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Blood Parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt

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Abstract

An epidemiological study was conducted on the occurrence of blood parasitic infection in local camels in three selected sites representing Northern West Coastal zone of Egypt using Giemsa-stain blood smears (GSBS) and Polymerase chain reaction (PCR). This is the first molecular diagnosis report, which gives a picture of blood parasites covering this areas in Egypt. Results revealed that GSBS examination stopped at genus level on contrary to PCR techniques that detected and identified DNAs of blood parasites. *Theileria* was the most common pathogen (50.8%, 71.9%), followed by *Anaplasma* (47.4%, 67.37%), *Trypanosoma* (20.24%, 67.06%), and a lesser extent *Babesia* (11.8%, 18.43%) by GSBS and PCR, respectively. Mixed infections were present in 68.9%, with at least two hemoparasites belonged to different genus. Statistical analysis showed considerable variation in values within locations and age category reflected in a high significant (p<0.001), and both sexes were at risk of parasitic infections, particularly females. Only *A. marginale* caused anaplasmosis in 51 (22.9%) of infected dromedaries, while the majority were having *A. marginale* together with *A. centrale* 172 (77.13%). This is the first time to record *B. bovis, B. bigemina*, *A. centrale* and *A. marginale* in camels in this area. We concluded that blood parasites infection is highly prevalent in this area which strengthens the need to control programs help to prevent the spread of these parasites. The present results can serve as the basis for subsequent studies in dromedaries in Egypt; particularly *Theileria* genotype needs further studies.

Keywords: *Anaplasma*; *Theileria*; *Trypanosoma*; *Babesia*; PCR; Camels; Egypt

Introduction

Camel is an important multipurpose animal and since the old times, it has been used for transportation and produce milk, wool and meat in arid and semi-arid areas of the world [1]. Although camels are hardy animals and can tolerate the harsh conditions of arid regions because of their unique adaptive physiological characteristics, these animals face a wide variety of diseases [2,3]. Gastrointestinal and blood parasites are known to affect the health of camels leading to anemia, wasting and death in heavy infection [4].

Trypanosomiasis is the most important and serious pathogenic protozoal disease of camel caused by *T. evansi* infecting a wide range of animals throughout tropical and sub-tropical regions of the world [5-8]. Theileriosis is an important hemoparasitic disease of animals inducing a variety of clinical manifestations ranging from a subclinical presentation to a fatal disease depending, in part, on the animal species, host, age and the species of the microorganism. Tropical theileriosis caused by species of the genus *Theileria* has a wider distribution extending from North Africa to China [9]. Piroplasmids belonging to the genera *Babesia* are suspected of infecting dromedaries [10], but data published so far are limited [2,11]. The significant effect of *Babesia* infections are reported in domestic animals, humans, and some wildlife species. These tick-borne apicomplexan were generally considered as highly specific for a given host species [12].

Anaplasmosis is an arthropod borne disease of ruminants caused by species of the genus *Anaplasma* (Rickettsiales: *Anaplasmataceae*) [13]. Of the known *Anaplasma spp*., *A. marginale* is the most virulent, characterized by a progressive hemolytic anemia, and is responsible for extensive economic losses in tropical and subtropical areas [14-16]. On the other hand, *A. centrale* is capable of producing a moderate degree of anemia, but clinical outbreaks in the field are extremely rare. It is used as a live vaccine for cattle against the pathogenic *A. marginale* in tropical and subtropical areas [17]. *A. marginale* can be distinguished from *A. centrale* by the location and the characteristics of the inclusion bodies in the erythrocytes [18].

