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Identification and molecular characterization of some blood parasites in camels

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ABSTRACT

Egyptian camels are vulnerable to blood parasitic infection, which is an economically important tick-borne diseases and compromise health and production of camels. Blood samples were collected for microscopic identification and molecular detection with phylogenetic analysis of piroplasms in apparently healthy camels ' number 82' of both sexes in Assiut, Egypt. The overall positive rates of *Babesia* was 62.2% and *Theileria* was 35.4% microsobically which revealed that *Babesia* infection is higher than *Theileria* in camels. The positive rates of *Babesia* and *Theileria* species were higher in females (71.7%) and (36.9%) than males (50%) and (33.3%), respectively. Likewise, the positive rates of *Babesia* and *Theileria* species were higher in camels > 3 years (68.8%) and (37.5%) than ≤ 3 years (52.9%) and (32.4%), respectively. Statistically, the positive rate of *Babesia* is significantly associated with gender (P< 0.05). PCR analysis targeted *Babesia* 18S rRNA and *Theileria annulata tams*1 genes. The phylogenetic analysis of PCR amplicons shown that analyzed isolates were *Babesia caballi* (*B. caballi*) with accession numbers (OR683438 and OR683439) and *Theileria annulata* (*T. annulata*) with accession numbers (OR682402 and OR682403) which closely related to global isolates present in NCBI. Genetically, *B. caballi* was related to isolates from South Africa, Cuba and Venezuela horses, while *T. annulata* was genetically related to isolates from United Kingdom, India and Turkey. We concluded that *B. caballi* and *T. annulata* is prevalent in camels with the need of early diagnosis and treatment to prevent further spread of infection with tick control program to reduce the risk of infection.

Introduction

Camel is a widely dispersed domestic animal in the semi-desert and deserts regions of different continents. The global population of camels is approximately '39 million' (FAOSTAT, 2023). It provides many areas of the world with an important source of milk and meat which is considered a high source for protein giving high nutritional value. There is a steadily rising in the number of slaughtered camels for meat in lots of developing countries, including Egypt (Abou El-Naga and Barghash, 2016). Even though camels are tough animals that can survive the severe desert environment as a result of their unique adaptive physiological individualities. They are exposed to a wide range of infectious diseases such as ecto and endoparasitic diseases that affect camel health (Swelum *et al*., 2014; Sazmand and Joachim, 2017; Selim *et al*., 2023).

Piroplasmosis in camel is an infectious disease spread globally and caused by hemoprotozoan parasites, that belong to the 'phylum Apicomplexa'. Haemoparasitic diseases have a significant effect on camels health causing a substantial economic burden to the camel owners and breeders (Aslam *et al*., 2022). It has adverse effects on camels leading to fever, wasting, anemia and death in case of intense infections (Abou El-Naga and Barghash 2016; Selim *et al*., 2023) and characterized by high morbidity and mortality, especially if untreated and it is responsible for significant financial losses (Faraj and Abd, 2018).

Ashour *et al*. (2023) and Salman *et al*. (2022) found that Egyptian camels infected with *B. bovis, B. bigemina, B. microti, Babesia* sp. Mymensingh, *T. equi*, *Theileria* sp. Yokoyama. Furthermore, camel infections have also been reported to be caused by *B. caballi*, *T. annulata*, *Theileria equi, Theileria mutans* and *Theileria ovis* (Qablan *et al*., 2012, Tomassone *et al*., 2012; Abd-Elmaleck *et al*., 2014; Lorusso *et al*., 2016; Aslam *et al*., 2022).

Babesiosis is a protozoan parasitic disease spread globally by vectors (Haghi *et al*., 2014). *B. caballi* was initially diagnosed in Sudanese camels (Abdelrahim *et al*., 2009). It is one of the most essential *Babesia* species that have been recorded in camels in a different parts of the world (Jasim *et al*., 2015; Ibrahim *et al*., 2017). Through the acute stage of the disease, it causes fever, anemia, hemoglobinuria, jaundice, edema, weakness, loss of appetite, depression, and gastrointestinal stasis which sometimes cause camel death (Swelum *et al*., 2014; Mirahmadi *et al*., 2022).

Theileriosis, resulted from hemoprotozoan infection of the 'genus *Theileria*' which has detrimental economic impact on the livestock sector in the majority of subtropical and tropical countries (Ismaeil *et al*., 2023). It has a substantial impact on camels production and reproduction causing wasting, fever, anemia and mortality in cases of serious infections (Hamed *et al*., 2011; Ismael *et al*., 2014; Selim *et al*., 2023).

The ordinary diagnostic method for confirming animals infected with blood parasites is microscopic identification of Giemsa stained blood smear which considered a rapid field test (Mirahmadi *et al*., 2022). The molecular diagnostic tests are specific and sensitive approach that results in the precise detection, identification and distingishing between *Babesia* and *Theileria* species, specifically in the status of low parasitemia (Mirahmadi *et al*., 2022; Selim *et al*., 2023). The DNA sequencing is considered a perfect diagnostic technique which recognized on PCR amplification consuming generic primers with exceptionally preserved ribosomal gene sequence amplification (Ullah *et al*., 2022).

There is scarce of information on theileriosis and babesiosis in camels in Assiut governorate, Egypt. Therefore, the current study was planned to determine their positive rate using direct microscope along with the sequence analysis of detected isolates to identify *Babesia* and *Theileria* species of the local camels.

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Materials and methods

Ethics approval

It was approved by The Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to The OIE standards for the use of animals in researches with (Approval No. 06/2023/0138).

Animals and blood sampling

A total of '82 blood samples' were collected from apparently healthy camels of different ages (2–6 years) and of both sexes. This study was carried out from April 2023 to December 2023 in Assiut governorate, Egypt. Approximately 3 mL of blood samples were collected from each animal in tubes containing ethylenediaminetetraacetic acid (EDTA) and immediately transported to the laboratory.

Microscopic examination

Thin and thick blood smears were prepared from whole blood on dry and clean slides, stained with Giemsa and examined for blood parasites identification by microscope at 1000× (oil immersion lens) based on morphological characteristics as described by Soulsby (1982) and Coles (1986). Then the rest of the blood samples were kept at -20°C untill DNA extraction and additional examination.

Molecular detection of blood parasites

Six samples was examined by PCR for identification of parasite species.

