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Molecular Detection of Pathogens in Ticks Infesting Camels in Matrouh Governorate, Egypt

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Abstract

Tick-borne pathogens become healthy important as the incidence of tick-borne diseases increases and the geographic areas in which they are found expand. A relatively little information is available on ticks infesting camels in Matrouh Governorate, Egypt, and the role of ticks as disease vectors. Thus rendering PCR assay the only viable alternative to demonstrate their presence. For this purpose, a surveillance was carried out from May 2011 to April 2013 to identify ticks parasitize camels, and tested part of them for the presence of parasitic, rickettsial and bacterial pathogens using specific primers targeting fragments of their genes. Out of 249 studied camels, 212 (85.14%) were infested by five species of ticks that increased in numbers during dry seasons. *Hyalomma dromedarii* was the predominant tick species (73.65%), followed by *H. rufipes* (12.03%), *H. truncatum* (6.62%), and low numbers were *H. anatolicum excavatum* (4.73%), and *H. impeltatum* (1.62%), besides 1.35% were belong to other species. PCR results revealed that the majority of samples were found co-infected with at least five pathogens. It evidenced the presence of *Trypanosoma evansi*, *Trypanosoma brucei*, *Babesia bovis*, *Babesia bigemina*, *Theileria camelensis* and *Anaplasma marginale*. *Borrelia burgdorferi*, *Rickettsial* DNA, and *Theileria annulata* were absent. *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma* sp. were detected in ticks DNAs, but not known if transmitted by ticks or not. We conclude that several pathogens are present in ticks in this area, phylogeny is required in order to validate the PCR results, and special attention should be given to tick control programs.

Keywords: Egypt; Tickborne pathogen; Camels; PCR

Introduction

The development of newly reclaimed areas has attracted attention to the problem of vectors and vector-borne diseases in particular ticks in many countries [1-5]. Ticks are second only to mosquitoes as vectors of rickettsial, bacterial, viral, and protozoan agents. Family *Ixodidae* (hard ticks) is the largest family of ticks species [6], some of them play the major role, as vectors, in spreading different diseases throughout the world, and transmit a great variety of pathogens to mammalian hosts, including human beings [7,8]. Ticks co-infected with multiple pathogens greatly increased the risk of co-infections to animals and humans, which would result in more complex clinical manifestation lead to misdiagnosed [9].

Babesiosis, theileriosis, and trypanosomiasis (Surra) are three economically important vector-borne diseases of tropical and subtropical parts of the world [10]. Apicomplexan haemoprotozoan parasites, *Babesia* sp. and *Theileria* sp. are transmitted by ticks, causing significant morbidity and mortality in animals, where *T. evansi* the causative agent of Surra is mechanically transmitted by biting insects, especially tabanids, and stomoxys, and possibly, by ticks [11]. The potential role of ticks as passive vectors of *T. evansi* by direct ingestion of contaminated blood and via engorged ticks could not be demonstrated [12].

Among tick-borne bacteria, extracellular spirochetes of the genus *Borrelia* are widely spread and most studied [13]. Some of these belong to the *Borrelia burgdorferi sensu lato* complex, are causative agents of Lyme borreliosis [14]. In addition, intracellular alpha-proteobacteria, which includes the families *Anaplasmataceae, Bartonellaceae*, and *Rickettsiaceae* could be transmitted by ticks [15]. *A. marginale* is the most prevalent tick-borne pathogen of animals worldwide [16,17]. It is transmitted by at least 20 ticks' species [18]. Other pathogenic bacteria, such as *Pasteurella* and *Haemophilus* (family: Pasteurellaceae), and *Mycoplasma* that infect animals, are not known if transmitted by ticks or not [19,20]. *Pasteurella multocida* causes disease in both humans and animals, including fowl cholera in poultry and bovine hemorrhagic septicemia in cattle and buffalo [21]. *Histophilus somni* (*H. somni*) is a parasitic organism that typically lives as a commensal organism in its host, in upper respiratory tract, prepuce, and vagina, and it can also cause many fatal diseases, especially in bovine [22].

In Egypt, few reports have been published in spite of ticks are often found on camels in large numbers. Some case reports are not considered reliable because they usually fail to give adequate taxonomic descriptions concerning tick-borne pathogens in camels [23,24]. In addition, most studies on the tick population dynamic were carried out on sheep, goats, cattle and on imported camels, not local Maghrabi camels, the most important domestic animals raised by local Bedouins in Matrouh Governorate, Egypt. The objectives of this study were to survey and identify ticks species were found on local camels, and to detect pathogens in ticks by different PCR assays

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to gain insight into transmission patterns of tick-borne diseases in camels in the study area.

Materials and Methods

Research area and study design

The study was carried in Matrouh governorate, the second largest province in terms of area in Egypt, with a special focus on remote sites areas. It represented the Northern West Coastal zone from Mersa-Matrouh to El-Salloum in the west. Three distinct regions were selected between latitude; north 31° 19'- 26° 00 and longitude; 27° 45'- 28° 00. Summer of this area is moderately hot and humid while its winter is mild and moderately wet. No tick control program is undertaken in this area, but owners occasionally treat camels using Ivomac injection and sometimes remove them manually. Therefore, any strategy intended to identify pathogens could be transmitted by tick infestation of camels should take into account the identified tick species and their abundance. For this purpose, 249 adult Maghrabi camels (*Camelus dromedaries*) belong to 33 different herds distributed all over study area were randomly selected. Ticks collection was carried out in seven different times from May 2011 to April 2013. Not all visible ticks were collected.

