

# Title

## Molecular Detection and Identification of *Babesia* spp., and *Trypanosoma* spp. in one-humped camel (*Camelus dromedarius*) in Halayeb and Shalateen, Egypt

### Running title

*Babesia* spp., and *Trypanosoma* spp. in camel in Egypt

### Authors

Shimaa Abd El-Salam El-Sayed <sup>1,2</sup>, Mohamed A. El-Adl <sup>1</sup>, Mayar O. Ali <sup>3</sup>, Mostafa Al-Araby <sup>4</sup>, Mosaab A. Omar <sup>5,6</sup>, Mohamed El-Beskawy <sup>7</sup>, Shimaa sobhy sorour<sup>8</sup>, Mohammad Ali Al-Wabel<sup>9</sup>, Mohamed Abdo Rizk <sup>2,10,\*</sup>, Magdy Elgioushy,<sup>11</sup>

### Addresses

<sup>1</sup> Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt. E-mail: abdo\_dr\_shimaa@yahoo.com (S.A.E.S.E.),

[drmohamedalymaher@hotmail.com](mailto:drmohamedalymaher@hotmail.com) (M.A.E.A.)

<sup>2</sup> National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-Cho, Obihiro, Hokkaido, Japan

<sup>3</sup> Department of Animal Genetics, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt. E-mail: [mayar.othman132@yahoo.com](mailto:mayar.othman132@yahoo.com)

<sup>4</sup> Department of Parasitology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt. E-mail: [alarabya@yahoo.com](mailto:alarabya@yahoo.com)

<sup>5</sup>Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, 51452 Qassim, Saudi Arabia. Email: mos.mohamed@qu.edu.sa

<sup>6</sup>Department of Parasitology, Faculty of Veterinary Medicine, South Valley University, 83523, Qena, Egypt

<sup>7</sup>Department of Internal Medicine (infectious diseases), Faculty of Veterinary Medicine, Matrouh University, Egypt. Email: melbeskawy@gmail.com

<sup>8</sup>Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt. Email: shsorour@yahoo.com

<sup>9</sup>College of Agriculture and Veterinary Medicine, Qassim University, 51452 Qassim, Saudi Arabia . email: mwabl@qu.edu.sa

<sup>10</sup> Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt. Email: dr\_moh\_abdo2008@mans.edu.eg

<sup>11</sup> Department of Animal Medicine, Faculty of Veterinary Medicine, Aswan University, Aswan, 37916, Egypt. Email: [gioushymagdy@yahoo.com](mailto:gioushymagdy@yahoo.com)

\*Correspondence: dr\_moh\_abdo2008@mans.edu.eg (M.A.Z)

### Simple Summary:

Camels provide the world by food with a high nutritional value. Taken together many studies reported the medicinal impact of camel's milk which help in recovery from several diseases affect human. In Egypt, vector transmitted diseases consider one of the critical obstacle hindering the camel production. Generally, identification and epidemiological screening of vector transmitted parasites including *Babesia* and *Trypanosoma* spp. help in improving the camels community. Therefore, in the current study we performed phylogenetic analysis of blood parasite infections including *Babesia (B.) bovis*, *Babesia microti* and *Trypanosoma (T.)* spp. in one-humped camel (*Camelus dromedarius*) breeds in Halayeb and Shalateen, in Upper Egypt. The results revealed 11.97 %, 2.81%, and 5.63% infection rates of *B. microti*, *B. bovis*, and *T. evansi* in the screened camels, respectively. This study provides the first evidence of *B. microti* in camel in Egypt and highlights the possible role of one-humped camels in maintaining the enzootic cycle of *Babesia* transmission in Egypt. These findings have economic importance and give an alert to the government to introduce an effective control strategies to minimize the prevalence of infection by blood parasites in camels.

## Abstract

Phylogenetic analysis of blood parasite infections including *Babesia (B.) bovis*, *Babesia microti* and *Trypanosoma (T.)* spp. in one-humped camel (*Camelus dromedarius*) (n= 142) breeds in Halayeb and Shalateen, in Upper Egypt were performed in the current study. Polymerase chain reaction (PCR) assays targeting the *Rhoptry Associated Protein-1 (RAP-1)*, *Babesia microti small subunit rRNA (ss-rRNA)* and *internal transcribed spacer 1 (ITS1)* genes were used to detect the prevalence of *B. bovis*, *B. microti* and *Trypanosoma* spp. in camels, respectively. Nested PCR assays were used for the detection of *Babesia* spp. (*B. bovis* and *B. microti*). While, KIN-multi species PCR reaction was employed to detect and identify trypanosome DNA in camels. *B. microti* was detected in (17/142) with infection rate (11.97 %). Sequencing and phylogenetic analyses revealed that *B. microti* detected in camel was closely related to the German strain in rats and voles in France. *B. bovis* was also detected in (4/142) with infection rate (2.81%). The sequence and phylogenetic analyses revealed that the isolated *B. bovis* was closely related to strains isolated from Argentina, USA and Brazil. Moreover, *T. evansi* was detected in (8/142) with infection rate (5.63%). Sequence and phylogenetic analyses revealed that isolated *T. evansi* was closely related to *T. theileri* that was detected from cattle in Brazil. This study provides the first evidence of *B. microti* in camel in Egypt and highlights the possible role of one-humped camels in maintaining the enzootic cycle of *Babesia* transmission in Egypt.