There has been a steady increase in the number of camels slaughtered for meat in Egypt. The camel's ability to utilize the scanty fodder resources of the arid and semiarid zones for body maintenance, growth and milk production makes this animal a potentially important source of food [19]. There is paucity of information on hemoparasites of camels and their significance on health and productivity in northern west coastal zone of Egypt (the main camel rearing area). Camels are largely kept without close association with other carrier animals in this area. Biting flies (*Stomoxys* and *Tabanus*), and hard ticks were noticed on several locations at the camel's body with large numbers; in particular *Hyalomma dromedarii*. No common use of a control program on a large scale is present. Molecular tools increasingly have become an integral part of studying the epidemiology of infectious agents. The current study was undertaken to verify the main blood parasites existing in dromedary Maghrabi camels in this area mainly by conventional PCRs.

Materials and Methods

Study design and study area

The present study was conducted on the occurrence of blood parasitic

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infection in local camels to detect and identify protozoan parasites, the etiological agents of trypanosomosis, theileriosis, babesiosis, and rickettsial anaplasmosis as being of economic importance. It was carried out during an epidemiological survey lasted from March 2012 to April 2015 within the frame of PROCAMED project, supported by the European Union (ENPI-Joint operational Programme of the Mediterranean Basin-IEVP-CT). For this purpose, 331 blood samples were collected randomly from local dromedary Maghrabi camels at different ages and both sexes. Diagnosis was performed primarily by Giemsa-stained blood smear (GSBS) and then analyzed mainly by different PCRs. Three sites in Matrouh governorate within the northern west coastal region of Egypt (NWC) between latitude; north 31°19- 26° 00 and longitude; 27° 45-28° 00 were selected (Figure 1).

Sample collection

Whole blood samples were collected from the jugular vein of each camel using clean sterile Vacutainer tubes containing ethylene di-amine tetra acetic acid (EDTA) for (a) microscopic examination and (b) DNA extraction as a target for PCR amplification. DNA samples were stored at −20°C until used. Cases of suspected trypanosomosis, theileriosis, anaplasmosis and babesiosis were investigated especially in *Tabanus*, *Stomoxys* and tick–infested camels with a fever, enlarged lymph nodes, anemia and jaundice, or hemoglobinuria due to *Babesia.*

Figure 1: Map of Egypt showing North western coast where blood samples were collected from camels reared in Matrouh Governorate. A (Sidi Barrany), B (El-Negeila), C (Mersa Matrouh): are the three selected sites where blood samples were collected.

Parasitological examination

Thin blood smears were prepared, air-dried, fixed in absolute methanol, stained with Giemsa-stain and examined microscopically for blood parasites with light microscopy (40X and oil immersion objectives) according to Hoare [20].

DNA extraction and PCR amplification

Genomic DNAs from 331 whole blood samples were extracted using The DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All samples were subjected to PCR based assays to detect blood parasites using species-specific primers, the details of which are shown in Table 1. PCRs were conducted in a total volume of 25 µL composed of 12.5 µL of commercial Master Mix (Bio-basic, Portugal), 10 pmol of each primer, ~25 ng of genomic DNA and sterile water. Genomic DNAs isolated from the PCR reactions were performed in an automatic DNA thermocycler (Bio-Rad, Hercules, CA, USA) as follows: one cycle of 94°C for 4 min. (Pre-denaturation), followed by 30-40 cycles of 94°C for 1 min. (denaturation). Annealing temperature was 57°C for 1 min. for each of *T. evansi*, *A. marginale, A. centrale*, *B. bigemina*, *B. bovis*, and 52°C for 1 min. for each of *T. brucei* and *Theileria* sp. Different annealing temperatures ranged from 47°C to 59°C were performed for *Theileria annulata*. Polymerization step with a final extension was one cycle of 72°C for 5-7 min. PCR products were separated by 1.5% agarose gel electrophoresis to assess the presence of specific bands indicative of different blood parasites spp. [21-28].

Data analysis

Data management was performed using SPSS V20.0, (IBM SPSS Statics 20, USA). Data were summarized by descriptive statistics for mean and standard deviation. Comparisons among groups were evaluated using an analysis of variance (ANOVA) test. All statistics were considered significant *at* $p \le 0.05$.