Tick collection

Tick specimens were collected from different predilection sites on the camel if present. They were counted and identified to determine the infestation and distribution of ticks in camels. Ticks sample from each camel were preserved in a separate vial containing 70% ethanol for further processing for pathogens DNAs identification and examined under a binocular stereomicroscope. The vials were labeled with regard to sites, date, animal number, sex, and age.

Samples preparation

Samples were washed twice with distilled water and dried on a bleached pulp. They were identified to the species level according to Hoogstraal [25] and Walker et al. [26], taking into consideration the recent valid names of the genus and species. The main identification features of the ticks are color, size, the shape of mouthparts, scutum, anal groove, festoon, punctuation, and legs. Ticks of each herd were pooled onto one PCR sample.

DNA extraction

Before DNA extraction, the ticks were divided into pools (maximum of 30 ticks/ pool) by collection camel herd. After the gut and a part of haemolymph of the collected tick species were homogenized in 180 µl ATL (Tissue lysis buffer) with a 5 mm steel bead in the Tissue Lyser (Qiagen, Hilden, Germany), DNA was extracted according to the manufacturer's blood and tissue DNeasy mini kit protocol (QIAamp DNA Mini Kit, Qiagen) in two elution steps of à 100 µl for the best quantitative DNA result. Recovery and purity of each DNA sample were estimated by spectrophotometer (NanoDrop®ND-1000, PeqLab, Erlangen, Germany) according to manufacturer's instructions (NanoDrop® User Manual, 2004), and stored in -20°C for used.

Molecular detection of pathogens

Ticks from the three sampling sites were screened for DNAs of parasitic and bacterial pathogens: *Babesia, Theileria, Trypanosoma, Rickettsia, Anaplasma, Borrelia, Histophilus, Pasteurella and Mycoplasma*. Conventional PCRs with previously published primers were used for the detection of DNAs of these pathogens expected to be found in extracted DNAs of collected ticks according to [27-38].

DNA amplification

Individually PCR amplification reaction was performed in a total reaction volume of 25 µl containing 100 ng of DNA, 10 pmol of each of forward and reverse primer, 1U Taq DNA polymerase, 2.5 mM $MgCl_2$ and 200 µM of dNTPS (Promega, Germany). All amplifications were carried out in a Biometra® thermocycler. PCR cycling conditions and specific primers were summarized in Table 1. After amplification, 10 µl of PCR product were loaded on 1.5% agarose gel; electrophoresis was

Pathogen	Primer sequence (5'-3')	PCR condition	(Bp)
Theileria sp.	F: AGTTTCTGACCTATCAG and R: TTGCCTTAAACTTCCTTG [27]	95°C for 3 min., 33 cycle at 94°C for 30 sec., 50°C for 30 sec., 72°C for 30 sec. and final extension (f. ext.) at 72°C for 5 min.	1100
Theileria annulata	F: ACT TTG GCC GTA ATG TTA AAC and R: CTC TGG ACC AACTGTTTGG [28]	95°C for 5 min., 33 cycle at 94°C for 30 sec., touchdown from 62°C-50°C for 30 sec., 72°C for 30 sec. and f. ext. at 72°C for 5 min.	312
Anaplasma marginale	F: GCT CTA GCA GGT TAT GCG TC and R: CTG CTT GGG AGA ATG CAC CT [29]	95°C for 3 min., 35 cycle at 94°C for 30 sec., 57°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 7 min.	265
Babesia bovis	F: TTTGGTATTTGTCTT GGTCAT and R: ACCACTGTAGTC AAACTCACC [30]	95°C for 3 min., 35 cycle at 94°C for 30 sec., 57°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 6 min.	448
Babesia bigemina	F: TAG TTG TAT TTC AGC CTC GCG and R: AAC ATC CAA GCA GCT AHT TAG [31]	95°C for 3 min., 35 cycle at 94°C for 30 sec., 57°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 6 min.	639
Trypanosoma brucei	F: GAA TAT TAA ACA ATG CGC AG and R: CCA TTT ATT AGC TTT GTT GC [32]	95°C for 3 min., 33 cycle at 94°C for 30 sec., 52°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 10 min.	164
Trypanosoma evansi	F: GCG GGG TGT TTA AAG CAA TA and R:ATT AGT GCT GCG TGT GTT CG [33]	95°C for 3 min., 33 cycle at 94°C for 30 sec., 52°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 10 min.	205
Borrellia burgdorferi	F: CCT GTT ATC ATT CCG AAC ACA G and R: TAC TCC ATT CGG TAA TCT TGG G [34]	95°C for 3 min., 33 cycle at 94°C for 30 sec., touchdown from 66°C-50°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 5 min.	411-452
Rickettsia 16S rRNA	F: GGG GGC CTG CTC ACG GCG G and R: ATT GCA AAA AGT ACA GTG AAC A [35]	95°C for 3 min., 33 cycle at 94°C for 30 sec., touchdown from 64°C-50°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 5 min.	380
Histophilus somni	F: AGA GTT TGA TCA TGG CTC AG and R: AGG GTT ACC TTG TTA CGA CTT [36]	95°C for 3 min., 33 cycle at 94°C for 30 sec., 52°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 7 min.	408
Pasteurella multocida	F: ATC CGC TAT TTA CCC AGT GG and R: GCT GTA AAC GAA CTCGCCAC [37]	95°C for 3 min., 35 cycle at 94°C for 30 sec., 50°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 10 min.	460
Mycoplasma spp.	F: GGGAGCAAACAGGATTAG AC CCT and R: TGCACCATC TGTCACT CTGTTAACC TC [38]	95°C for 3 min., 30 cycle at 94°C for 30 sec., 52°C for 30 sec., 72°C for 30 sec. and f. ext.at 72°C for 5 min.	270