DNA extraction

It was done using the 'QIAamp DNA Mini kit (Qiagen, Germany, GmbH)' and was modified according to the manufacturer's guidances. 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at '56°C' for 10 min. 200 µl of 100% ethanol was added to the lysate after the incubation. The sample was centrifuged after washing following the manufacturer's guidances. Elution of the nucleic acid was done using 100 µl of elution buffer which supplied by the kit.

Oligonucleotide Primer

The used primers were provided from 'Metabion (Germany)' are listed in Table 1.

PCR amplification

Primers were utilized in a 25 µl reaction that contain 12.5 µl of 'EmeraldAmp Max PCR Master Mix (Takara, Japan)', 1 µl of each primer at a concentration of 20 pmol, 4.5 µl of water, and 6 µl of DNA template. The positive control samples of PCR were obtained from 'Biotechnology unit in Animal Health Research Institute'. This reaction was implemented in an 'Applied biosystem 2720 thermal cycler'.

Analysis of the PCR Products

The PCR products were separated via electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer' using gradients of 5V/cm at room temperature. 15 µl of the products was loaded in each gel slot for gel analysis. The determination of the fragment sizes was done by using a generuler 100 bp ladder (Fermentas, Germany). The gel was photographed using 'a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed by means of computer software.

DNA Sequencing and phylogenetic analysis

Four positive PCR products were chosen then purified via 'QIAquick PCR Product extraction kit (Qiagen, Valencia)'. The sequence reaction was performed using 'Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer)' and then was purified by Centrisep spin column. DNA sequences were gained by 'Applied Biosystems 3130 genetic analyzer (HITACHI, Japan)'. A BLAST® analysis (Basic Local Alignment Search Tool) was at first performed to create sequence identity to accessions in GenBank (Altschul *et al*., 1990). The phylogenetic tree was established by the 'MegAlign module of LasergeneDNAStar version 12.1' (Thompson *et al*., 1994). The phylogenetic analyses was performed via 'maximum likelihood, neighbour joining and maximum parsimony' in MEGA6 (Tamura *et al*., 2013).

Statistical analysis

Microsoft Excel was used to record and categorize all of the data. After that, SPSS software ver. 16.0 (IBM, USA) was used to analyze the data. The possible correlation between age, sex and infection rate was investigated using the chi-square ($\chi 2$) test. The results are deemed significant if the '*P* value' is less than 0.05 (Nakayima *et al*., 2017).

Results

The examination of '82 camels' for the presence of blood parasites showed that the positive rates of *Babesia* and *Theileria* species were 62.2% (51/82) and 35.4% (29/82) by microscope, respectively. This findings revealed higher infection with *Babesia* than *Theileria* in camels. The positive rates of *Babesia* species were higher in females (71.7%) than males (50%) with a significant difference ($P<0.05$). Likewise, the positive rates of *Theileria* species were greater in females (36.9%) than males (33.3%). While, the positive rates of *Babesia* and *Theileria* species were higher in camels of age group >3 years (68.8%) and (37.5%) than of age group ≤ 3 years (52.9%) and (32.4%), respectively. There was no significant difference between gender of camels and *Theileria* positive rate and between the age of camels and *Theileria* and *Babesia* positive rate (Table 2).

Giemsa stained blood smears of camels showed the presence of intraerythrocytic *Babesia* and *Theileria* species. Intraerythrocytic trophozoites of *Babesia* appeared pear-shaped (2.1–3.7 µm in length) commonly occurring single, round or oval forms (2.2–2.7 µm in diameter) (Figure

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions used for PCR amplifications.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Babeisa 18S rRNA	GTCTTGTAATTGGAATGATGGTGAC ATGCCCCCAACCGTTCCTATTA	340	94˚C 5 min.	94˚C 30 s.	55˚C 40 s.	72˚C 40 s.	72˚C 10 min.	Salem and Farag (2014)
Theileria annulata tams1	GTAACCTTTAAAAACGT GTTACGAACATGGGTTT	721	94˚C 5 min.	94˚C 30 s.	55˚C 40 s.	72˚C 45 s.	72˚C 10 min	Nourollahi-Fard *et al*. (2015)
Theileria equi 16S rRNA	CATCGTTGCGGCTTGGTTGG CCAAGTCTCACACCCTATTT	664	94˚C 5 min.	94˚C 30 s.	54˚C 45 s.	72˚C 45 s.	72˚C 10 min	Bashiruddin *et al*. (1999)

1). While, intraerythrocytic *Theileria* trophozoites were mainly round (0.66–1.25 µm) to oval (1.58x0.66 µm), rod or comma-shaped (1.41x0.58 µm). *Theileria* Koch's blue bodies were detected in the lymphocytes comprising micromeronts which contain chromatin granules (0.11–0.67 µm in diameter) (Figure 2). The specific amplications of *Babesia* 18S rRNA gene (340 bp) and *T. annulata tams*1 gene (721 bp) was discovered by PCR assays with no existence of *T. equi* (Figure 3 A, B and C).

Every PCR-positive sample was subjected to sequencing. The phylogenetic analysis of PCR amplicons showed that analyzed isolates were closely related to *B. caballi* which belongs to genotype A and *T. annulata* existing in NCBI from various global locations. *Babesia caballi* is avilable in the GenBank with the accession numbers (OR683438 and OR683439). The *B. caballi* OR683438 sequence was identified and showed 100% correspondence with reported from South Africa horses (EU642512), Cuba horse (MT463343) and Venezuela horse (MZ298263). While, OR683439 sequence revealed 75.2% similarity with OR683438 sequence that mentioned in this study and from South Africa horse (EU642512)), Cuba horse (MT463343) and Venezuela horse (MZ298263) (Figure 4, 5).

In the current study, *T. annulata* is avilable in the GenBank with the accession numbers (OR682402 and OR682403). The *T. annulata* OR682402

Table 2. Positive rate of *Babesia* and *Theileria* species in camels in relation to gender and age.