Results

Clinical examination

Field clinical examination to signs related blood parasites of 331 camels revealed 256 (77.34%) were asymptomatic and apparently healthy while 75 (22.66%) camels showing some clinical abnormalities

Pathogen	Nucleotide sequences of primers	(Bp)	Reference
Anaplasma marginale	MAR1bB2F: 5′-GCT CTA GCA GGT TAT GCG TC-3′ and MAR1bB2R: 5′-CTG CTT GGG AGA ATG CAC CT-3′, were based on Major surface protein–1β encoding gen	265	[22]
Trypanosoma evansi	TR3: 5′-GCGCGGATTCTTTGCAGACGA-3′ and TR4: 5′- TGC AGA CAC TGG AAT GTTACT-3′, were derived from repetitive nucleotide sequences.	257	[23]
Babesia bovis	Bb1: 5′-TTTGGTATTTGTCTTGGTCAT -3′ and B. bovis Bb2: 5′- ACC ACT GTA GTC AAA CTCACC-3′, were derived from the sequence of the gene encoding the enzyme carbamoyl phosphate synthetase II.	446	[24]
Babesia bigemina	Bg3: TAG TTG TAT TTC AGC CTC GCG and Bg4: AAC ATC CAA GCA GCT AHT TAG, were based on their small subunit ribosomal RNA sequences.	689	[25]
Theileria annulata	In the case of *T. annulata*, the cytochrome b gene was selected and cytob1 primer set: Forward: 5′-ACT TTG GCC GTA ATG TTA AAC–3′/Reverse: 5′-CTC TGG ACC AAC TGT TTGG–3′ was used to amplify a 312 bp variable region.	312	[26]
Theileria sp.	989: 5′-AGT TTCTGA CCT ATC AG–3′ and 990: 5′- TTG CCT TAA ACT TCC TTG–3′, were based on their small subunit ribosomal RNA sequences.	1100	[27]
Anaplasma marginale	Am3: GTGGCAGACGGGTGAGTAATG A and Am4: CATGTCAAGAAGTGGTAAGGT, were derived from the sequence of the gene encoding the surface protein.	160	[27]
Trypanosoma brucei	TBR1.2F: 5'-GAA TAT TAA ACA ATG CGC AG-3' and TBR1.2R: 5`-CCA TTT ATT AGC TTT GTT GC-3' were based on the highly repeated sequence of mini-chromosome satellite DNA.	164	[28]

Table 1: Nucleotide sequences of species-specific primers were used for different blood parasites detection and their expected sizes.

varied in their manifestation from subclinical (52/75) to clinical (23/75) (Table 2). These include weakness, depression, rough coat, emaciation, atrophy of the hump and some camels remained in sterna recumbence. The reported clinical cases are mostly associated with the recrudescence of existing infections due to stress or with the introduction of native animals raised in tick free areas into tick-infested areas. Of the 256 asymptomatic camels, 98 (38.3%) and 232 (90.63%) were positive by GSBS and PCR respectively, where 24 (9.38%) were negative by either tests. None of the camel samples positive by GSBS were negative by PCR. All clinical and subclinical camels were positive by GSBS and PCR except for 5 camels were negative by microscopic examination.

Parasitological findings

Initial diagnoses by GSBS do not meet our requirements and its ability stopped at genus level, revealing four genera of parasites including *Trypanosoma*, *Theileria*, *Babesia* and *Anaplasma*. Of the 331 camels were examined for presence of blood parasites, 168 (50.8%), 157 (47.4%), 67 (20.24%), and 39 (11.8%) were harboring theileriosis, anaplasmosis, trypanosomosis and babesiosis, respectively (Table 3). *Anaplasma* was detected in two forms in examined camels according to the location of the inclusion bodies in the erythrocytes, while *Babesia* had Pear shaped like and arranged in pairs with acute or wide angles near the margin of infected erythrocytes. *Theileria* were detected in erythrocytes in most cases and somewhat in schizont forms.