Table 1: Nucleotide sequences of specific primers, PCR conditions and the targeted size for pathogens transmitted by ticks.

done at 120 V for 1 h, with 100 bp DNA ladder (Qiagen®, Germany) as a standard molecular weight marker. Gels were stained with ethidium bromide and photographed by gel documentation machine for bands analysis.

Data management and analysis

Data obtained in this survey was entered in MS Excel and analyzed using SPSS version 20.0 (IBM SPSS Statics 20, USA). Chi-square test was applied to compare the difference in results. All statistics were considered significant at $P \leq 0.05$.

Results

Ticks and ticks species

In the current study, results in Tables 2 and 3 revealed that 740 ticks were collected from 249 camels (75 male + 174 female) and identified. Out of those animals, 212 (85.14%) were found to be infested with ticks and females harbored more ticks (93.69%) than males (65.33%). The majority of camels were apparently healthy during tick collection period. Most of the ticks belonged to the genus *Hyalomma*. *H. dromedarii* was the predominant species 545 (73.65%) followed by *H. rufipes* 89 (12.03%), *H. truncatum* 49 (6.62%), *H. anatolicum excavatum* 35 (4.73 %), *H. impeltatum* 12 (1.62%), and others 10 (1.35%). Ticks significantly preferred to attach to the lower parts of the camel's body under the tail, scrotal/ udder and nose for feeding than the back/side of the animal.

Parameter		Total no. of ticks	No	%
Tick species	*Hyalomma dromedarii*	740	545	73.65
	H. marginatum rufipes		89	12.03
	H. truncatum		49	6.62
	H. anatolicum excavatum		35	4.73
	H. impeltatum		12	1.62
	Others		10	1.35
Date of infestation	Winter	740	0	0
	Spring		398	53.78
	Summer		292	39.46
	Autumn		50	6.76
Site of infestation	Lower parts	740	474	64.05
	Ear and Nose		187	25.27
	Ventral and Back /side		79	10.68

*A Significant difference (P<0.05) in prevalence was observed between tick species, in the date of infestation, and the site of infestation.

Table 2: Prevalence of ticks infested camels on the basis of tick species, date of infestation and site of infestation.

Item		No.	Infested camels	Frequency
Location	Mersa-Matrouh	111	89	80.18
	El- Negeila	68	57	83.82
	Sidi-Barrany	70	66	94.29
	Total	249	212	(85.14)
Sex	Males	75	49	65.33
	Females	174	163	93.68
	Total	249	212	(85.14)
Age groups	X ≤ 4	116	84	72.41
	X>4	133	128	96.24
	Total	249	212	(85.14)

*No significant difference (P>0.05) in prevalence between the three studied areas, but significance difference (p<0.05) was observed between both sexes and among age groups of studied camels.

Table 3: Prevalence of infested camels with ticks on the basis of location, sex and age.

There was a significance difference in tick infestation rate between sex groups and within age groups (P<0.05), while there was not significance difference (P>0.05) variation detected between locations. Based on the date of tick collection, spring and summer seasons recorded the highest rate of infestation, whereas infestation rate was not observed during the winter season (Figure 1).

Detection of pathogens by PCR assays

In the present study, results of tick-borne parasites revealed three genera of pathogens, namely *Theileria, Babesia* and *Trypanosoma*, were detected in the three locations. Parasites were detected using five species-specific primers as summarized in Table 1. The PCR amplified fragments for *T. evansi, T. brucei, B. bovis*, *B. bigemina* and *Theileria sp*. at 205, 164, 446, 689 and 1100 bp respectively, while *Theileria annulata* product was absent in ticks parasitizes camels (Figure 2). Also, *B. burgdorferi* and *Rickettsia* were not detected in ticks in this area. The prevalence of *Anaplasma spp*. in ticks was estimated from the number of PCR-positive samples based on the major surface protein–1β encoding gene. Out of 33 herds were examined for Anaplasmosis, 25 (75.76%) were harbored *Anaplasma* amplified at 519 bp instead of 265 bp, the expected size, showing a lack of specificity to confirm the occurrence of only *A. marginale* in ticks (Figure 3). The presence of bacteria in *Hyalomma* was screened by individually PCR assays targeting 408 bp, 460 bp and 270 bp for *Histophilus somni, Pasteurella multocida* and *Mycoplasma* spp., respectively (Figure 4).

