_ribosomal_RNA_gene_partial_sequence	0.71	0.72	0.62	0.43	0.67	0.71	0.72	0.23	0.29	0.42	0.71	0.51	0.72	0.00													
16 KF410826.1_Babesia_microti_isolate_TC-2012-B99_18S_ribosomal_RNA_gene_partial_sequence	0.71	0.72	0.62	0.43	0.67	0.71	0.72	0.23	0.29	0.42	0.71	0.51	0.72	0.00	0.00												
17 KF410827.1_Babesia_microti_isolate_TC-2012-C1_18S_ribosomal_RNA_gene_partial_sequence	0.74	0.75	0.68	0.78	0.73	0.74	0.75	0.70	0.68	0.71	0.11	0.66	0.04	0.73	0.73	0.73											
18 KF908775.1_Babesia_microti_isolate_B33_18S_ribosomal_RNA_gene_partial_sequence	0.74	0.75	0.68	0.78	0.73	0.74	0.75	0.70	0.68	0.71	0.11	0.66	0.04	0.73	0.73	0.73	0.00										
19 KM051827.1_Babesia_microti_isolate_8099_small_subunit_ribosomal_RNA_gene_partial_sequence	0.74	0.75	0.68	0.78	0.73	0.74	0.75	0.70	0.68	0.71	0.11	0.66	0.04	0.73	0.73	0.73	0.00	0.00									
20 KM051833.1_Babesia_microti_isolate_Z_small_subunit_ribosomal_RNA_gene_partial_sequence	0.75	0.76	0.68	0.77	0.74	0.75	0.76	0.71	0.67	0.72	0.12	0.67	0.05	0.72	0.72	0.72	0.01	0.01	0.01								
21 KT271759.1_Babesia_microti_strain_S1_18S_ribosomal_RNA_gene_partial_sequence	0.76	0.77	0.71	0.79	0.74	0.76	0.77	0.68	0.71	0.69	0.00	0.69	0.15	0.71	0.71	0.71	0.11	0.11	0.11	0.12							
22 KU204794.1_Babesia_microti_strain_HLJ97_18S_ribosomal_RNA_gene_partial_sequence	0.66	0.67	0.35	0.48	0.65	0.66	0.67	0.54	0.02	0.63	0.73	0.24	0.68	0.31	0.31	0.31	0.69	0.69	0.69	0.68	0.73						
23 KX758442.1_Babesia_microti_18S_ribosomal_RNA_gene_partial_sequence	0.75	0.74	0.74	0.00	0.47	0.75	0.74	0.66	0.48	0.70	0.79	0.70	0.77	0.43	0.43	0.43	0.78	0.78	0.78	0.77	0.79	0.48					
24 LC005771.1_Babesia_microti_gene_for_18S_ribosomal_RNA_partial_sequence_isolate_BmSSR218-2	0.01	0.00	0.36	0.74	0.30	0.01	0.00	0.50	0.67	0.32	0.77	0.44	0.76	0.72	0.72	0.72	0.75	0.75	0.75	0.76	0.77	0.67	0.74				
25 LC127372.1_Babesia_microti_gene_for_18S_ribosomal_RNA_partial_sequence_isolate_lpSG13-18-1	0.01	0.00	0.36	0.74	0.30	0.01	0.00	0.50	0.67	0.32	0.77	0.44	0.76	0.72	0.72	0.72	0.75	0.75	0.75	0.76	0.77	0.67	0.74	0.00			
26 LC314655.1_Babesia_microti_gene_for_18S_ribosomal_RNA_partial_sequence_isolate_US-Bm2	0.01	0.00	0.36	0.74	0.30	0.01	0.00	0.50	0.67	0.32	0.77	0.44	0.76	0.72	0.72	0.72	0.75	0.75	0.75	0.76	0.77	0.67	0.74	0.00	0.00		
27 MF737082.1_Babesia_microti_18S_ribosomal_RNA_gene_our_strain_partial_sequence	0.75	0.74	0.74	0.00	0.47	0.75	0.74	0.66	0.48	0.70	0.79	0.70	0.77	0.43	0.43	0.43	0.78	0.78	0.78	0.77	0.79	0.48	0.00	0.74	0.74		
28 MG674832.1_Babesia_microti_isolate_Bm2017_18S_ribosomal_RNA_gene_partial_sequence_(human_china)	0.71	0.72	0.62	0.43	0.67	0.71	0.72	0.23	0.29	0.42	0.71	0.51	0.72	0.00	0.00	0.00	0.73	0.73	0.73	0.72	0.71	0.31	0.43	0.72	0.72	0.42	