Camels	Clinical examination		Blood Film		PCR	
	No.	%	No.	%	No.	%
Asymptomatic	256	77.34	98	38.3	232	90.63
Clinical	23	6.95	23	100.0	23	100.0
Sub-clinical	52	15.70	47	90.38	52	100.0
Total	331		168	50.75[a]	307	92.75[b]

Table 2: Detection of blood parasites in camels by microscopy and PCR based assay based on repetitive nucleotide sequences.

PCR results

PCR was the powerful method used, not only when products were not detected in DNA free samples but also describe what *Trypanosoma, Anaplasma, Theileria and Babesia* subspecies present. Moreover, it allowed the accurate diagnosis of mixed infections which could not be detected by GSBS. Upon using their specific primers, the expected fragments of size 257 bp, 164 bp, 446 bp, and 689 bp were obtained from TR3/TR4 for *T. evansi*, TBR1/TBR2 for *T. brucei*, Bb1/Bb2 for *B. bovis* and Bg3/Bg4 for *B. bigemina*, respectively (Figures 2a-2c and 3). In the current study, two different species-specific primers were evaluated to detect *A. marginale*: Am3/Am4 amplified 160 bp as expected size, whereas MAR1bB2F/MAR1bB2R could not gave the expected fragment at 265 bp, but amplified strong fragments at 519 bp with mixed infections of *A. marginale* and *A. centrale* (Figure 2b). While, 989/990 the species-specific primer for *Theileria* detected fragments at 1100 bp, Cytob1F/Cytob1R evidenced the specificity of piroplasms infection in camels when failed to detect *Th. annulata* in this area (Figure 4). The presence and the percentage of blood parasites infection in Maghrabi camels in each of the three locations, both sexes and different ages are shown in Tables 2 and 3. PCR results revealed that the majorities of the detected infections were due to *Theileria* (71.9%), followed by *Anaplasma* (67.37%) and *Trypanosoma* (67.06%) with no significant difference, then *Babesia* (18.43%). The prevalence of these parasites was significantly varied between and within age groups, gender and origin of the animals ($p<0.001$), except for *Theileria* ($p<0.05$) (Tables 2-5). However, the higher incidence of the infected camels with protozoan parasites was found in Mersa Matrouh, the main entrance for camels from neighboring governorates, followed with slightly difference by El Negeila, and Sidi barrany that have a plenty of rains and plants than Mersa Matrouh (Table 4).

Parasite	Blood smear (Frequency)	PCR		
		Prevalence	Parasite identification (Frequency)	
Trypanosoma	67 (20.24%)	222 (67.06%)	194 (87.39%) *T. evansi*	28 (12.61%) *T. brucei*
Anaplasma	157 (47.4%)	223 (67.37%)	51 (22.9%) *A. marginale*	172 (77.13%) (*A. marginale* & *A. centrale*)
Theileria	168 (50.8%)	238 (71.9%)	238 (100%) *Th. camelensis*	0 (0.0%) *Th. annulata*
Babesia	39 (11.8%)	61 (18.43%)	36 (59.1%) *B. bovis*	25 (40.9%) *B. bigemina*

Table 3: Prevalence and identification of blood parasites in camels by Giemsa-stained blood smear and PCR based assay (n=331).