Discussion

The economic importance of tick infestation on camels is important as they are important meat and milk producer animals, and their health and production are greatly affected by the high tick infestation [39]. *Hyalomma* spp. are increasingly being recognized as playing a role in human and animal diseases [40,41]. *H. marginatum* is distributed in a wide range of Arabia, parts of Northeastern and South Africa, and Central Asia, whereas the distribution of *H. anatolicum excavatum* is somewhat more limited [42]. Most parts of Egypt especially deserts offer favorable environmental conditions for ticks, which can infect a variety of hosts and transmit diseases to livestock animals [43]. Epidemiological studies

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

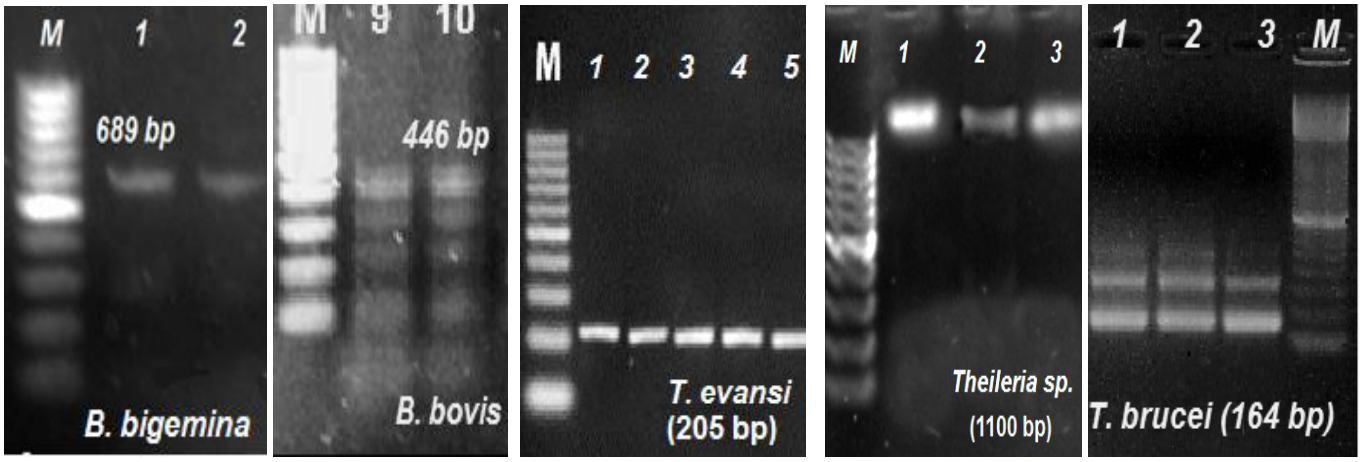

Figure 2: Agarose gel electrophoresis of amplified DNAs from different parasitic pathogens (*B. bigemina, B. bovis T. evansi, T. brucei,* and *Theileria* sp.), using five sets of species specific primers. M, 100 bp molecular size marker (Qiagen®, Germany)

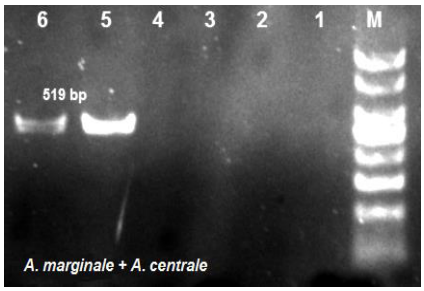

Figure 3: Agarose gel electrophoresis of PCR based assay products of 519 bp were amplified from *A. marginale* in lanes 5 and 6. Lanes from 1 to 4 represent negative results. M, 100 bp molecular size marker (Qiagen®, German).

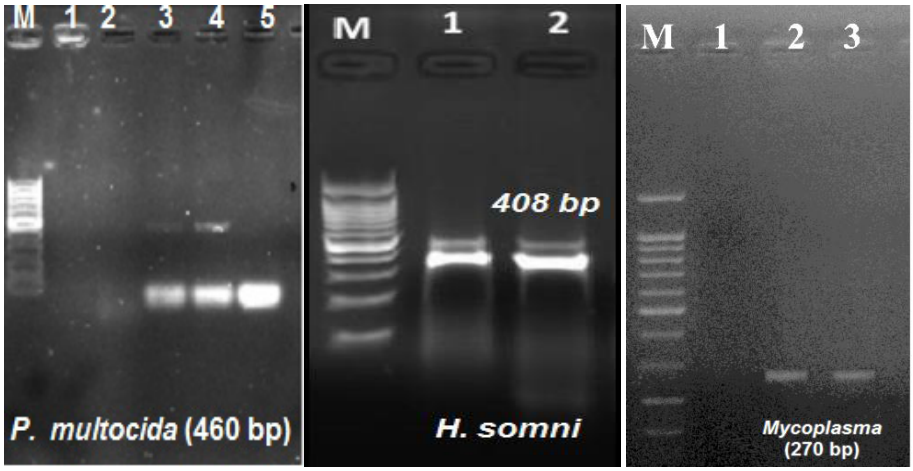

Figure 4: Agarose gel electrophoresis of PCR based assays of different bacterial pathogens DNAs from *P. multocida, H. somni* and *Mycoplasma* sp. M, 100 bp molecular size marker (Qiagen®, Germany)