**Keywords:** *Babesia* spp.; *Trypanosoma* spp.; Camel; Egypt; Epidemiology

### 1. Introduction

The world's event of desertification has highlighted the important role of camel in socio-economic aspects and the need of proper management system and appropriate disease control measures [1]. In several regions of the world, camels provide an important source of food and milk which is considered a high source of protein of high nutritional value. Moreover, the unique survival capability of camels and production in adverse environmental conditions permit the breeders for adopting semi-intensive farming systems for camels' production [2].

It was thought that camels are resistant to various pathogenic diseases [3]. Recently, different literatures described the susceptibility of camels to large number of pathogenic agents such as bacterial, fungal, parasitic and viral diseases [4].

In Egypt, vector transmitted diseases cause various clinical manifestations in farm animals [5]. Members of piroplasms are efficiently found in several species of equines, cattle and camel in Egypt, where specific DNA fragments were detected of those piroplasms in blood stream in apparently healthy camels of *Babesia* and *Theileria* species [6]. One of the famous piroplasms is *Babesia* spp. which is efficiently affected humans through ticks infestation [7]. *Babesia microti* has been identified in rodents and can efficiently affect human [8,9]. Camel babesiosis is an acute to chronic infectious disease that is distributed all over the world [2]. The disease is responsible for its deteriorative effects, high morbidity rate and higher economic losses, caused by the tick-borne hemo-parasitic protozoan [2,6].

*Trypanosoma evansi* is one of the famous parasite that affect animals and transmitted mechanically by tabanids species causing weight loss, anemia and abortion in several portions of the world such as South America, Asia and Africa [10]. In certain cases of trypanosomiasis, subclinical cases and other chronic infection can be found in hosts infected by low virulence strains [11].

Clinical signs of vector borne diseases are similar and is not sufficient for the ultimate diagnosis [12]. Moreover, the accurate diagnosis of piroplasms requires a wide range of laboratory techniques such as serological tests, blood smears and most definitely molecular diagnosis [13]. ELISA technique can be highly efficient in detecting both clinical and carrier animal, but lack specificity in determining recent and old infection [11]. Blood smear is considered a rapid field test for diagnosis of blood parasites definitely, but it has a low sensitivity against chronic and subclinical infections [14]. The molecular technique can offer a more accurate results in sensitivity and specificity in diagnosis of blood parasites and can be used frequently as a diagnostic approach for identifying active infections [5]. Therefore, the current study is aimed to use molecular diagnostic techniques for detecting blood parasitic infection (*B. bovis*, *B. microti*, and *Trypanosoma* spp.) in camels breeds in Halayeb and Shalateen that located at Lower Egypt and conducting phylogenetic analysis for all identified parasites.

## 2. Material and Methods

**Ethical Statement:** All experimental protocols in this study were approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt (Approval No. 07-55). 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). All experiments were conducted in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Egypt. An informed written consent was obtained from the owner.

### 2.1. Animals and blood sampling

A total of 142 blood samples were collected from one-humped camels during 2017. Blood samples were collected from camels reared in Halayeb and Shalateen, which located in Lower Egypt at the border with Sudan (Fig. 1). All the collected blood samples were investigated for infection with *B. bovis*, *B. bigemina*, *B. microti* and *Trypanosoma* spp.. All animals were apparently healthy during the sampling period.



**Figure 1.** Map of the study sites of the blood parasites molecular epidemiology study in Egypt. Blood samples were collected from animals reared in Halayeb and Shalateen as indicated by bullet points.

### 2.2. DNA extraction and PCR detections of hemoparasites

Blood samples were collected from the jugular veins of the camels under study. Approximately 2 ml of whole blood was collected from each animal into a Vacutainer tube containing EDTA. The blood samples were labeled and stored at  $-20^{\circ}\text{C}$  until the DNA extractions were conducted. DNA samples were extracted from 300  $\mu\text{l}$  of the blood samples using a commercial kit (Promega, Madison, WI, USA) following the manufacturer's instructions, and then stored at  $-20^{\circ}\text{C}$  until further use. *B. bovis*, *B. microti* and *Trypanosoma* spp. were detected in the DNA samples using previously described diagnostic PCR assays targeting the *Rhoptry Associated Protein-1 (RAP-1)*, *B. microti small subunit rRNA (ss-rRNA)* and *internal transcribed spacer 1 (ITS1)* genes, respectively [15-17]. Nested PCR assays were used for the detection of *Babesia* spp. (*B. bovis* and *B. microti*). While, KIN-multi species PCR reaction was employed to detect and identify trypanosome DNA in camels. A KIN-multi species PCR amplifies the *ITS1* and allows for the simultaneous detection of three major trypanosome species (*T. evansi*, *T. congolense* and *T. vivax*) [16]. Primer sequences and annealing temperatures were shown in (Table 1). The enzyme activation and denaturation used for the amplification conditions for *B. bovis*, *B. microti* and *Trypanosoma* spp. were  $95^{\circ}\text{C}$  for 3 min and  $95^{\circ}\text{C}$  for 30 sec,  $95^{\circ}\text{C}$  for 5 min and  $95^{\circ}\text{C}$  for 30 sec, and  $94^{\circ}\text{C}$  for 3 min and  $94^{\circ}\text{C}$  for 1 min, respectively [15-17]. The product was then chilled to  $4^{\circ}\text{C}$ . After that gel electrophoresis of the PCR products was performed on a 1.5% agarose gel with TBE buffer and stained with ethidium bromide. Then, the final PCR product was visualized under UV light.