Variable	Variable levels	No. of examined animals	No. of *Babesia* Positive samples	*Babesia* positive rate (%)	Chi2 (χ2)	P- value	No. of *Theileria* Positive samples	*Theileria* positive rate (%)	Chi2 (χ2)	P- value
Gender	Female	46	33	71.7	4.059*	0.04	17	36.9	0.12	0.73
	male	36	18	50			12	33.3		
Age	≤ 3 years	34	18	52.9	2.12	0.15	11	32.4	0.23	0.63
	>3 years	48	33	68.8			18	37.5		
Total		82	51	62.2			29	35.4		

*: Significant difference (P<0.05)

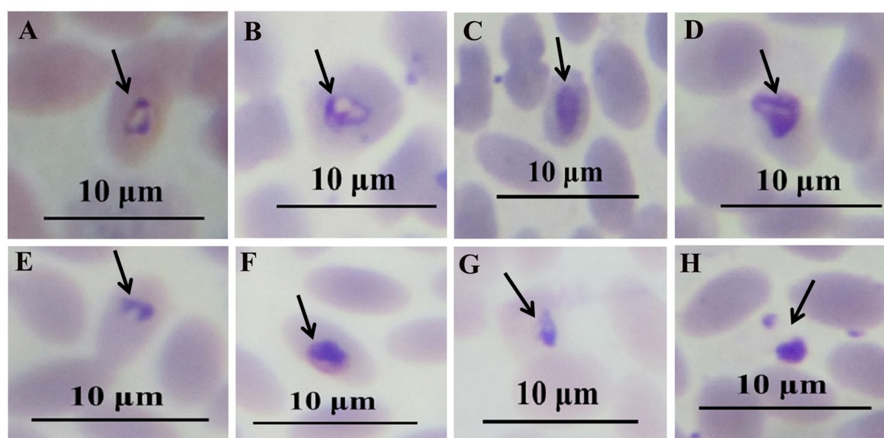

Figure 1. Microscopic image of Giemsa stained blood smears of camels showing a presence of *Babesia* spp. indicated by arrows A, B, C, D, E: intra-erythrocytic pear shape, F: intra-erythrocytic round shape and G, H: free bear shape outside erythrocytes at 1000x (oil immersion lens) (scale bar 10 µm).

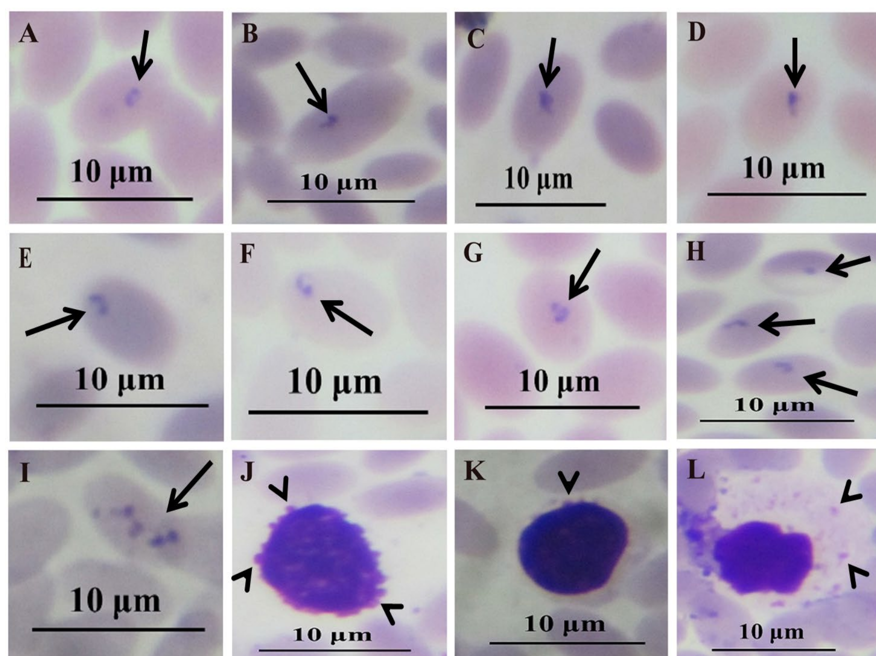

Figure 2. Microscopic image of Giemsa stained blood smears of camels showing intra-erythrocytic pear, oval, ring and comma shaped *Theileria annulata* indicated by arrows. A, B, C, D, E, F, G: single intra-erythrocytic *Theileria*, while H, I: multiple intra-erythrocytic *Theileria* and J, K, L: lymphocytes contain chromatin granules of Koch,s blue bodies (arrow heads) at 1000x (oil immersion lens) (scale bar 10 µm).

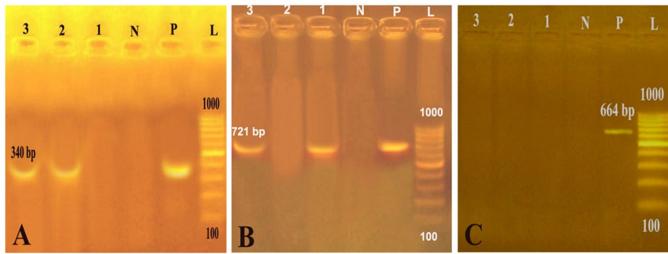

Figure 3. Gel electrophoresis pattern of PCR amplicon of camel blood samples showing: L: 100bp DNA ladder; P: Positive control; N: Negative control (A): using *B. caballi*-specific primer; 1: Negative samples; 2, 3: Positive samples (340 bp PCR product). (B): using *T. annulata*-specific primer; 1, 3: Positive samples (721 bp PCR product); 2: Negative samples. (C): using *T. equi*-specific primer; 1, 2, 3: Negative samples.

sequence was identified and showed 100% correspondence with that recorded from United Kingdom (KX981037) and showed 99.8% similarity with detected from United Kingdom (KX981038). While, OR682403 sequence were revealed 97.3% similarity with OR682402 that found in the current work and demonstrated 100% similarity with recorded from India (MF346012), United Kingdom (KX981026) and Turkey (MW412254) (Table 3 and Figures 6,7).