on simultaneous detection of tick-borne pathogens in local Maghrabi dromedary camels are rare. In the present study, results revealed the presence of five species of tick infesting camels belong to subgenus *Hyalomma* contains ticks of veterinary and public health importance. *H. dromedarii* was the predominant species on camels followed by *H. rufipes, H. truncatum, H. anatolicum excavatum* and *H. impeltatum*. This finding is in agreement with results were reported by Diab et al. [44], Mazyad and Khalaf [45], Van Straten and Jongejan [46] in Egypt, Al Waer [47] in Libya, El Khalifa et al. [48] in Saudi Arabia and Nazifi et al. [39] in Iran. Additionally, females of *H. dromedarii* were found to be engorged on camels till fall on the soil around water resources, in stables, and weedy or fallow fields, while the females of the other tick species were found either flat or partially engorged, indicating the host specificity of camels to *H. dromedarii*. This finding on host preference

is in agreement with Elghali [49], and El Tigani and Mohammed [50] in Sudan, who speculated that *H. dromedarii* competes for other tick species and lessen their chance to engorge.

Regarding the date of infestation in the present study, ticks were found on camels throughout the year, except rainy season in December and January, and increased in numbers during dry seasons from March to October. Our result was observed also by Diab et al. [44] who reported high tick infestation in Egypt occurs during March to November, but it is in contrary to Zeleke and Bekele, [51]. The effect of age on tick load was shown by the fact that, the older camels carried significantly more tick load than the younger. This finding is in agreement with Abdalla [52] and Al Waer [47], who explained that the majority of moving camels fall within this age, and thus are more exposed. Because of the three selected regions are nearly the same in eco-types, and no clear differences in their characters, their susceptibility to tick infestation are not significantly different. This was probably related to an insufficient tick control strategy in these areas. The high tick load was recorded in Sidi-barrany compared to El-Negeila and Mersa-Matrouh may be due to regular migration of local camels from neighboring areas to Sidi-Barrany for grazing in drying seasons [43], and imported camels from Libya across Sidi-Barrany. The highest infestation level of ticks was observed on the lower parts of the camel's body under the tail, scrotal/ udder, and nose, and the lowest was observed on the back and side of the animal's body region in agreement with Taddese and Mustefa [53]. In addition, the infestation rate of ticks in females was high comparing with males. This may due to the majority of collected samples were from female-camels, whereas nomads keep only one or two males for reproduction purpose [43].

Concerning to the presence of hemoparasitic, rickettsial and bacterial diseases, it is broadly related to the presence and distribution of their vectors. It could be diagnosed with difficulty by using in vitro cultivation, blood smear microscopy, and serological methods. Because ticks can harbor more than one disease-causing agent, the camel can be infected with more than one pathogen at the same time, compounding the difficulty in diagnosis and treatment. Few studies have been previously reported the presence of theileriosis (caused by *Th. annulata*), babesiosis (*B. bovis*), and anaplasmosis (*A. marginale*) to affect both small ruminants and cattle in the study area [54,55]. In our study, *T. evansi, Th. camelensis* and *A. marginale* were the most frequently detected pathogens from the three locations, wherever, *T. brucei and H. somni* were detected in a few herds (Table 4 and Figures 2-4). Additionally, we detected two pathogenic *Babesia* species (*B. bovis and B. bigemina*) which were also detected in camels in this area with low prevalence. On the other hand, no detectable amplified products were seen using primers for *Th. annulata* despite the presence of this pathogen in small ruminants and cattle were kept in the same governorate [56]. Those results are in agreement with an epidemiological data related to blood parasites in camels in the study area [57]. To our knowledge, small ruminants and cattle were kept in the same area, but camels were rearing in open fields apart from other animals. So, it is not necessary that the same pathogen infected camel strains that afflict other ruminants. This result indicates that *Th. annulata* and *Borrelia burgdorferi*, if present, might be rare in camels in this area in agreement with Hvidsten et al. [58]. Furthermore, the amplified product of *A. marginale* was detected at 519 bp, not 265 bp, the expected size identified by Bilgiç et al. [27]. This coincides with a parallel study of camel anaplasmosis caused by mixed infections of *A. marginale* and *A. centrale* gave the same result in this area [59]. On the other hand, no amplified products of *Rickettsia* sp. were detected by PCR in spite of ticks belong to *Hyalomma* could transmit *Rickettsia* sp., *Anaplasma*, and several protozoa that infect wildlife, livestock, and people [59-61].

