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

Safaa M Barghash 1*, Amani A Hafez 2, Ahmed M Darwish 2 and Tarek R Abou El-Naga 3,4

1 Doctor in Parasitology, Parasitology Unit, Animal Health Department, Animal Production and Poultry Division, Desert Research Center; 1-Mathaf El-Mataria St. 11753, El-Naam, Cairo, Egypt
2 Doctor in Bacteriology, Infectious Diseases Unit, Animal Health Department, Animal Production and Poultry Division, Desert Research Center; 1-Mathaf El-Mataria St. 11753, El-Naam, Cairo, Egypt
3 Doctor in Genetics, Cell Biology Department, Genetic Engineering and Biotechnology Division, National Research Centre, Tahrir St. 12622, Dokki, Giza, Egypt
4 Professor in Infectious Diseases, Infectious Diseases Unit, Animal Health Department, Animal Production and Poultry Division, Desert Research Center, Cairo, Egypt

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 (6.82%), 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 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 haemoproteozooan 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 also known to spread among wildlife, 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.
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 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 a 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 MgCl2, 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 performed.

| Pathogen              | Primer sequence (5’-3’) | PCR condition (Bp) |
|-----------------------|------------------------|--------------------|
| Theileria sp.         | F: AGTTTTGCTACATGAG and R: TGGCCCTAAACCTTG    | 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 anatoliana   | F: ACTTGGCCTAGTTAACC and R: TTCGCAACTGTTTGG    | 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: GCTCTGAAGTGGTTGACAG and R: GAGAATCTGCTTGG    | 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: TTTGTTATTGCCCTTGTAGCAT and R: ACCACTGAATCTAACC    | 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 8 min. 448 |
| Babesia bigemina      | F: TACCTAGAAGTAGCCTG and R: AACATATCCATGTTTGG    | 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: GAAATACGCTAATGCTGCGAGG and R: CCAATTATTAGCTTGTGC    | 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: GCGGGTTTAACTGATAGG and R: ATTATCTGCATGTTGG    | 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 |
| Borrelia burgdorferi  | F: CTGTCTTGGATACCGAC and R: TACCTGGCCTGCTTGG    | 95°C for 5 min., 33 cycle at 94°C for 30 sec., touchdown from 46°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: GGGAGCTCTACGACGCGG and R: ATGCATGAAAGAATCGTGAGG    | 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: AGATTGGATCCTGCTTGG and R: ATGCATCTGCTTGG    | 95°C for 5 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: ATGCATCTGCTTGG and R: TTCAGATGCCTCTGCTTGG    | 95°C for 3 min., 35 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. 460 |
| Mycoplasma spp.        | F: GAACTTGTTCAGGATTGCAGC and R: TGGCAATCTGCTTGG    | 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 Stastics 20, USA). Chi-square test was applied to compare the difference in results. All statistics were considered significant at P ≤ 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.

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

| 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.*

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

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, 466, 689 and 1100 bp respectively, while *Theileria annullata* 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).

Disease transmission in the desert environment offered favorable conditions for ticks to transmit pathogens to livestock animals [44]. A variety of tick-borne pathogens infect livestock animals [45]. 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](image_url)
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-Negelia 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 Mustefá [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 Bilièg 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 Ricketttsia sp. were detected by PCR in spite of ticks belong to Hyalomma could transmit Rickettsia sp., Anaplasmata, and several protozoa that infect wildlife, livestock, and people [59-61].

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 to 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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