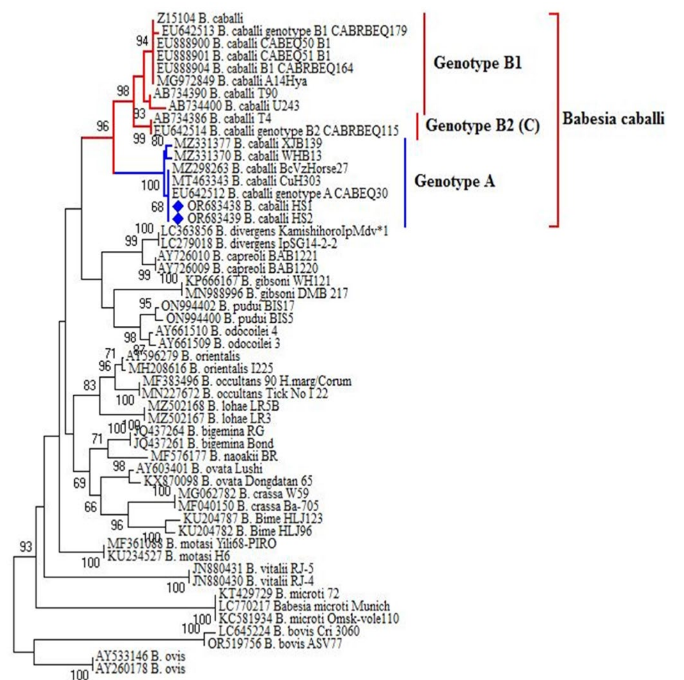

Figure 4. Phylogenetic relationship of 18s rRNA gene of *B. caballi* two isolates identified in current study with closely related *Babesia* species recorded in the GenBank already detected worldwide. New sequences isolates were 'OR683438 *B. caballi* HS1 and OR683439 *B. caballi* HS2' marked with blue diamond.

Percent Identity (upper part) / Divergence (lower part)

	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	28		
1		77.2	77.7	72.0	72.8	76.6	73.6	75.5	75.8	77.7	76.6	63.6	72.3	76.9	76.9	77.2	76.9	76.4	75.0	75.8	75.8	75.3	75.3	74.7	75.0	75.3	75.3	58.3	1	LC645224 B. bovis Cri_3060
2	21.9		93.8	83.7	84.0	88.3	82.1	88.6	89.1	89.1	90.2	71.5	83.7	86.4	86.4	86.1	86.4	86.1	84.8	87.0	87.0	86.7	86.7	86.1	86.1	86.7	86.7	63.8	2	MG062782 B. crassa W59
3	21.4	7.2		82.3	83.7	87.5	81.2	88.0	87.0	87.8	88.6	72.0	83.4	86.4	86.4	86.1	86.4	85.6	84.2	86.4	86.4	85.9	85.9	85.3	85.3	85.9	85.9	63.5	3	KU204787 B. Bime HLJ123
4	24.4	13.9	15.5		92.7	83.2	79.9	84.0	84.0	86.4	84.0	73.6	90.5	87.8	87.8	87.5	87.8	87.5	86.1	88.0	88.0	88.3	88.3	87.8	88.3	88.3	88.3	65.1	4	LC279018 B. divergens IpSG14-2-2
5	22.6	12.5	12.6	7.1		85.6	80.2	84.8	85.6	87.0	84.5	75.0	92.7	88.0	88.0	87.8	88.0	87.0	85.6	87.5	87.5	87.8	87.8	87.2	87.5	87.8	87.8	64.6	5	ON994400 B. pudui BIS5
6	20.4	11.3	12.1	15.4	11.7		79.6	94.6	94.6	88.9	89.7	72.3	84.5	87.8	87.8	87.5	87.8	87.2	85.9	87.8	87.8	87.0	87.0	86.7	86.7	87.0	87.0	64.3	6	MZ502168 B. lohae LR5B
7	24.2	16.9	17.8	17.6	16.9	18.4		81.0	80.4	84.5	82.3	69.0	79.1	80.4	80.4	80.4	80.4	80.4	79.1	81.0	81.0	80.7	80.7	80.4	81.0	80.7	80.7	60.2	7	JN880430 B. vitalii RJ-4
8	21.6	10.3	10.7	12.9	11.5	5.3	15.7		97.8	89.4	88.6	71.5	84.0	88.3	88.3	88.0	88.3	88.3	87.0	88.9	88.9	88.9	88.9	88.6	88.6	88.9	88.9	66.2	8	AY596279 B. orientalis
9	21.5	9.9	12.6	13.2	10.7	5.6	16.9	2.3		88.9	89.1	72.6	84.5	87.8	87.8	87.5	87.8	87.8	86.4	88.3	88.3	87.2	87.2	87.0	87.0	87.2	87.2	64.6	9	MN227672 B. occultans Tick No, I 22
10	20.8	10.0	11.5	10.7	9.6	10.3	11.7	8.3	9.3		89.4	72.6	85.6	90.5	90.5	90.2	90.5	90.2	88.9	91.8	91.8	91.0	91.0	90.5	90.5	91.0	91.0	67.3	10	KU234527 B. motasi H6
11	21.3	8.8	10.7	11.5	9.7	8.8	14.4	9.7	9.3	7.6		72.6	83.2	87.0	87.0	86.7	87.0	86.4	85.1	87.5	87.5	86.4	86.4	85.9	85.9	86.4	86.4	63.2	11	JQ437264 B. bigemina RG
12	27.3	21.5	20.2	20.8	18.4	18.6	25.9	19.4	18.0	17.4	17.7		74.5	71.7	71.7	71.7	71.7	71.5	70.7	72.8	72.8	70.9	70.9	70.9	70.4	70.9	70.9	51.2	12	KT429729 B. microti 72
13	24.5	12.9	13.0	9.2	8.1	13.2	17.8	12.6	12.2	11.5	11.7	18.5		87.0	87.0	86.7	87.0	86.1	84.8	86.4	86.4	86.1	86.1	85.6	85.6	86.1	86.1	63.5	13	KP666167 B. gibsoni WH121
14	21.2	12.2	11.8	10.8	10.2	11.0	16.8	8.9	10.0	8.5	9.4	19.9	11.6		100.0	99.7	100.0	98.9	97.6	97.3	97.3	94.3	94.3	94.3	94.3	94.3	94.3	70.6	14	EU888900 B. caballi CABEQ50_B1
15	21.2	12.2	11.8	10.8	10.2	11.0	16.8	8.9	10.0	8.5	9.4	19.9	11.6	0.0		99.7	100.0	98.9	97.6	97.3	97.3	94.3	94.3	94.3	94.3	94.3	94.3	70.6	15	Z15104 B. caballi
16	20.7	12.5	12.2	11.2	10.6	11.4	16.8	9.3	10.4	8.9	9.7	19.9	12.0	0.3	0.3		99.7	98.6	97.3	97.0	97.0	94.0	94.0	94.0	94.0	94.0	94.0	70.3	16	EU642513 B. caballi B1_CABRBEQ179
17	21.2	12.2	11.8	10.8	10.2	11.0	16.8	8.9	10.0	8.5	9.4	19.9	11.6	0.0	0.0	0.3		98.9	97.6	97.3	97.3	94.3	94.3	94.3	94.3	94.3	94.3	70.6	17	MG972849 B. caballi A14Hya
18	22.1	12.5	13.0	11.2	11.7	11.7	16.8	8.9	10.0	8.9	10.1	20.4	12.8	1.3	1.3	1.6	1.3		98.6	98.4	98.4	93.8	93.8	93.8	93.8	93.8	93.8	70.0	18	AB734390 B. caballi T90
19	23.5	13.7	14.1	12.3	12.8	12.9	18.0	10.0	11.1	10.0	11.3	20.8	13.9	2.2	2.2	2.6	2.2	0.9		97.0	97.0	92.4	92.4	92.4	92.4	92.4	92.4	68.7	19	AB734400 B. caballi U243
20	22.6	11.7	12.2	10.8	11.2	11.3	16.3	8.5	9.6	7.1	9.0	18.6	12.7	2.9	2.9	3.2	2.9	1.6	2.6		100.0	94.6	94.6	94.0	94.0	94.6	94.6	70.0	20	AB734386 B. caballi T4
21	22.6	11.7	12.2	10.8	11.2	11.3	16.3	8.5	9.6	7.1	9.0	18.6	12.7	2.9	2.9	3.2	2.9	1.6	2.6	0.0		94.6	94.6	94.0	94.0	94.6	94.6	70.0	21	EU642514 B. caballi B2_CABRBEQ115
22	23.5	12.1	12.9	10.4	10.9	12.5	16.7	8.5	11.1	8.1	10.4	21.5	13.1	6.6	6.6	6.9	6.6	7.2	8.3	6.5	6.5		100.0	99.5	99.5	100.0	100.0	75.2	22	MZ298263 B. caballi BcVzHorse27
23	23.5	12.1	12.9	10.4	10.9	12.5	16.7	8.5	11.1	8.1	10.4	21.5	13.1	6.6	6.6	6.9	6.6	7.2	8.3	6.5	6.5	0.0		99.5	99.5	100.0	100.0	75.2	23	MT463343 B. caballi CuH303
24	24.5	12.9	13.7	11.1	11.6	12.9	17.1	8.9	11.5	8.8	11.2	21.5	13.8	6.6	6.6	6.9	6.6	7.2	8.3	7.2	7.2	0.6	0.6		99.5	99.5	99.5	74.9	24	MZ331377 B. caballi XJB139
25	24.0	12.9	13.7	10.4	11.2	12.8	16.3	8.9	11.5	8.8	11.2	22.4	13.8	6.5	6.5	6.9	6.5	7.2	8.3	7.2	7.2	0.6	0.6	0.6		99.5	99.5	74.7	25	MZ331370 B. caballi WHB13
26	23.5	12.1	12.9	10.4	10.9	12.5	16.7	8.5	11.1	8.1	10.4	21.5	13.1	6.6	6.6	6.9	6.6	7.2	8.3	6.5	6.5	0.0	0.0	0.6	0.6		100.0	75.2	26	EU642512 B. caballi A_CABEQ30
27	23.5	12.1	12.9	10.4	10.9	12.5	16.7	8.5	11.1	8.1	10.4	21.5	13.1	6.6	6.6	6.9	6.6	7.2	8.3	6.5	6.5	0.0	0.0	0.6	0.6	0.0		75.2	27	OR683438 B. caballi HS1
28	58.1	52.6	52.9	49.4	50.7	52.6	54.4	46.5	50.7	46.8	51.4	60.9	53.0	43.2	43.2	43.7	43.2	44.3	46.1	44.7	44.7	34.5	34.5	35.0	35.5	34.5	34.5		28	OR683439 B. caballi HS2
	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	28		