Pathogen	Sampling location			
	Mersa-Matrouh (n=11)	El-Negeila (n=9)	Sidi-barrany (n=13)	Total herds (n=33)
Theileria camelensis	7 (63.64%)	8 (88.89%)	10 (76.92%)	25 (75.76%)
Theileria annulata	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Babesia bovis	2 (18.18%)	3 (33.3%)	1 (7.69%)	6 (18.18%)
Babesia bigemina	3 (27.27)	4 (44.44%)	2 (15.39%)	9 (27.27%)
Trypanosoma brucei	1 (9.09%)	1 (11.11%)	4 (30.77%)	6 (18.18%)
Trypanosoma evansi	9 (81.81%)	6 (66.67%)	8 (61.54%)	23 (69.70%)
Anaplasma sp.	9 (81.81%)	8 (88.89%)	8 (61.53%)	25 (75.76%)
Rickettsia sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Borrellia burgdorferi	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Histophilus somni	1 (9.09%)	2 (22.22%)	1 (7.69%)	4 (12.12%)
Pasteurella multocida	1(9.09%)	3 (33.3%)	5 (38.46%)	9 (27.27%)
Mycoplasma spp.	5 (45.45%)	3 (33.33%)	3 (23.08%)	11 (33.33%)

Table 4: Prevalence of detected pathogens in different camel herds in relation to sampling location.

Despite the increasing information available on ticks, there are no recent reviews on tick-borne bacterial diseases except Lyme disease. In the present study, 9 pooled samples out of 33 (27.27%), 11 (33.33%) and 4 (12.12%) were having *P. multocida, Mycoplasma* sp. and *H. somni* amplified fragments at 460 bp, 270 bp, and 408 bp, respectively. The presence of *Mycoplasma* sp. was coincided with Abou-Elnaga et al. [54], who applied PCR for identification of *Mycoplasma* isolated from aborted she-camel samples in Maryout region, Egypt. We did not know the role of ticks, is it play as a vector or not? The same result was recorded by Quan et al. [62], who reported that most human infections with *P. multocida* are due to animal bites, and ticks may contain *P. multocida*, but they are not considered to act as a vector, thus further studies are needed for answering this question.

Conclusion

This is the first molecular study evaluated and evidenced the presence of *Anaplasma* and three bacterial pathogens in ticks in Egypt. Of the pooled samples were examined for tick-borne pathogens, only two were free rendering high problem in rearing camels. It is important to point out that *Th. annulata, Rickettsia* sp. and *Borrelia burgdorferi* not exist in *Hyalomma* ticks in the study area. Until now, we are still not sure that ticks have a role in transmitting *T. evansi, P. multocida, Mycoplasma*, and *H. somni* or hosted it. We recommended that pathogens in ticks in this area requires further genotyping investigation.

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References

1. Uspensky I (2008) Ticks (acari: Ixodoidea) as urban pests and vectors with special emphasis on ticks outside their geographical range. In: Proceedings of the Sixth International Conference on Urban Pests William H Robinson and Dániel Bajomi (editors), Printed by OOK-Press Kft., H-8200 Veszprém, Pápai út 37/a, Hungary.

2. Comer JA, Paddock CD, Childs JE (2001) Urban zoonoses caused by Bartonella, Coxiella, Ehrlichia, and Rickettsia species. Vector Borne Zoonotic Dis 1: 91-118.

3. Dautel H, Kahl O (1999) Ticks (Acari: Ixodoidea) and their medical importance in the urban environment. In: Proceedings of the 3rd International Conference on Urban Pests, Prague, Czech Republic: Czech University of Agriculture pp.73-82.

4. Gratz NG (1999) Urbanization, arthropod and rodent pests and human health. In: Proceedings of the 3rd International Conference on Urban Pests. Prague, Czech Republic: Czech University of Agriculture pp.51-58.

5. Steere AC (1994) Lyme disease: a growing threat to urban populations. Proc Natl Acad Sci USA 91: 2378-2383.

6. Barker SC, Murrell A (2004) Systematics and evolution of ticks with a list of valid genus and species names. Parasitology 129 Suppl: S15-36.

7. Kakar MN, Kakarsulemankhel Jk (2008) Re-description of Hyalomma anatolicum excavatum Koch, 1844 (Metastigmata, Ixodidae). Pak Entomol 30: 141-146.

8. Parola P, Raoult D (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 32: 897-928.

9. Chen Z, Liu Q, Liu JQ, Xu BL, Lv S, et al. (2014) Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. Parasit Vectors 7: 237.

10. Homer MJ, Aguilar-Delfin I, Telford SR 3rd, Krause PJ, Persing DH (2000) Babesiosis. Clin Microbiol Rev 13: 451-469.

11. Camargo RE, Uzcanga GL, Bubis J (2004) Isolation of two antigens from Trypanosoma evansi that are partially responsible for its cross-reactivity with Trypanosoma vivax. Vet Parasitol 123: 67-81.

12. Vergne T, Kamyingkird K, Desquesnes M, Jittapalapong S (2011) Attempted transmission of Trypanosoma evansi to rats and mice by direct ingestion of contaminated blood and via engorged ticks. Acta Protozool 50: 133-136.

13. Korenberg EI, Kovalevsky YV, Gorelova NB (2002) Ecology of Borrelia burgdorferi sensu lato in Russia. In: Gray G, Kahl O, Lane RS, editors. Lyme borreliosis epidemiology and control. Oxford (UK): CAB International pp. 175-200.

14. Parola P, Raoult D (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 32: 897-928.

15. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, et al. (2001) Reorganization of genera in the families' Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophilum. Int J Syst Evol Microbiol 51: 2145-2165.

16. Jacomo V, Kelly PJ, Raoult D (2002) Natural history of Bartonella infections (an exception to Koch's postulate). Clin Diagn Lab Immunol 9: 8-18.