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. bovis</i>	PCR	F-CACGAGCAAGGAACCTACCGATGTTGA R- CCAAGGACCTTCAACGTACGAGGTCA	$55^{\circ}\text{C}$	45	360 bp	<i>RAP-1</i>	14-22
	nPCR	F- TCAACAACGTACTCTATATGGCTACC R- CTACCGACCAGAACCCTTCTTACCAT	$55^{\circ}\text{C}$	35	298 bp		
<i>B. microti</i>	PCR	F-CTTAGTATAAGCTTTTATACAGC R- ATAGGTCAGAACTTGAATGATACA	$55^{\circ}\text{C}$	35	238 bp	<i>ss-rRNA</i>	18
	nPCR	F-GTTATAGTTTATTTGATGTTTCGTTT R-AAGCCATGCGATTCGCTAAT	$55^{\circ}\text{C}$	50	154 bp		
<i>Trypanosoma</i> spp.	KIN-PCR	F- GCGTTCAAAGATTGGGCAAT R- CGCCCGAAAGTTCACC	$60^{\circ}\text{C}$	40	540 bp	<i>ITS1</i>	[16]

### 2.3. Cloning and sequencing PCR products

Amplicons of PCR samples that exhibited high band intensities were extracted from agarose gels using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), and cloned into a plasmid vector (PCR 2.1-TOPO, Invitrogen, Carlsbad, CA, USA). Sequencing was done for two colonies for each amplicon, using an AB1 PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

### 2.4. Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method [18]. The optimal tree with the sum of 3 branch length = 56.08480007 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [19]. The evolutionary distances were computed using the Maximum Composite Likelihood method [20] and are in the units of the number of base substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 201 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [20].

### 2.5. Statistical analyses

The upper and lower limits of the confidence intervals of the positive rates were calculated for *B. bovis*, *B. microti* and *T. evansi* parasites using the Open Epi program (<http://www.openepi.com/v37/Proportion/Proportion.htm>).

## 3. Results

The genomic DNA sequences of the detected blood parasite in camel's blood were subjected to PCR with universal primers for all possible strains of *Babesia* and *Trypanosoma* species that was directed to amplify small ribosomal subunit in those parasites in order to conduct molecular diagnostic approach for blood parasite in camels. The infection rates with *B. bovis*, *B. microti*, and *T. evansi* were 2.81%, 11.97 %, and 5.63%, respectively (Table 2). Surprising, the highest infection rate in the present study was achieved by *B. microti* (Table 1). The species-specific PCR assays detected the surveyed *Babesia* (*B. bovis*, and *B. microti*) and KIN-multi species PCR reaction detected

*Trypanosoma* species in the camel populations (Supplementary Fig. 1). Seven samples harbored mixed infections from *Babesia* and *Trypanosoma* parasites species. In details, one sample was infected with *B. bovis*, *B. microti* and *Trypanosoma* species, two samples were positive for *B. bovis* and *B. microti*, and four samples were positive for *B. microti* and *Trypanosoma* species.

Table 2. PCR detection of blood parasites in camels

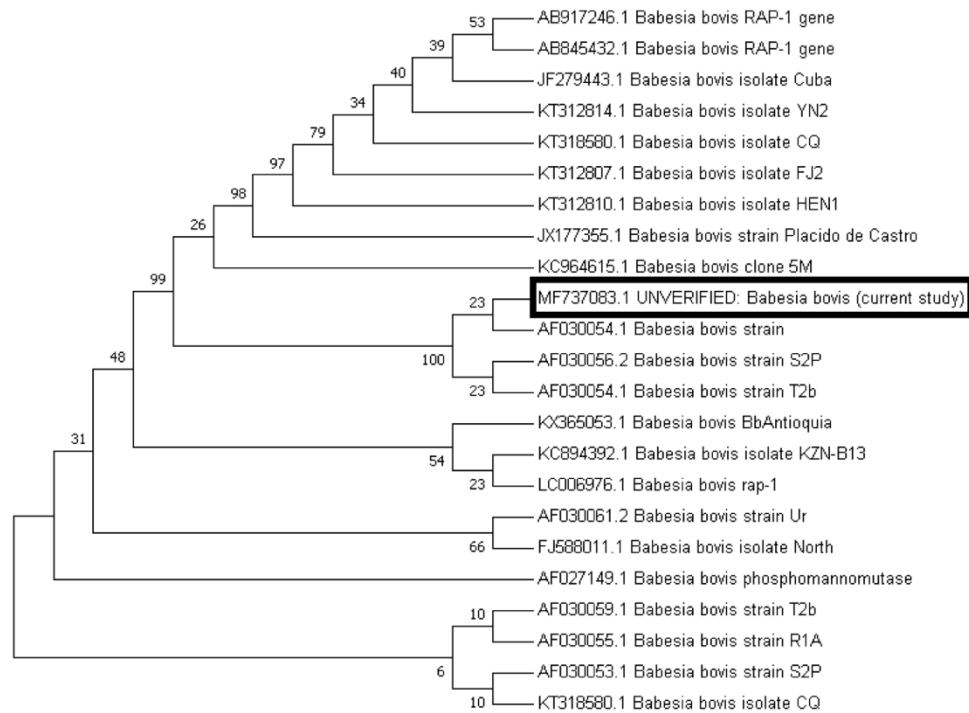
Species	Positive No.	% CI <sup>a</sup>
<i>B. bovis</i>	4	2.81 (1.10-7.01)
<i>B. microti</i>	17	11.97 (7.61-18.34)
<i>T. evansi</i>	8	5.63 (2.88-10.72)

<sup>a</sup> 95% confidence interval.