Figure 5. Nucleotides alignment and distance matrix of two isolates of 18s rRNA gene of *Babesia caballi* in camel 'OR683438 *B. caballi* HS1 and OR683439 *B. caballi* HS2' showing (A): Nucleotides alignment. (B): Distance matrix computed sequence distance for 18s rRNA gene of *B. caballi* detected in camel. The upper part of the matrix provides the (percent identity) between the percentage-scaled alignments. The lower part provides the alignments divergence.

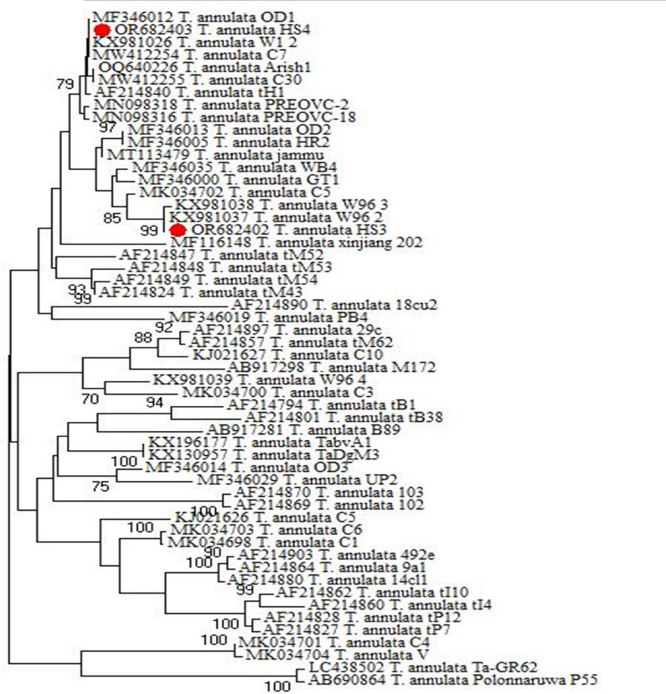

Figure 6. Phylogenetic relationship of *Tams* 1 gene of *Theileria annulata* two isolates identified in current study with closely related *T. annulata* recorded in the GenBank already detected worldwide. Isolates were 'OR682402 *Theileria annulata* HS3 and OR682403 *Theileria annulata* HS4'. New sequences are marked with red circles.