17. Zeaiter Z, Fournier PE, Raoult D (2002) Genomic variation of Bartonella henselae strains detected in lymph nodes of patients with cat scratch disease. J Clin Microbiol 40: 1023-1030.

18. Hubbard MJ, Baker AS, Cann KJ (1998) Distribution of Borrelia burgdorferi s.l. spirochaete DNA in British ticks (Argasidae and Ixodidae) since the 19th century, assessed by PCR. Med Vet Entomol 12: 89-97.

19. Larsen B, Hwang J (2010) Mycoplasma, Ureaplasma, and adverse pregnancy outcomes: a fresh look. Infect Dis Obstet Gynecol 2010.

20. Howard MD, Cox AD, Weiser JN, Schurig GG, Inzana TJ (2000) Antigenic diversity of Haemophilus somnus lipo-oligosaccharide: phase-variable accessibility of the phosphorylcholine epitope. J Clin Microbiol 38: 412-4419.

21. Kokotovic B, Friis NF, Ahrens P (2007) Mycoplasma alkalescens demonstrated in bronchoalveolar lavage of cattle in Denmark. Acta Vet Scand 49: 2.

22. Alton CSW, Glen CW, Bruce CA, Patrick JC, Karen FA, et al. (2005) Haemophilus somnus (Histophilus somni) in bighorn sheep. University of Idaho, College of Agriculture, Caine Veterinary Teaching Center, 1020 East Homedale Road, Caldwell, Idaho, 83607-8098, USA (Ward, Weiser, Anderson).

23. Alim MA, Das S, Roy K, Masuduzzaman M, Sikder S, et al. (2012) Prevalence of haemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Pak Vet J 32: 221-224.

24. Hamed MI, Zaitoun AMA, El-Allawy TAA, Mourad MI (2011) Investigation of Theileria camelensis in camels infested by Hyalomma dromedarii ticks in Upper Egypt. J Adv Vet Res 1: 4-7.

25. Hoogstraal H (1956) African Ixodidae, ticks of the Sudan with special reference to equatoria province and preliminary reviews of the genera Boophilus, Margaropus and Hyalomma. Research Report NM 00505029, Navy, Bureau of Medical Surgery, Washington DC., USA pp. 1-1101.

26. Walker AR, Bouattour A, Camicas J (2003) Ticks of domestic animals in Africa-a guide to identification of species. Unpublished Bioscience Report 42, Coniston Drive Edinburgh, Scotland, UK pp: 1-221.

27. Allsopp BA, Baylis HA, Allsopp MTEP, Cavalier-Smith T, Bishop RP, et al. (1993) Discrimination between six species of Theileria using oligonucleotide probes which detect small subunit ribosomal RNA sequences. Parasitol 107: 157-165.

28. Bilgic HB, Karagenç T, Shiels B, Tait A, Eren H, et al. (2010) Evaluation of cytochrome b as a sensitive target for PCR based detection of T. annulata carrier animals. Vet Parasitol 174: 341-347.

29. Bilgiç HB, Karagenç T, Simuunza M, Shiels B, Tait A, et al. (2013) Development of a multiplex PCR assay for simultaneous detection of Theileria annulata, Babesia bovis and Anaplasma marginale in cattle. Exp Parasitol 133: 222-229.

30. Chansiri K, Bagnara AS (1995) The structural gene for carbamoyl phosphate synthetase from the protozoan parasite Babesia bovis. Mol Biochem Parasitol 74: 239-243.

31. Ellis J, Hefford C, Baverstock PR, Dalrymple BP, Johnson AM (1992) Ribosomal DNA sequence comparison of Babesia and Theileria. Mol Biochem Parasitol 54: 87-95.

32. Masiga DK, Smyth AJ, Hayes P, Bromidge TJ, Gibson WC (1992) Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int J Parasitol 22: 909-918.

33. Claes F, Radwanska M, Urakawa T, Majiwa PAO, Goddeeris B, et al. (2004) Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. Kinetoplastid Biol Dis 3: 3.

34. Rar VA, Fomenko NV, Dobrotvorsky AK, Livanova NN, Rudakova SA, et al. (2005) Tickborne pathogen detection, Western Siberia, Russia. Emerg Inf Dis 11: 1708 -1715.

35. Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 173: 1576-1589.

36. Angen O, Ahrens P, Tegtmeier C (1998) Development of a PCR test for identification of Haemophilus somnus in pure and mixed cultures. Vet Microbiol 63: 39-48.

37. Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ (1998) Development of PCR assays for species- and type-specific identification of Pasteurella multocida isolates. J Clin Microbiol 36: 1096-1100.

38. Ossewaarde JM, De Vries A, Bestebroer T, Angulo AF (1996) Application of a Mycoplasma group-specific PCR for monitoring decontamination of Mycoplasma-infected Chlamydia species strains. Applied Env Microbiol 62: 328-331.

39. Nazifi S, Tamadon A, Behzadi MA, Haddadi S, Raayat-Jahromi AR (2011) One-humped camels (Camelus dromedarius) hard ticks infestation in Qeshm Island, Iran. Vet Res Forum 2: 135-138.

40. Apanaskevich DA (2003) [Towards a diagnostic view of Hyalomma (Hyalomma) aegyptium (Acari, Ixodidae)]. Parazitologiia 37: 47-59.