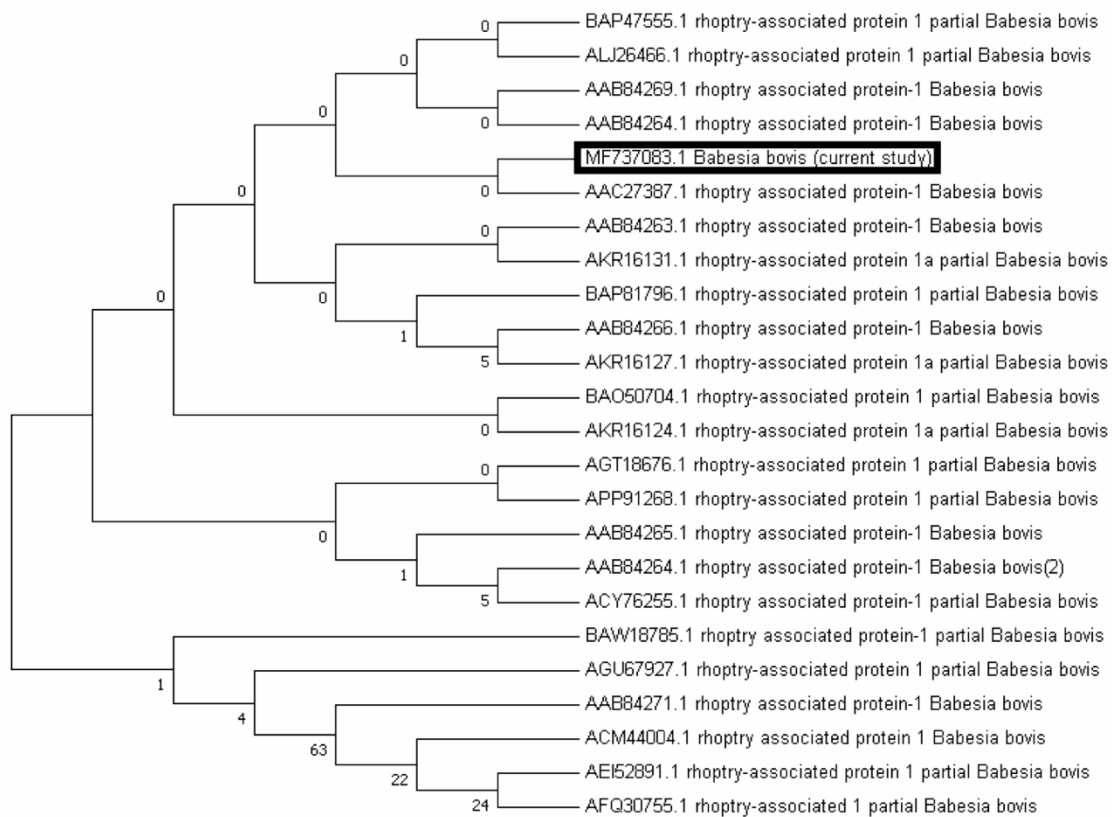
The nucleotide sequences of PCR-amplified *RAP-1*, and *ss-rRNA* genes of *Babesia* species and *ITS1* gene of *Trypanosoma* species determined in the present study have been registered and assigned the following GenBank accession numbers: *B. bovis* (MF737083.1), *B. microti* (MF737082.1), and *T. evansi* (MF737081.1). For phylogenetic analysis, nucleotide sequences of target genes from other species of *Babesia* and *Trypanosoma* were included for comparison (Tables S1 and S2).

The detection of *B. bovis* in blood of camel was determined with primer pairs flanking *RAP-1*. The polymerase chain reaction of *RAP-1* produced an amplicon size of 298 bp that was checked on GenBank for further confirmation of the desired sequence. The currently checked sequence was deposited on GenBank under accession number of MF737083.1, where similarity index was tested among other related *B. bovis* species on GenBank. Phylogenetic analysis was constructed between our obtained species and other related species of *RAP-1* gene in *B. bovis*. The phylogenetic analysis revealed the significant decrease in genetic distance between our obtained species and other species isolated from Argentina (AF030056), USA (AF030054) and Brazil (KC964615) (Fig. 2). Inter-species genetic distance analysis was conducted between our obtained *RAP-1* gene sequences and other related taxa and a close genetic distance was detected between our isolated strain and other taxa from Argentina (AF030056.2) and USA (AF030054.1) in cattle (Supplementary Fig. 2). For further confirmation, amino acids sequence was deduced from our obtained sequence and aligned with other related sequence of *RAP-1* protein (Supplementary Fig. 2). The results of protein's phylogenetic analysis showed a close genetic relationship with other strain from Mexico (AAC27387.1) (Fig. 3). Pairwise genetic distance was performed between our obtained amino acid sequence and other related species of *RAP-1* genes and revealed a close relationship with all aligned species except the isolated species from Brazilian bovine species (AFQ30755.1) (Supplementary Fig. 3).