Discussion

Babesia and *Theileria* are parasites that commonly extent by ticks and have a substantial effect on many domestic animals comprising camels (Schnittger *et al*., 2022). In spite of several investigations on piroplasmid host specificity in domestic animals, *Theileria* and *Babesia* species identification in camels may indicate species recognized from other hosts that have been transferred to camels through shared ticks (Qablan *et al*., 2012)

According to our results, the total positive rate of *Babesia* and *Theileria* 63% microscopically. Which greater than reported by (Hekmatimoghaddam *et al*., 2012) was 15.79% in camels using microscope in Iran; (Bahrami *et al*., 2017) 6.45% piroplasms via pcr in Iran; (Ibrahim *et al*., 2017) 43.6% and 74.5% by microscopic and molecular examinations, respectively in Sudan; (Aslam *et al*., 2022) 12% by microscope in Pakistan and (Mahdy *et al*., 2023) 11% and 38% via microscopic and PCR, respectively in the camel in Egypt. These alterations in the infection rates amongst all these studies may be attributed to a difference in the prevalence of tick between different districts, where climatic agents such as humidity, temperature and rainfall affect the ticks habitat, the number of examined animals, nutritive, camel health status and the presence of parasite reservoirs (Oncel *et al*., 2007; Aslam *et al*. 2022; Mahdy *et al*., 2023; Selim, *et al*., 2023). The high positive rates in our study might be attributable to close interaction of native camels with imported camels of nearby nations including Sudan, population growth, seasonal distribution and tick geographic expansion as mentioned (Gratz 2006; Kim *et al*., 2021; Mirahmadi *et al*., 2022).

The present finding exposed that the positive rate of *Babesia* of camel was 62.2% microscopically. The recorded results were greater than that reported in Egypt 46.9% by microscope in Assiut (Abd-Elmaleck *et al*., 2014) 11.9% microscopically and 18.43% by PCR in Northern West Coast (Abou El-Naga and Barghash, 2016) 4.7% by microscope in Qalubia (Ab-

A

Percent Identity (upper part); Divergence (lower part)

	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	28		
1		97.9	97.7	92.8	96.0	95.8	96.5	92.1	93.7	92.3	97.7	97.6	97.6	97.6	97.7	97.1	97.6	96.6	96.6	96.9	96.3	96.0	95.5	94.4	91.6	91.0	96.0	97.7	1	AF214847 T. annulata tM52
2	2.1		99.7	93.7	97.4	97.3	97.9	93.4	94.5	93.2	99.7	99.5	99.5	99.5	99.7	98.9	98.4	98.4	98.4	98.2	98.1	96.9	95.2	94.0	92.8	91.5	97.4	99.7	2	MN098318 T. annulata PREOVC-2
3	2.3	0.3		93.7	97.3	97.1	97.9	93.2	94.4	93.1	100.0	99.8	99.8	99.8	100.0	98.9	98.2	98.4	98.4	98.1	98.1	97.1	95.0	93.9	92.6	91.3	97.3	100.0	3	MW412254 T. annulata C7
4	7.6	6.6	6.6		92.6	92.4	93.2	91.9	96.3	90.5	93.7	93.9	93.9	93.6	93.7	93.6	93.6	93.1	93.1	92.8	92.8	93.7	92.9	91.5	93.1	89.7	92.6	93.7	4	AB917298 T. annulata M172
5	4.1	2.6	2.8	7.8		99.8	99.0	92.1	93.6	94.4	97.3	97.1	97.1	97.4	97.3	98.1	96.5	97.6	97.6	98.4	98.2	96.3	95.0	93.1	93.4	91.0	100.0	97.3	5	KX981037 T. annulata W96_2
6	4.3	2.8	3.0	8.0	0.2		98.9	91.9	93.4	94.2	97.1	96.9	96.9	97.3	97.1	97.9	96.3	97.4	97.4	98.2	98.1	96.1	94.8	92.9	93.2	91.1	99.8	97.1	6	KX981038 T. annulata W96_3
7	3.6	2.1	2.1	7.1	1.0	1.1		92.8	94.0	94.5	97.9	98.1	98.1	98.1	97.9	99.0	97.3	98.6	98.6	99.2	99.2	96.3	95.0	93.4	93.7	91.3	99.0	97.9	7	MK034702 T. annulata C5
8	8.4	6.9	7.1	8.5	8.4	8.5	7.6		91.6	89.9	93.2	93.4	93.4	93.1	93.2	92.9	94.2	92.6	92.6	93.1	92.9	91.3	91.0	90.7	90.0	91.5	92.1	93.2	8	MK034701 T. annulata C4
9	6.6	5.7	5.9	3.8	6.7	6.9	6.2	8.9		93.1	94.4	94.2	94.2	94.2	94.4	94.0	94.0	93.7	93.7	93.6	93.2	94.5	92.4	91.8	94.2	89.9	93.6	94.4	9	AF214897 T. annulata 29c
10	8.2	7.1	7.3	10.2	5.9	6.0	5.7	10.9	7.3		93.1	93.2	93.2	93.2	93.1	93.9	93.1	93.7	93.7	94.4	93.7	95.5	93.9	93.7	95.5	88.6	94.4	93.1	10	AF214794 T. annulata tB1
11	2.3	0.3	0.0	6.6	2.8	3.0	2.1	7.1	5.9	7.3		99.8	99.8	99.8	100.0	98.9	98.2	98.4	98.4	98.1	98.1	97.1	95.0	93.9	92.6	91.3	97.3	100.0	11	KX981026 T. annulata W1_2
12	2.5	0.5	0.2	6.4	3.0	3.1	2.0	6.9	6.0	7.1	0.2		100.0	99.7	99.8	99.0	98.4	98.6	98.6	98.2	98.2	96.9	95.2	94.0	92.8	91.5	97.1	99.8	12	OQ640226 T. annulata Arish1
13	2.5	0.5	0.2	6.4	3.0	3.1	2.0	6.9	6.0	7.1	0.2	0.0		99.7	99.8	99.0	98.4	98.6	98.6	98.2	98.2	96.9	95.2	94.0	92.8	91.5	97.1	99.8	13	MW412255 T. annulata C30
14	2.5	0.5	0.2	6.7	2.6	2.8	2.0	7.3	6.0	7.1	0.2	0.3	0.3		99.8	99.0	98.1	98.6	98.6	98.2	98.2	97.3	94.8	93.7	92.4	91.5	97.4	99.8	14	AF214840 T. annulata tH1
15	2.3	0.3	0.0	6.6	2.8	3.0	2.1	7.1	5.9	7.3	0.0	0.2	0.2	0.2		98.9	98.2	98.4	98.4	98.1	98.1	97.1	95.0	93.9	92.6	91.3	97.3	100.0	15	MF346012 T. annulata OD1
16	3.0	1.1	1.1	6.8	2.0	2.1	1.0	7.5	6.2	6.4	1.1	1.0	1.0	1.0	1.1		97.9	99.5	99.5	99.2	99.2	96.3	94.7	93.6	93.1	91.3	98.1	98.9	16	MT113479 T. annulata jammu
17	2.5	1.6	1.8	6.7	3.6	3.8	2.8	6.0	6.2	7.3	1.8	1.6	1.6	2.0	1.8	2.1		97.4	97.4	97.7	97.1	96.1	94.7	93.6	92.4	92.1	96.5	98.2	17	AF214824 T. annulata tM43
18	3.5	1.6	1.6	7.3	2.5	2.6	1.5	7.8	6.6	6.6	1.6	1.5	1.5	1.5	1.6	0.5	2.6		100.0	98.7	99.0	95.8	94.2	93.4	92.9	91.1	97.6	98.4	18	MF346013 T. annulata OD2
19	3.5	1.6	1.6	7.3	2.5	2.6	1.5	7.8	6.6	6.6	1.6	1.5	1.5	1.5	1.6	0.5	2.6	0.0		98.7	99.0	95.8	94.2	93.4	92.9	91.1	97.6	98.4	19	MF346005 T. annulata HR2
20	3.1	1.8	2.0	7.6	1.6	1.8	0.8	7.3	6.7	5.9	2.0	1.8	1.8	1.8	2.0	0.8	2.3	1.3	1.3		99.4	96.0	95.0	93.4	93.4	91.6	98.4	98.1	20	MF346035 T. annulata WB4
21	3.8	2.0	2.0	7.6	1.8	2.0	0.8	7.5	7.1	6.6	2.0	1.8	1.8	1.8	2.0	0.8	3.0	1.0	1.0	0.6		95.5	94.5	92.9	92.9	91.1	98.2	98.1	21	MF346000 T. annulata GT1
22	4.1	3.1	3.0	6.6	3.8	4.0	3.8	9.3	5.7	4.7	3.0	3.1	3.1	2.8	3.0	3.8	4.0	4.3	4.3	4.1	4.7		96.3	94.8	93.2	90.2	96.3	97.1	22	MF116148 T. annulata xinjiang 202
23	4.7	5.0	5.2	7.5	5.2	5.4	5.2	9.6	8.0	6.4	5.2	5.0	5.0	5.4	5.2	5.5	5.5	6.0	6.0	5.2	5.7	3.8		96.6	93.9	89.0	95.0	95.0	23	KX130957 T. annulata TaDgM3
24	5.9	6.2	6.4	9.1	7.3	7.5	6.9	10.0	8.7	6.6	6.4	6.2	6.2	6.6	6.4	6.8	6.7	6.9	6.9	6.9	7.5	5.3	3.5		93.2	88.1	93.1	93.9	24	MF346029 T. annulata UP2
25	8.9	7.6	7.8	7.3	6.9	7.1	6.6	10.7	6.0	4.7	7.8	7.6	7.6	8.0	7.8	7.3	8.0	7.5	7.5	6.9	7.5	7.1	6.4	7.1		88.2	93.4	92.6	25	AF214903 T. annulata 492e
26	9.1	8.6	8.7	10.6	9.1	8.9	8.8	8.6	10.4	11.9	8.7	8.6	8.6	8.6	8.7	8.8	7.8	8.9	8.9	8.4	8.9	10.0	11.4	12.5	12.3		91.0	91.3	26	LC438502 T. annulata Ta-GR62
27	4.1	2.6	2.8	7.8	0.0	0.2	1.0	8.4	6.7	5.9	2.8	3.0	3.0	2.6	2.8	2.0	3.6	2.5	2.5	1.6	1.8	3.8	5.2	7.3	6.9	9.1		97.3	27	OR682402 T. annulata HS3
28	2.3	0.3	0.0	6.6	2.8	3.0	2.1	7.1	5.9	7.3	0.0	0.2	0.2	0.2	0.0	1.1	1.8	1.6	1.6	2.0	2.0	3.0	5.2	6.4	7.8	8.7	2.8		28	OR682403 T. annulata HS4
	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	28		