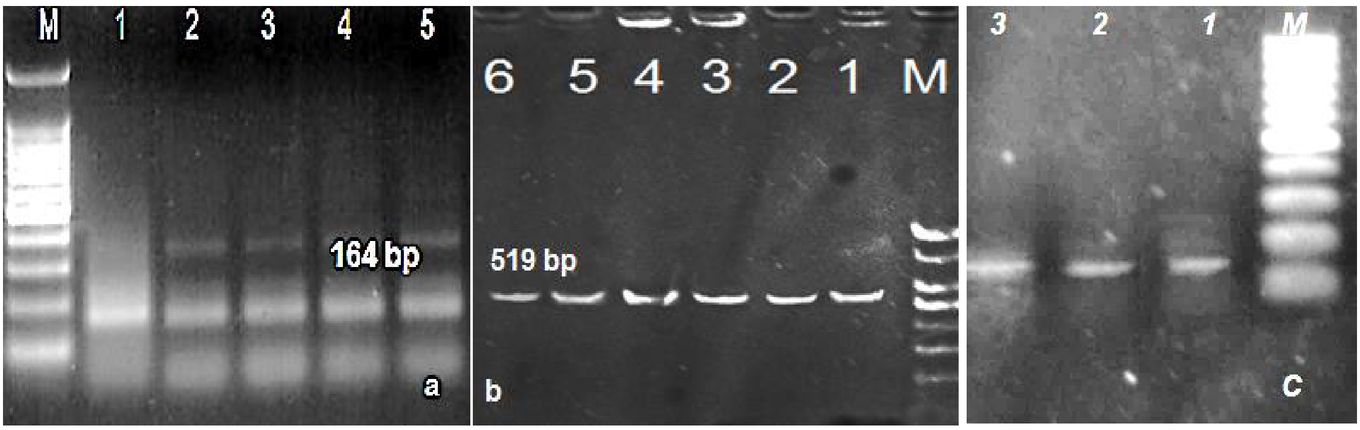

Figure 2 (a, b & c): Results of agarose (1.5%) gel electrophoresis of 164 bp of PCR-product for *T. brucei* (a), 519 bp of PCR-product for *A. marginale* together with *A. centrale* (b), and 160 bp of PCR-product for *A. marginale* only (c). Lane M, DNA molecular size marker (Qiagen, Germany).

Blood parasites	Sex				Age						Location					
	Female (n= 241)		Male (n= 90)		X ≤ 6 (n= 152)		12 ≥ X > 6 (n= 84)		X > 12 (n= 95)		Mersa Matrouh (n= 138)		El Negeila (n=73)		Sidi Barrany (n= 120)	
	Inf.	%	Inf.	%	Inf.	%	Inf.	%	Inf.	%	Inf.	%	Inf.	%	Inf.	%
T. evansi	162	67.2	32	35.6	81	53.3	64	76.2	49	51.6	108	78.3	36	49.3	50	41.7
T. brucei	22	9.13	6	6.7	12	7.9	4	4.8	12	12.6	4	2.9	10	13.7	14	11.7
Anaplasma	152	63.1	71	78.9	105	69.1	63	75	55	57.9	108	78.3	53	72.6	62	51.7
Theileria	172	71.4	66	73.3	106	69.7	65	77.4	67	70.5	103	74.6	53	72.6	82	68.3
Babesia	47	19.5	14	15.6	47	30.9	4	4.8	10	11.1	44	31.9	13	17.8	4	3.33

Table 4: Prevalence of hemoparasites were detected in camels by PCR based on sex, age and site of collection (n=331).

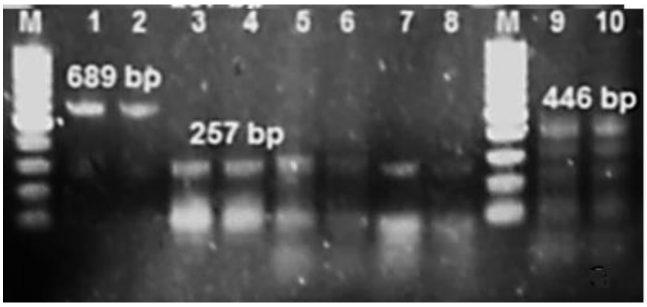

Figure 3: Ethidium bromide-stained 1.5% agarose gel electrophoresis of PCR products, Lanes M, M contained 100-bp DNA marker (Qiagen, Germany). Lanes 1, 2 contained PCR products of *B. bigemina* from camels. Lanes from 3 to 8 correspond to positive PCR products of *T. evansi*. Lanes 9, 10 were PCR positive due to *B. bovis*.