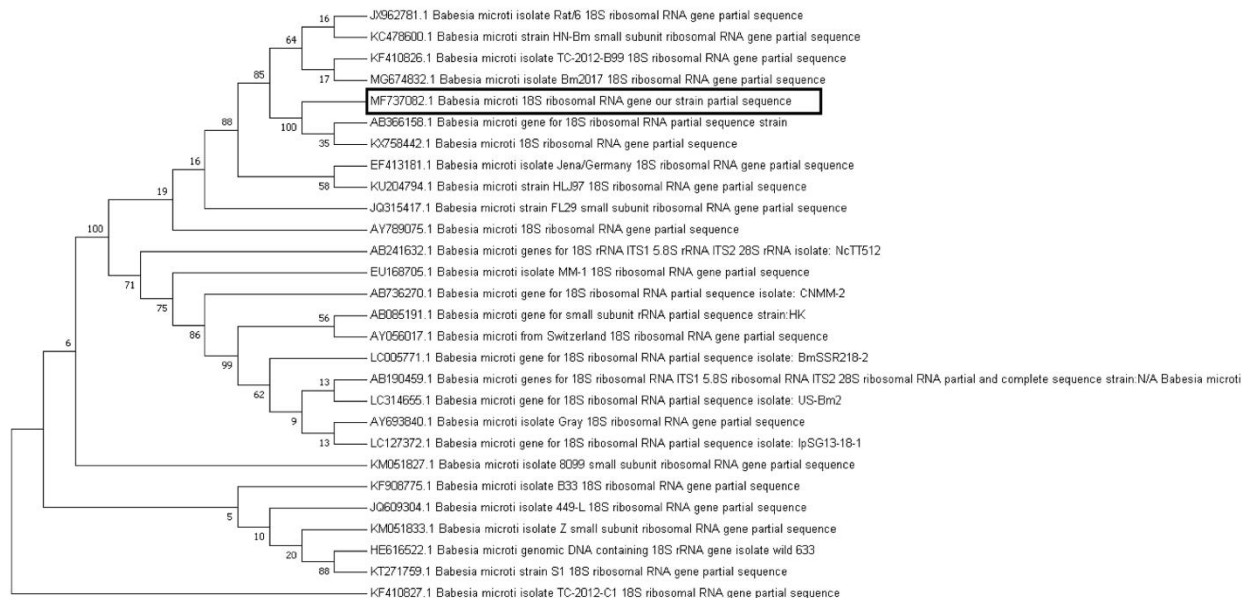


**Figure 2. Phylogenetic tree of the *B. bovis* RAP-1 gene.** The nucleotide sequences determined in this study are shown in boldface type letters. Bootstrap values are provided at the beginning of each branch. The sequence identified from Egypt in this study is boxed in black.



**Figure 3. Protein's phylogenetic tree of the *B. bovis* RAP-I gene.** The amino acids 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.

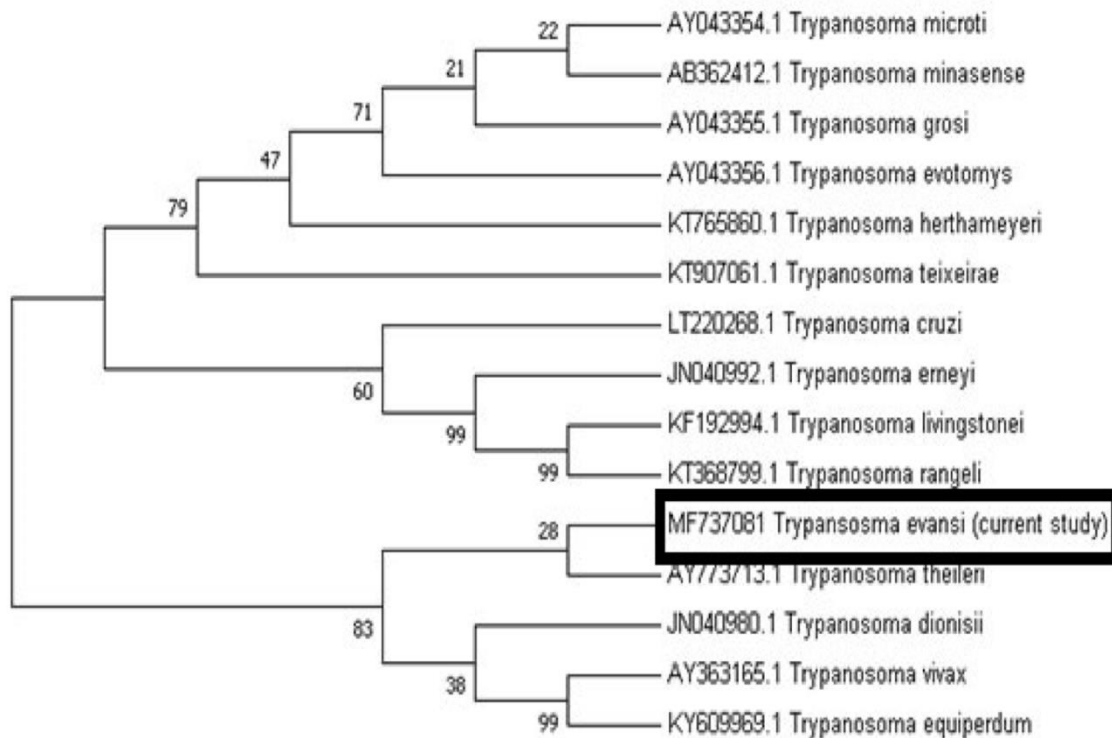
Amplification of small ribosomal subunit of *B. microti* produced an amplicon size of 154 bp that was excised, sequenced and check for similarity index on GenBank to construct a phylogenetic tree for evaluating closely related strains of *B. microti* that was genetically related to our isolated species. A GenBank accession number was granted for the deposited sequence (MF737082.1). The result of phylogenetic analysis showed the closely related genetic relationship of our isolated species of *B. microti* with German strain in rat (AB366158.1) and voles in France (KX758442.1) (Fig. 4). The interspecies distance between our isolated sequence of small ribosomal subunit of *B. microti* and other related species of *B. bovis* and revealed a close genetic relationship with (AB366158.1) and (KX758442.1) from Germany in rats and France in voles, respectively (Supplementary Fig. 4).