B

Figure 7. Nucleotides alignment and distance matrix of two isolates of *Tams* 1 gene of *Theileria annulata* in camel 'OR682402 *Theileria annulata* HS3, OR682403 *Theileria annulata* HS4' showing (A): Nucleotides alignment. (B): Distance matrix computed sequence distance for *Tams* 1 gene of *T. annulata* detected in camel. The upper part of the matrix provides the (percent identity) between the percentage-scaled alignments. The lower part provides the alignments divergence.

Table 3. Identity percentages of the *Theileria annulata tams*1 sequences obtained from isolates of camels with other sequences isolates deposited in GenBank.

Accession no.	OR682403 Camel Egypt (present isolate) (Identity %)	KX981037 United Kingdom (Identity %)	KX981038 United Kingdom (Identity %)
OR682402 camel Egypt (present isolate)	97.30%	100%	99.80%
Accession no.	MF346012 Cattle India (Identity %)	KX981026 United Kingdom (Identity %)	MW412254 Cattle Turkey (Identity %)
OR682403 camel Egypt (present isolate)	100%	100%	100%

del Gawad, 2018) and 5.4% of *B. caballi* by pcr in Egypt (Mahdy *et al*., 2023). Likewise, It was higher than that found in other nations such as 13.17% in Saudi Arabia (Swelum *et al*., 2014); 53% in Iraq (Al-Naily and Jasim, 2018); 17.5% and 54.16% by microscope and PCR, respectively in Iraq (Faraj and Abd, 2018) and 10% via microscope and 19.28% by PCR in Iran (Mirahmadi *et al*., 2022). The high *Babesia* positive rate of apparent healthy camels in our study could be resulted from the subclinical form, as reported by Nakayima *et al*. (2017) who stated that *B. caballi* can persist subclinically for at least one to four years before being eradicated.