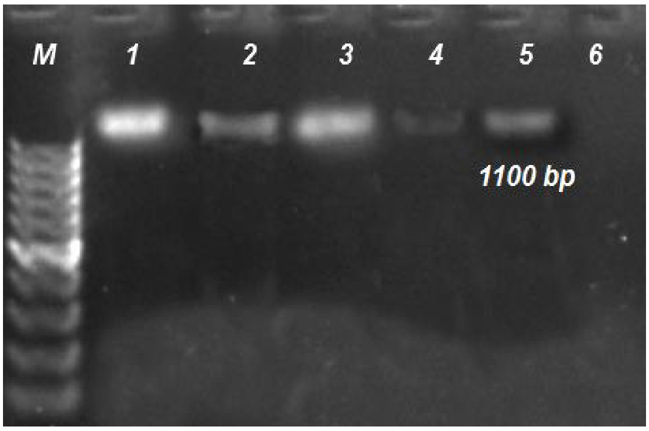

Figure 4: Agarose gel electrophoresis of amplified DNA from different *Theileria* species at 1100 bp by using primer set 989/990. Lanes M, molecular size marker (Qiagen, Germany). Lanes from 2 to 5 correspond to positive PCR products of *Theileria sp.* Lanes 1, 6 represent positive and negative controls, respectively.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Gender	Between Groups	3.609	2	1.804	9.558	0.000
	Within Groups	61.920	328	0.189		
	Total	65.529	330			
Age	Between Groups	17.059	2	8.530	12.710	0.000
	Within Groups	220.125	328	0.671		
	Total	237.184	330			
T. evansi	Between Groups	9.405	2	4.702	21.756	0.000
	Within Groups	70.892	328	0.216		
	Total	80.296	330			
T. brucei	Between Groups	.751	2	0.375	4.947	0.008
	Within Groups	24.881	328	0.076		
	Total	25.631	330			
Ana-plasma	Between Groups	4.796	2	2.398	11.572	0.000
	Within Groups	67.965	328	0.207		
	Total	72.761	330			
Theileria	Between Groups	.260	2	0.130	0.639	0.528
	Within Groups	66.610	328	0.203		
	Total	66.870	330			
Babesia	Between Groups	5.236	2	2.618	19.286	0.000
	Within Groups	44.523	328	0.136		
	Total	49.758	330			

Table 5: Statistical analysis for blood parasites infections in camels based on PCR results.

Prevalence	Single infestation	Mixed infestations			Free
		Two	Three	Four	
No.	89	85	93	40	24
Frequency	26.9%	25.7%	28.1%	12.1%	7.25%

Table 6: Mixed infestations of blood parasites were detected in camels by PCR.

Parasite-parasite interactions (Co-infections)

Infection with one type of disease agent can reduce or increase the incidence of infection with another. In this study, mixed infestation with different parasites from 2 to 4 in the same camel was common with a prevalence of 65.9%. The overall PCR rate of mixed infection was 25.7% for two parasites, 28.1% for three parasites and 12.1% for four parasites, while 26.9% of camels were harbored one parasite and 7.25% were free from examined blood parasites as representative in Table 6.

Discussion

Parasitic diseases have severely hindered development of livestock production in many Countries. Egypt is one of OIE Member Countries reporting blood parasitic diseases, trypanosomosis, theileriosis and babesiosis, of which trypanosomosis is the most widely distributed. The bulk of these diseases are caused by vector-borne Protozoa and Rickettsia. In the present study, camels are largely kept without close association with other carrier animals, such as cattle, sheep and goats. Consequently, transmission of blood parasites could take place at any time by biting flies and hard ticks which were noticed in large numbers; particularly *H. dromedarii* [8]. Females were-positive compared to males due to stress during gestation and milk production rendering them more susceptible to blood parasites infection [29,30]. Blood parasites infection was present in all age groups, but a group of 12 ≥ X<6 recorded the maximum rate of infection in all detected parasites (Table 3). This is due to owners and nomads prefer to graze their animals in open fields' where they become more exposed to vector bites, and this age group had the most performance and activity.