**Figure 4. 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.

Internal transcript spacer 1 was used for simultaneous identification of detection of three major trypanosome species (*T. evansi*, *T. congolense* and *T. vivax*)- camel in Upper Egypt with a product size of 540 bp using a KIN-multi species PCR assay. The sequence results indicated that *T. evansi* is the causative agent of trypanosomiasis in camels under study. An accession number was deposited on GenBank for our obtained sequence (MF737081) (Fig. 5). Phylogenetic tree was constructed between our isolated species of *T. evansi* and other related species where the higher similarity index species were aligned with our sequence and a lower genetic distance was observed between our isolated sequence and *T. theileri* that was isolated from cattle in Brazil. The phylogenetic analysis revealed the presence of two clades (mammalian and non-mammalian clades), where *T. evansi* clustered with the mammalian group

(Fig. 5). Genetic distance analysis of our isolated *T. evansi* and other related trypanosome species and the result revealed a close genetic distance with (AY0433156.1) from United kingdom in Bank vole, (AY043355.1) from United kingdom in wood mouse and (AY043354.1) from United kingdom in field vole with 0.69 value in comparison with other included taxa (Supplementary Fig. 5).



**Figure 5. Phylogenetic tree of the *T. evansi* ITS1 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.

#### 4. Discussion

A phylogenetic analysis was designed for our isolated sequence and others with high similarity index using neighbor joining method for statistical analysis of phylogenetic analysis with 500 replicates. A close genetic

relationship was observed between our strain and other strains from Argentina, USA and Brazil. Similar result was investigated by a research work conducted by [21], they found that nested PCR technique conducted to amplify rhoptry-associated protein 1 (*RAP-1*) produced a close genetic relationship of South African strain of *B. bovis* strains with other strains from Uruguay, Argentina, Brazil and USA. However, the presence of polymorphism can adequately discriminate between a species and another [22]. Similarly, the conservation of *Babesia* spp. sequence between species was observed between Brazilian strains of *B. bovis* (98-100%) [23].

The amplification of small ribosomal subunit of *B. microti* was conducted which is considered a first record identification of *B. microti* in camels. *B. microti* was first discovered in the united states among human patients infecting mature erythrocytes [24]. In the current study, phylogenetic analysis of *18s rRNA* gene of *B. microti* was revealed a close relationship between our isolated species and other species in Germany and France with a low genetic distance which was isolated from rodents. In Mongolia, a phylogenetic characterization of *B. microti* was executed and revealed a higher similarity index between isolated species of *B. microti* and other strains from united states, where the small ribosomal subunit gene was clustered with the United States -type of *B. microti* [25]. In fact, human babesiosis was detected in Egypt by El Bahnasawy and Morsy [26]. The symptoms, complete blood count, liver and kidney functions tests and all other serologic tests did not give concert diagnosis for *B. microti* in study performed by El Bahnasawy and Morsy [26]. Additionally, the patients were sero-negative for malaria infection. The patients were critically diagnosed by the demonstration of the typical ring forms of *Babesia* species in stained blood smears. These clinical cases were successfully treated with quinine and clindamycin, and were discharged from the hospital after the clinical and parasitological improvement. As this study demonstrated the infection of camels reared in Halayeb and Shalateen, in Lower Egypt by *B. microti*, future critical concerns must be taken to clarify the possible role of camels and its infested tick spp. in the zoonotic infection of babesiosis in Egypt. Importantly, the samples used in the present study were collected from apparently healthy camels, highlighting the fact that camels in Egypt might act as a silent reservoir for the infection of human by *Babesia* spp.

Internal transcript spacer 1 was used for simultaneous detection of three major trypanosome species (*T. evansi*, *T. congolense* and *T. vivax*) in circulating blood of camels. Phylogenetic analysis was conducted to identify the genetic relationship between our isolated species and other species of *Trypanosma* species. In the current study, *T. evansi* was identified by sequencing of the PCR products and was closely related to *T. theileri* that was detected from cattle in Brazil. *Trypanosome* is classified into several clades in a study in 1996 by Maslov et al., [27] and are as

follows: mammalian, bird group, elasmobranch and are basically classified into vertebrate and in-vertebrates parasite. Hughes and Piontkivska, [28] classified trypanosome species according to amino acid sequence of small ribosomal subunit into 42 protein families. They also classified *Trypanosoma* families according to host and location into American, African and bonoidae. In Egypt, internal transcript spacer 1 was used to investigate the phylogenetic relationship between trypanosome species and showed a genetic diversity among those species that can be used as a diagnostic tool for the identification of trypanosome species [12].