In the present work, the positive rate of *Theileria* species of camel was 35.4% based on microscopy. Our results more than that found in upper Egypt (6.8%) by (Hamed *et al*., 2011); 9.18% in Assiut (Abd-Elmaleck *et al*., 2014); 30.86% in Egypt (Youssef *et al*., 2015); 0.4% in Qalubia (Abdel Gawad, 2018) and 21.1% of *T. annulata* by pcr in Egypt (Selim *et al*., 2023). Also, it was higher than that of previous results in other countries as 5.8% in Iraq (Jasem *et al*., 2015); 0.6% in Iran (Bahrami *et al*., 2016); 10% of *T. annulata* by PCR in Iraq (Al-Naily and Jasim, 2018); 28.88% by microscope in Iraq (Alsaadi and Faraj, 2020a); 34% of *T. annulata* by PCR in Iraq (Alsaadi and Faraj, 2020b); 12% by microscope and 13.5% of *T. annulata* by PCR in Pakistan (Aslam *et al*., 2022); 22.94% microscopically in Iraq (Tamimi, 2022) and 8.5% in United Arab Emirates (Ismaeil *et al*., 2023). Even so, our findings lower than that found by Abou El-Naga and Barghash (2016) who reported 71.9% positive cases by pcr in Egypt and by Al-Naily and Jasim (2018) who recorded 46.5% in Iraq. Positive examined camels with *Theileria* species in our study displayed apparent healthy without any clinical signs specific for theileriosis. This in concur with Youssef *et al*. (2015) who clarified that camels *Theileria* infection is subclinical or chronic infection.

The current investigation recorded higher positive rate of *Babesia* and *Theileria* species in females (71.7%) and (36.9%) than males (50%) and (33.3%), respectively. These findings come in accord with that reported by Al-Naily and Jasim (2018) who enumerated that prevalence of Babesiosis and Theileriosis in female was (60.8%) and (55.2%) more than in males (40%) and (32%), respectively in Iraq. As well, Alsaadi and Faraj (2020b) and Selim *et al*. (2023) who established high prevalence of *T. annulata* in females compared with males and Selim *et al*. (2023) and Aslam *et al*. (2022) who documented that infection was 2.5 times higher in females than in males. This disproportion in prevalence could be due to the decrease of females immunity during pregnancy and lactation, making them more susceptible to the disease (Nijhof *et al*., 2007; Al-Hosary *et al*., 2018; Selim *et al*., 2023). In contrary, Ibrahim *et al*. (2017) and Tamimi (2022) noted that both genders similarly vulnerable to the disease.

Following our findings, the positive rate of *Babesia* and *Theileria* species was greater in camels of age group > 3 years (68.8%) and (37.5%) than of age group ≤ 3 years (52.9%) and (32.4%), respectively. Similar results were reported by Farhan and Hameed (2017); Alsaadi and Faraj (2020a) and Aslam *et al*. (2022) who confirmed that older camels may be more susceptible to infection than the younger ones. This could be due to repeated exposure to parasites throughout time or by the existence of persistently infected animals. Furthermore, maternal immunity plays a crucial role in protecting newly born animals against most infections (Ullah *et al*. 2022; Selim, *et al*., 2023). In contrast with, Mohsin *et al*. (2022) who recorded that the high infection rate of *Theileria* occurs in young animals.

In our study, Giemsa stained blood smears of camels revealed the presence of intraerythrocytic *Babesia* and *Theileria* trophozoites. The *Babesia* trophozoites within erythrocytes are pear, round or oval shaped. While, intraerythrocytic *Theileria* trophozoites are mainly round to oval but may also be rod or comma-shaped. Koch's blue bodies of *Theileria* were detected in the lymphocytes. This was in agreement with Taylor *et al*. (2007) who described *B. caballi* trophozoites as pear-shaped (2–5 μm) in length and *T. annulata* intraerythrocytic trophozoite forms are mostly round (0.5–2.7 μm) to oval (2 × 0.6 μm) and Mirahmadi *et al*. (2022) who noted various shapes as pear and amoeboid form with a length of '1–2.5 or 2.5–5 μm' within erythrocytes.

PCR analysis targeting the *T. annulata tams*1 gene in the current research revealed the presence of *T. annulata* with negative detection of *T. equi* by PCR. These findings are in accordance with Omer *et al*. (2021) who identified *T. annulata* in camels with no evidence of *T. equi*. Unlike to prior studies in Egypt (Youssef *et al*., 2015; Abou El-Naga and Barghash, 2016) which reported that tested positive camels for *Theileria* species through PCR were negative for *T. annulata*. Also, Bahrami *et al*. (2016) verified that all the sequences of 18S rRNAs from the isolates had more than '99%' homology to Iranian *T. equi* isolates in the GenBank and Mahdy *et al*. (2023) found that *T. equi* is the most prevalent agent in Egyptian camels. This may be due to geographical distribution of camels and presence of other neighboring domestic animals in addition to the ticks which is responsible for its transmission and play a significant role in the spread of different *Theileria* species between camels and other domestic animals (Tamimi, 2022).

The results of phylogenetic analysis in the current research by PCR amplicons showed the existence of *B. caballi* and *T. annulata* sequences in camels in Egypt which are closely related to isolates found in NCBI from numerous areas of the world. *B. caballi* was genetically related to isolates from South Africa, Cuba and Venezuela horses. Similar to that noticed by Mirahmadi *et al*. (2022) who demonstrated that *B. caballi* found in all positive camels infected with babesiosis by DNA sequencing in Iran. Also, phylogenetic analysis of *T. annulata tams*1 gene in current study was genetically correlated with isolates from United Kingdom, India and Turkey. Like to that observed by Alsaadi and Faraj (2020b) who recorded that DNA sequence of all camel isolates of *Theileria* were *T. annulata* in middle of Iraq and Aslam *et al*. (2022) who indicated that DNA sequencing and BLAST analysis of all piroplasmosis positive samples were infested with *T. annulata* in camels from Pakistan and added that *T. annulata* is a silent killer with destructive effects on immune system. The existence of *B. caballi* and *T. annulata* in camels may be owing to their co-inhabitance with equines or bovines and contact with ticks orginated from infected other species, which agree with Alanazi *et al*. (2019). Also, Youssef *et al*. (2015) and Nakayima *et al*. (2017) mentioned that piroplasms have low host specificity and the camels could serve as accidental parasites hosts or reservoirs since they are in similar environmental setting. Therefore, the vertebrate host-specificity of these tick-borne blood parasites is likely wider than expected (Mahdy *et al*., 2023).

Conclusion

Based on our findings we concluded that an apparently healthy local camels infected with the only prevailing identified species *B. caballi* and *T. annulata* in Assiut. This proved the presence of equine and bovine piroplasmids in camels. The molecular diagnosis followed by sequencing and genetic characterization is more sensitive and specific than microscopic and PCR only. Consequently, there is a pressing need to develop early diagnosis, therapeutic and preventive measures regarding this parasites throughout Egypt to avoid spread of infection and improve camel's health conditions and production.

Conflict of interest

The authors declare that they have no conflict of interest.

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