Furthermore, the distribution of blood parasites in the three sites of the study area reflected their characteristic. The effective diagnosis of parasitic infections requires highly sensitive and specific tests [31]. In many cases the identification of parasites concerns their epidemiology and it is important to distinguish between species and subspecies. Piroplasms occasionally occur in blood of carrier animals but in many cases they can't be detected by direct examination, the diagnosis must be confirmed by detecting schizont [32]. Many authors did not clarify the incidence and the exact species of the blood parasite investigated camels [33]. PCR-based methods of detection have allowed prevalence data of parasites to be obtained with far greater accuracy than conventional microscopy and in some instances has taken over as the 'gold standard' for the diagnosis of parasitic infections [34].

In the present study, 60 out of 331 camels examined by GSBS, were positive for *Trypanosoma* (20.24%). Nearly the same rate of infection was recorded in 16.9% of local camels and 20.9% of imported camels from Sudan at Matrouh and Aswan governorates, respectively [8,35]. Lower rates were recorded in different countries from time to time viz., 3.5% in Nigeria [1], 5.7% in Egypt [31], and 4% in Kenya [36]. On the other hand, the prevalence of *T. evansi* by GSBS was 29.17% at Darwa quarantine, Aswan, Egypt [37]. This variation in rates could be due to climatic variation, the diverse farming systems, abundance of vectors, and lack of health care and lack of veterinary services. Molecularly, PCR results showed that 222 out of 331 camels tested were having trypomastigotes 67.06% [194 (87.39%) *T. evansi*; 28 (12.6%) *T. brucei*], compared to a rate of 65.9% was recorded in the same area using RoTat1.2 amplified 205 bp [8]. Sex are likely to be risk factors for trypanosomiasis in camels in contrast to other blood parasites examined, however 162 out of 241 (67.2%) females and 32 out of 90 (35.6%) males were *T. evansi*-positive. This is coincides with two reported studies [8,38]. Those results were nearer to that previously recorded: 73.5% in Halaib, Shalateen and Abu-Ramad Triangle, 90% in Siwa Oasis and 46.7% in Maryout, Egypt [30]. In addition, our present study showed that where Mersa Matrouh recorded the highest rate of *T. evansi* infection 78.3%, Sidi Barrany the border area between Libya and Egypt recorded the highest rate of *T. brucei* infection evidencing the presence of *T. brucei* in Egypt in 22 (9.13%) females and 6 (6.7%) males. Based on the available information, the presence of *T. brucei* in Egypt was monitored for the first time [39], and the presence of mixed genotyping between *T. evansi* isolates with *T. brucei* in frontiers was discussed in detail [30,40].

Concerning theileriosis, it is considered to be the second most important haemoprotozoan disease following trypanosomosis affecting dromedary camels in tropical and subtropical countries [41]. In the present study, the parasite is thought to be transmitted by several species of ticks of the genus *Hyalomma*. However, a parallel study on tick-borne pathogens in camels (not published yet) by the same scientific team had confirmed *Hyalomma species*, the principal vector in the study area, has a big role in *Theileria camelensis* transmission among camel population. The current work indicated 50.8% of the examined camels by GSBS harbored *Theileria* with various developmental stages of different shapes and forms inside erythrocytes and schizont [42,43]. By using PCR assay, 71.9% of investigated camels were having theileriosis caused by *Theileria sp.* and the incidence of infection was 71.4% in females and 73.3% in males in agreement with those previously reported [44-47]. Higher and lower rates of *Theileria camelensis* ranged from 6.9% to 75% were recorded in Egypt by different authors [43,46-49]. In our opinion, these variations may be attributed to nature of the study area, climatic condition, diagnosis method and animal influences. Moreover, the specificity of piroplasms infection in camels has been achieved in the present study due to absence of *Th. Annulata* infection despite of presence in other ruminants in the study area [50]. This is in contrast to a study provided an evidence of low host specificity of piroplasmids and the possibility that dromedaries are capable of hosting other host-specific piroplasms [45].