It should be noted that there are some limitations to the present study. Although this study report the first molecular evidence of *Babesia microti* in camel, the microscopy examination of the screened samples was not performed. Therefore, further investigation of *Babesia microti* in camels in Egypt by microscopy examination is needed. Also, to investigate the possible role of camels and its infesting tick as reservoir host for maintaining an enzootic cycle of *Babesia* transmission in Egypt, the simultaneous detection of *B. microti* prevalence in the tick species infesting camels, camels and human in the same area are urgently required. The small sample size used in this study may not allow obtaining a concrete conclusion about the incidence of different hemoparasites included in the study. Moreover, this study was carried out in Upper Egypt only makes the need for further investigation in other areas in Egypt are needed.

## 5. Conclusions

This study provides the first evidence of *B. microti* in camel in Egypt and highlights the possible role of one-humped camels in maintaining the zoonotic cycle of *Babesia* transmission in Egypt. While, the prevalence of *B. microti* infection in the most prevalent tick species infesting camels need to be further identified which may imply its possible role served as a reservoir host for maintaining an enzootic cycle of *Babesia* 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 infection by blood parasites in camels.

## Acknowledgments

The authors would like to thank Prof. Ikuo Igarashi, and Prof. Naoaki Yokoyama (both from National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-Cho,

Obihiro, Hokkaido, Japan) for providing the authors by the positive control DNA samples. Also, the authors would like to thank owners and staff of the study farms in Egypt.

### Author Contributions

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

### Funding

This research received no external funding

### Competing interests

The authors declare that they have no competing interests.

### References

1. Zarrin, M.; Riveros, J.L.; Ahmadpour, A.; de Almeida, A.M.; Konuspayeva, G.; Vargas-Bello-Pérez, E.; Faye, B.; Hernández-Castellano, L.E. Camelids: new players in the international animal production context. *Trop Anim Health Prod* **2020**, *52*, 903-913, doi:10.1007/s11250-019-02197-2.
2. Mihic, T.; Rainkie, D.; Wilby, K.J.; Pawluk, S.A. The Therapeutic Effects of Camel Milk: A Systematic Review of Animal and Human Trials. *J Evid Based Complementary Altern Med* **2016**, *21*, Np110-126, doi:10.1177/2156587216658846.
3. Hirad, A.H.; Ahmad, J.; Alkhedairy, A.A.; Bahkali, A.H.; Khan, S.T. Bacterial isolates exhibiting multidrug resistance, hemolytic activity, and high 16S rRNA gene similarity with well-known pathogens found in camel milk samples of Riyadh region. *Apmis* **2018**, *126*, 215-226, doi:10.1111/apm.12802.
4. Abbas, B.; Agab, H. A review of camel brucellosis. *Prev Vet Med* **2002**, *55*, 47-56, doi:10.1016/s0167-5877(02)00055-7.
5. Elsify, A.; Sivakumar, T.; Nayel, M.; Salama, A.; Elkhtam, A.; Rizk, M.; Mosaab, O.; Sultan, K.; Elsayed, S.; Igarashi, I., et al. An epidemiological survey of bovine Babesia and Theileria parasites in cattle, buffaloes, and sheep in Egypt. *Parasitol Int* **2015**, *64*, 79-85, doi:10.1016/j.parint.2014.10.002.