**Figure 3.** Pairwise genetic distance based on *18s rRNA* gene sequences of *B. microti* detected in camel in Egypt and compared with other related species on GenBank.

ring-like structure was found in blood smears. Those patients were successfully treated with clindamycin and quinine.

This study demonstrated the infection of camels reared in Halayeb and Shalateen, in Upper Egypt by *B. microti*. It drives our concern the role of camels and ticks in provoking zoonotic infection of babesiosis. The other important matter was that the positively infected species of camels were apparently healthy individuals with no apparent clinical signs which draw our attention of potential role of camels as reservoir host for *Babesia* spp.

Despite of the first report of molecular evidence of *B. microti* in camels, several limitations were detected; such as the absence of microscopical examination of babesiosis in camels, which require further investigation of camels in Egypt. Moreover, this study did not provide a clear hypothesis about the role of camel's in babesiosis infection and required a further investigations on ticks, the reservoir host for sustaining enzootic cycle of babesiosis in Egypt and human individuals in the same region.

## CONCLUSION AND RECOMMENDATION

The present study gives the primary proof of evidence on the presence of *B. microti* in camels in Egypt and draws our intention to the role of camels and prevalent tick species (reservoir host) in this area in providing enzootic cycle for babesiosis transmission in Egypt. These findings have economic significance and indicate the importance of introducing effective prevention and control strategies throughout Egypt to minimize the prevalence of *B. microti* in camels.

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### Conflict of Interest

The authors declare that they have no conflict of interest.



### Statement of animal rights

The Animal Care and Use Committee at the Obihiro University of Agriculture and Veterinary Medicine approved all of the study's experimental protocols (Approval No. 27-65). All Institutional and National Guidelines for the care and use of animals were followed according to the Egyptian Medical Research Ethics Committee (No. 14-126). Informed written permission was gotten from the owner.

### Author Contributions

Conceived and designed the experiments: MAR, SAEE, MAE. Performed the experiments: SAEE, MAR, MAE, MOA, MA. Analyzed the data: MAR, MAE. Contributed reagents/materials/analysis tools: MAR, SAEE, MAE, MA, MEB. Wrote the manuscript: MAR, MAE, NAG. All authors reviewed the manuscript.

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## الملخص العربى

التشخيص الجزيئى لطفيل البابيزيا ميكروتى (*Babesia microti*) فى الجمال ذات السنم الواحد بمنطقة

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هناك ندرة فى الدراسات الوبائية لطفيل البابيزيا ميكروتى (*Babesia microti*) طفيل الدم فى الجمال بمصر و الذى له اهمية كبيرة حيث انه يمكنه اصابة الانسان. و لذلك فقد تم فحص عدد 142 عينة دم من الجمال ذات السنم الواحد من منطقة حلايب و شلاتين لاكتشاف اصابتها بطفيل البابيزيا ميكروتى من عدمه و كذلك فقد تم تحليل النشوء و التطور لمعزولات هذه الدراسة. و قد تم العثور على عدد 17 عينة موجبة (11.97%) لطفيل البابيزيا ميكروتى بعد فحص عدد 142 عينة باستخدام تفاعل البلمرة المتسلسل (PCR) و توظيف العلامة الجينية (ss-rRNA). و من الجدير بالذكر انه بمقارنة تحليل النشوء و التطور لمعزولات هذه الدراسة مع معزولات الدراسات السابقة عالميا قد وجد انها تتطابق مع المعزولات الالمانية التى تم عزلها من الفئران و الجرذان فى فرنسا. و تعتبر هذه الدراسة الاولى بمصر التى تشير الى اصابة الجمال بطفيل الدم بابيزيا ميكروتى .