Regarding anaplasmosis, there is such dearth of research on camel anaplasmosis. *A. marginale* is considered capable of infecting dromedaries [51], and the occurrence of subclinical anaplasmosis was addressed in dromedaries' camels [52]. In the present study, 47.4% of examined camels were harbored anaplasmosis by GSBS, while the overall infection rate recorded by PCR was 67.4% (223/331), of them, 78.9% and 63.1% were found in males and females, respectively. *Anaplasma* was detected in two forms belonging to *A. marginale* and *A. centrale* which were screened together in 172 (77.13%) of positive samples, wherever only *A. marginale* was detected in 51 (22.9%). These results were lower than those reported the presence of *A. marginale* in 83.8% and 95.5% of examined camels [51,53], respectively. A recent study in Nigeria showed the prevalence of hemoparasites in camels was 21.5%, *Anaplasma* sp. was the common hemoparasites seen in examined camels, and the infection was higher in females than males [54,55].

Few papers have reported *Babesia* sp. in camels; *B. caballi* was recorded for the first time in Sudanese camel [55], and the infection of *Camelus dromedaries* by *Babesia* was recorded in Egypt [47]. In the present study, 39/331 (11.8%) and 61/331 (18.43%) were harbored the infection with *Babesia* using GSBS and PCR-based assay respectively, and relatively low infections were recorded in 19.5% females and in 15.56% males. When *Babesia* positive samples identified by PCR-based assays, 36 (59.01%) and 25 (40.9%) of *Babesia* infection were found belong to *B. bovis* and *B. bigemina*, respectively. This means that we face problematic to cure these infected animals as they become carriers of the parasite and serve as reservoirs for transmission; in particular *B. bovis* is more dangerous than *B. bigemina* because it is less sensitive to some babesiacidal compounds [55]. These findings are contrary to previous studies that recorded an overall prevalence of 29% for *Babesia*, using PCR in Pakistan, whereby 11% were positive for *B. bovis* and 18% for *B. bigemina* [55]. In Nigeria, *Babesia* and *Anaplasma* species were the common hemoparasites seen in camels examined either singly or in combination, and more females (44.5%) than males (34.5%) were positive for various parasitic infections with no significant difference [54]. A recent study recorded an infection rate of 13.2% in camels in Saudi Arabia [2]. On the other hand, more recent study in Iran indicated 6.56% of camels were positive for *Babesia* spp. and the infection rate in males and females was 6.76% and 5.17%, respectively [56].

Combined data of blood parasites infection in our study revealed that 307 out of 331 examined camels were positive (92.7%), while 24 camels (7.25%) were free. Mixed infection was common in the same camel (65.9%), while 26.9% were having the infection with one parasite. A percentage of 25.7% and 28.1% were having 2 and 3 different parasites respectively, while 12.1% were infected with 4 different parasites. In our opinion, we suggest that the capacity to mount immune response against *Theileria, Anaplasma and Babesia* may be immunosuppressed in *T. evansi* infected camels in agreement with a study demonstrated that *T. evansi* infection lowers the immune-responsiveness of camels to concurrent immunizations [57].

In conclusion, it is the first time to expand our knowledge of the molecular epidemiology of parasitic infections in camels in this region except for *Trypanosoma*. Camels appeared to succumb to the infection with *Theileria, Anaplasma and Trypanosoma* with high rates. *A. marginale* and *A. centrale* were the main cause of anaplasmosis in dromedaries. This is the first report to show the presence of *Babesia* DNA in camels in this area. The source of infection of camels with *A. marginale*, *B. bovis and B. bigemina* might be as a result of animal movement to neighboring governorates. In addition to climates and tests, the vectors "particularly ticks" are of importance in transmission and disease management in this area. From the present study, it is clear that we face a big problem needed to reevaluated, especially the first infection with blood parasite may make the host more likely to acquire the second.

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