6. Qablan, M.A.; Sloboda, M.; Jirku, M.; Obornik, M.; Dwairi, S.; Amr, Z.S.; Horin, P.; Lukes, J.; Modry, D. Quest for the piroplasms in camels: identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR. *Vet Parasitol* **2012**, *186*, 456-460, doi:10.1016/j.vetpar.2011.11.070.
7. Rizk, M.A.; El-Sayed, S.A.E.; Nassif, M.; Mosqueda, J.; Xuan, X.; Igarashi, I. Assay methods for in vitro and in vivo anti-Babesia drug efficacy testing: Current progress, outlook, and challenges. *Vet Parasitol* **2020**, *279*, 109013, doi:10.1016/j.vetpar.2019.109013.
8. Rizk, M.A.; El-Sayed, S.A.E.; AbouLaila, M.; Eltaysh, R.; Yokoyama, N.; Igarashi, I. Performance and consistency of a fluorescence-based high-throughput screening assay for use in Babesia drug screening in mice. *Sci Rep* **2017**, *7*, 12774, doi:10.1038/s41598-017-13052-5.
9. Chen, X.R.; Ye, L.I.; Fan, J.W.; Li, C.; Tang, F.; Liu, W.; Ren, L.Z.; Bai, J.Y. Detection of Kobe-type and Otsu-type *Babesia microti* in wild rodents in China's Yunnan province. *Epidemiol Infect* **2017**, *145*, 2704-2710, doi:10.1017/s0950268817001686.
10. Desquesnes, M.; Dargantes, A.; Lai, D.H.; Lun, Z.R.; Holzmuller, P.; Jittapalapong, S. *Trypanosoma evansi* and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *Biomed Res Int* **2013**, *2013*, 321237, doi:10.1155/2013/321237.
11. Elhaig, M.M.; Youssef, A.I.; El-Gayar, A.K. Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia, Egypt. *Vet Parasitol* **2013**, *198*, 214-218, doi:10.1016/j.vetpar.2013.08.003.
12. Amer, S.; Ryu, O.; Tada, C.; Fukuda, Y.; Inoue, N.; Nakai, Y. Molecular identification and phylogenetic analysis of *Trypanosoma evansi* from dromedary camels (*Camelus dromedarius*) in Egypt, a pilot study. *Acta Trop* **2011**, *117*, 39-46, doi:10.1016/j.actatropica.2010.09.010.
13. Arjmand Yamchi, J.; Tavassoli, M. Survey on infection rate, vectors and molecular identification of *Theileria annulata* in cattle from North West, Iran. *J Parasit Dis* **2016**, *40*, 1071-1076, doi:10.1007/s12639-014-0636-y.
14. El-Sayed, S.; Rizk, M.A.; Terkawi, M.; Igarashi, I. Cocktail Babesia bovis antigens for global detection of Babesia bovis infection in cattle. *Exp Parasitol* **2019**, *206*, 107758, doi:10.1016/j.exppara.2019.107758.
15. Mtshali, M.S.; Mtshali, P.S. Molecular diagnosis and phylogenetic analysis of *Babesia bigemina* and *Babesia bovis* hemoparasites from cattle in South Africa. *BMC Vet Res* **2013**, *9*, 154, doi:10.1186/1746-6148-9-154.
16. Mossaad, E.; Salim, B.; Sukanuma, K.; Musinguzi, P.; Hassan, M.A.; Elamin, E.A.; Mohammed, G.E.; Bakhiet, A.O.; Xuan, X.; Satti, R.A., et al. *Trypanosoma vivax* is the second leading cause of camel trypanosomosis in Sudan after *Trypanosoma evansi*. *Parasit Vectors* **2017**, *10*, 176, doi:10.1186/s13071-017-2117-5.
17. Rizk, M.A.; AbouLaila, M.; El-Sayed, S.A.E.; Guswanto, A.; Yokoyama, N.; Igarashi, I. Inhibitory effects of fluoroquinolone antibiotics on *Babesia divergens* and *Babesia microti*, blood parasites of veterinary and zoonotic importance. *Infect Drug Resist* **2018**, *11*, 1605-1615, doi:10.2147/IDR.S159519.
18. Saitou, N.; Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **1987**, *4*, 406-425, doi:10.1093/oxfordjournals.molbev.a040454.



19. Felsenstein, J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* **1985**, *39*, 783-791, doi:10.1111/j.1558-5646.1985.tb00420.x.
20. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **2013**, *30*, 2725-2729, doi:10.1093/molbev/mst197.
21. Mtshali, P.S.; Tsotetsi, A.M.; Thekiso, M.M.; Mtshali, M.S. Nested PCR detection and phylogenetic analysis of *Babesia bovis* and *Babesia bigemina* in cattle from Peri-urban localities in Gauteng Province, South Africa. *J Vet Med Sci* **2014**, *76*, 145-150, doi:10.1292/jvms.13-0197.
22. Fisher, T.G.; McElwain, T.F.; Palmer, G.H. Molecular basis for variable expression of merozoite surface antigen gp45 among American isolates of *Babesia bigemina*. *Infect Immun* **2001**, *69*, 3782-3790, doi:10.1128/IAI.69.6.3782-3790.2001.
23. Ramos, C.A.; Araújo, F.R.; Alves, L.C.; de, S., II; Guedes, D.S., Jr.; Soares, C.O. Genetic conservation of potentially immunogenic proteins among Brazilian isolates of *Babesia bovis*. *Vet Parasitol* **2012**, *187*, 548-552, doi:10.1016/j.vetpar.2012.01.020.
24. Skariah, S.; Arnaboldi, P.; Dattwyler, R.J.; Sultan, A.A.; Gaylets, C.; Walwyn, O.; Mulhall, H.; Wu, X.; Dargham, S.R.; Mordue, D.G. Elimination of *Babesia microti* Is Dependent on Intraerythrocytic Killing and CD4(+) T Cells. *J Immunol* **2017**, *199*, 633-642, doi:10.4049/jimmunol.1601193.
25. Tuvshintulga, B.; Sivakumar, T.; Battsetseg, B.; Narantsatsaral, S.O.; Enkhtaivan, B.; Battur, B.; Hayashida, K.; Okubo, K.; Ishizaki, T.; Inoue, N., et al. The PCR detection and phylogenetic characterization of *Babesia microti* in questing ticks in Mongolia. *Parasitol Int* **2015**, *64*, 527-532, doi:10.1016/j.parint.2015.07.007.
26. El-Bahnasawy, M.M.; Morsy, T.A. Egyptian human babesiosis and general review. *J Egypt Soc Parasitol* **2008**, *38*, 265-272.
27. Maslov, D.A.; Lukes, J.; Jirku, M.; Simpson, L. Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. *Mol Biochem Parasitol* **1996**, *75*, 197-205, doi:10.1016/0166-6851(95)02526-x.
28. Hughes, A.L.; Piontkivska, H. Molecular phylogenetics of Trypanosomatidae: contrasting results from 18S rRNA and protein phylogenies. *Kinetoplastid Biol Dis* **2003**, *2*, 15, doi:10.1186/1475-9292-2-15.

1