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Research Article
Tick-Borne Pathogens in Ticks and Blood Samples Collected from Camels in Riyadh Province, Saudi Arabia

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Abstract

Background and Objective: Domestic animals, including camels, in Saudi Arabia suffer from various diseases, among which tick-borne infections are important because they reduce the productivity of these animals. However, knowledge of tick-borne pathogens in camels in Saudi Arabia is very limited, so the aims of this study were to quantify the abundance and distribution of tick species infesting camels from different districts of Riyadh province and use molecular tools to detect tick-borne pathogens in both the ticks and blood samples. **Materials and Methods:** A total of 218 ticks were collected from 116 camels from the 5 districts of Riyadh. The ticks and camel blood samples were analyzed for *Borrelia*, *Babesia* and *Theileria* pathogens using conventional and real-time PCR. **Results:** The results showed that five different tick species were identified. Majority of the ticks were *Hyalomma dromedarii* (70.6%), which were collected from camels in all 5 districts. This was followed by *Hyalomma impeltatum* species (25.2%), which was again found in all the districts. The other species found were *Hyalomma anatolicum*, *Haemaphysalis* sp. and *Rhipicephalus turanicus*. The only one *H. dromedarii* tick was positive for *Theileria* sp. DNA. Although the sample size and the area of tick collection were limited, the data suggest that the prevalence of pathogens in the Riyadh province, Saudi Arabia is relatively low. **Conclusion:** The study provides useful preliminary data to inform future full-scale country-wide surveys.

Key words: Tick-borne diseases, *Theileria* sp., qPCR, *Hyalomma dromedarii* tick

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ticks are important vectors of pathogens of both humans and animals and transmit a broad range of bacterial, protozoal, rickettsia and viral pathogens[1-3]. In addition to acting as vectors, ticks also affect the well being of their host directly through irritating bites, blood loss, damage to the skin and anorexia leading to reduced growth[4,5].

In Saudi Arabia, camel, sheep, goat and cattle production makes an important economic contribution to agricultural production; in 2016, the livestock sector contributed 2.7% to the Gross Domestic Product (GDP) of Saudi Arabia (General Authority for Statistics 2016). Camels in particular also play an important cultural role; estimates by the General Authority for Statistics suggested that the number of indigenous camels in Saudi Arabia exceeded 1, 356, 7290 in 2016. In addition, thousands of live camels are imported annually from neighbouring countries such as Sudan and Arabian Gulf countries (General Authority for Statistics 2015). Many rural communities are dependent on this animal species for their livelihood since camels are a good source of meat, milk, leather and wool. Camels are vital working animals under the arid and semi-arid conditions because of their unique adaptive physiological and anatomical characteristics[6]. Camels can survive and be productive under limiting environmental conditions and can utilize marginal areas[7], where they are able to feed on plants not often eaten by the smaller livestock species; this reduces the competition and helps in better resource utilisation[8]. These animals also support tourism, are used in sports and for transportation through the deserts and rural areas[9].

Camel production is severely affected by various diseases and inadequate veterinary services. Several internal and external parasites affect their health, productivity and performance including ticks. The feeding activity of ticks causes blood loss and anaemia, but they also transmit various disease pathogens[1-3]. Ticks can cause irritation, inflammation, hypersensitivity and damage to the hide, leading to production losses. Ticks also reduce the quality of hides[4.] Animal transport and globalization has led to the spread and establishment of various tick species to new environments along with the pathogens they carry[10].

In recent decades, there have been enormous advancements in the field of agriculture in the Kingdom of Saudi Arabia, which has transformed large areas of the desert in to cultivable land, where humans and livestock thrive. These livestock are usually infested by ticks and tick-borne diseases at low prevalence[11,12], but can cause epidemics under certain circumstances[13,14].

Previous studies on ticks in Saudi Arabia showed that there are at least 15 ixodid species and subspecies infesting domestic animals[11,12,15,16]. Eight species and subspecies belong to the genus *Hyalomma*, three to the genus *Rhipicephalus*, two to *Amblyomma* and one each to *Haemaphysalis* and *Boophilus*. *Hyalomma dromedarii* is considered to be the most prevalent tick species infesting camels with a reported prevalence of 51%, whereas *Rhipicephalus turanicus* is the most prevalent tick species infesting sheep and goats with reported prevalence of 41.2 and 55.6%, respectively[11]. However, knowledge of tick-borne pathogens in camels in Saudi Arabia is very limited with few molecular epidemiological studies being undertaken[17,18]. Hence, there is a need to validate the previous tick abundance records and apply more sensitive molecular diagnostics.