41. Wood OL, Moussa MI, Hoogstraal H, Büttiker W (1982) Kadam virus (Togaviridae, flavivirus) infecting camel-parasitizing Hyalomma dromedarii ticks (Acari: Ixodidae) in Saudi Arabia. J Med Entomol 19: 207- 215.

42. Capek M, Literak I, Kocianova E, Sychra O, Najer T, et al. (2014) Ticks of the Hyalomma marginatum complex transported by migratory birds into Central Europe. Ticks Tick Borne Dis 5: 489-493.

43. Barghash SM, El-Naga TR, El-Sherbeny EA, Darwish AM (2014) Prevalence of Trypanosoma evansi in Maghrabi camels (*Camelus dromedarius*) in Northern-West Coast, Egypt using molecular and parasitological methods. Acta Parasitol Glob 5: 125-132.

44. Diab FM, El-Kady GA, Shoukry A (2001) Bionomics of ticks collected from Sinai. 2. Abundance, attachment sites and density of ticks infesting Arabian camels. J Egypt Soc Parasitol 31: 479-489.

45. Mazyad SA, Khalaf SA (2002) Studies on theileria and babesia infecting live and slaughtered animals in Al Arish and El Hasanah, North Sinai Governorate, Egypt. J Egypt Soc Parasitol 32: 601-610.

46. van Straten M, Jongejan F (1993) Ticks (Acari: Ixodidae) infesting the Arabian camel (Camelus dromedarius) in the Sinai, Egypt with a note on the acaricidal efficacy of ivermectin. Exp Appl Acarol 17: 605-616.

47. Alwaer OR (2004) Epidemiology study on camel ticks infestation in Libya and vaccine trials using Boophilus derived vaccine against Hyalomma dromedarii ticks. Unpublished MSC Thesis, Institut Agronomique et Vétérinaire. HASSAN II, Morocco.

48. Al-Khalifa MS, Al-Asgah NA, Diab FM (1984) Ticks (Acari: Ixodidae) infesting common domestic animals in Al-Qasim Province, Saudi Arabia. J Med Entomol 21: 114-115.

49. Elghali A (2005) Studies on camel ticks in River Nile State, Sudan with particular reference to ecology and behavior of Hyalomma dromedarii, Acari: Ixodidae. Unpublished Ph.D. thesis, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

50. El Tigani MA, Mohammed AS (2010) Ticks infesting camels in El Butana area Mid-Central Sudan. Sudan J Vet Res 25: 51-54.

51. Zeleke M, Bekele T (2004) Species of ticks on camels and their seasonal population dynamics in Eastern Ethiopia. Trop Anim Health Prod 36: 225-231.

52. Abdalla MM (2007) Studies on ticks and tick-borne diseases of cattle in South Darfur State, Sudan', MVSc thesis, Department of Parasitology, University of Khartoum.

53. Taddese A, Mustefa M (2013) A Study on camels ticks in and around Dire Dawa, Eastern Ethiopia. Acta Parasitol Glob 4: 64-70.

54. Abou-El-Naga TR, Gouda ASA, Kamel YM, Mahmoud MA, Osman WA (2012) Application of the polymerase chain reaction for identification of Mycoplasma isolated from aborted she-camel. Glob Vet 8: 386-392.

55. Gebely MAM (2004) Prevalence of some parasitic diseases in small ruminants in Siwa Oasis. MVSc. Thesis (Infectious Diseases). Faculty of Veterinary Medicine, Beni-suef, Cairo University, Egypt.

56. Abou-El-Naga TR, Abdou TA, Mahmoud MA (2005) Clinicopathological studies on Theileria annulata infection in Siwa Oasis, Egypt. Beni-Suef Vet Med J 15: 40-46.

57. Abou El-Naga TR, Barghash SM (2016) Blood Parasites in Camels (Camelus dromedarius) in Northern West Coast of Egypt. J Bacteriol Parasitol 7: 258.

58. Hvidsten D, Stordal F, Lager M, Rognerud B, Kristiansen BE, et al. (2015) Borrelia burgdorferi sensu lato-infected Ixodes ricinus collected from vegetation near the Arctic Circle. Ticks Tick Borne Dis 6: 768-773.

59. Kumsa B, Socolovschi C, Raoult D, Parola P (2015) Spotted fever group rickettsiae in ixodid ticks in Oromia, Ethiopia. Ticks Tick Borne Dis 6: 8-15.

60. Sparagano OA, Allsopp MT, Mank RA, Rijpkema SG, Figueroa JV, et al. (1999) Molecular detection of pathogen DNA in ticks (Acari: Ixodidae): a review. Exp Appl Acarol 23: 929-960.

61. Ul-Hasan M, Abubakar M, Muhammad G, Khan MN, Hussain M (2012) Prevalence of tick infestation (Rhipicephalus sanguineus and Hyalomma anatolicum anatolicum) in dogs in Punjab, Pakistan. Vet Ital 48: 95-98.

62. Quan TJ, Tsuchiya KR, Carter LG (1986) Recovery and identification of Pasteurella multocida from mammals and fleas collected during plague investigations. J Wildl Dis 22: 7-12.