Piroplasma including *Theileria* and *Babesia* are known as worldwide haemoparasites for ruminants including camels. For example, *Babesia caballi* and *Theileria equi* were detected in camels by PCR and sequencing of 18S rRNA marker in Jordan and Iran[19], *Theileria annulata* was detected in camels by a traditional method in Egypt[20] and by molecular tools in Egypt[21]. Studies on such parasites in Saudi Arabia are very limited. Camels were free found more *Theileria* infection in Saudi Arabia[12,22]. Furthermore, PCR assay using specific primer for *Babesia bovis* in camels in Saudi Arabia revealed that the prevalence of *Babesia bovis* was 6.25% for camels[23]. Although many studies detected *Borrelia* in camels all over the world, the studies on the infection of camels with *Borrelia* are lack in Saudi Arabia. In Egypt, Helmy[24] detected antibody *Borrelia* sp. with the highest infection rate in camel (47.8%). Furthermore, it detected the spirochetes in soft tick *Ornthodoros savignyi* associated with the investigated camels. Later, this *Borrelia* was identified by molecular analysis as *Borrelia burgdorferi*[25].

The aims of this study were to quantify the abundance and distribution of tick species infesting camels from different districts of Riyadh province and use molecular tools to detect tick-borne pathogens in both the ticks and blood samples collected from camels.

MATERIALS AND METHODS

Study area: The investigation was conducted from October, 2017 to June, 2018. Riyadh province was selected for this study since it is one of the most densely populated parts of the country and has a number of distinct climatic zones. The investigation was conducted in five different regions of Riyadh province (Fig. 1): Riyadh city (Central Riyadh province), Ad-Dawadimi district (Western Riyadh province), AL-Kharj district (Southern Riyadh province), Rumah district (Eastern Riyadh province) and AL-Majmaah district (Northern Riyadh province).

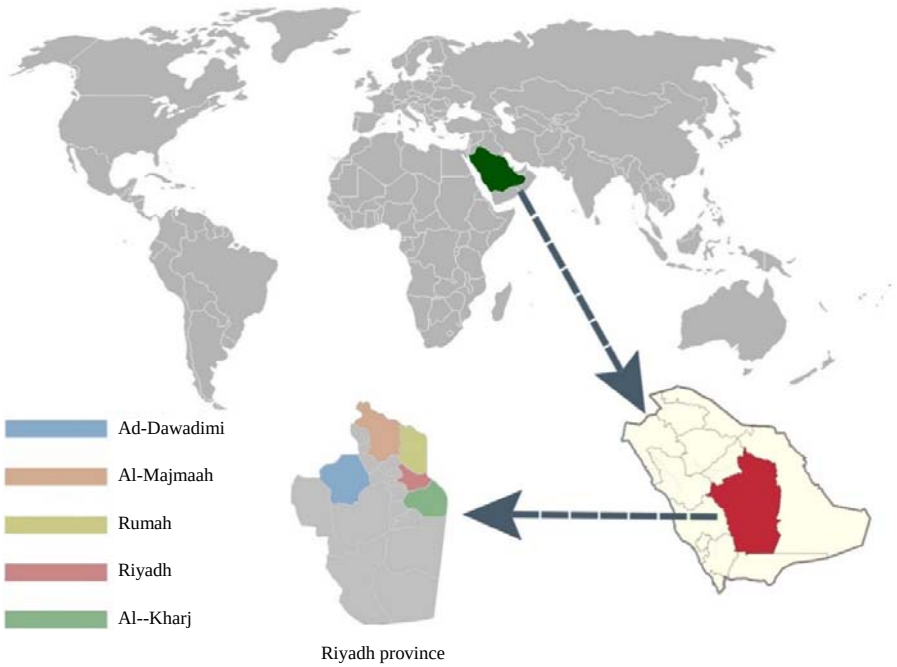

Fig. 1: Map of Saudi Arabia showing the study regions

Table 1: Number of camels examined, their sex and the number and percentage infested by ticks

Location	No of camels examined	Sex of camels examined		Sex of camels infested	
		Male	Female	Male	Female
Riyadh	23	9	14	3/9 (33.3%)	10/14 (71.4%)
Majmaah	19	7	12	5/7 (71. 4%)	9/12 (75%)
AL-Kharj	25	6	19	3/6 (50%)	14/19 (74%)
Dawadimi	31	3	28	3/3 (100%)	19/28 (68%)
Rumah	18	2	16	2/2 (100%)	11/16 (69%)
Total	116	27	89	16/27(59.3%)	63/89 (71%)

Tick collection and identification: Ticks were collected from 116 animals, with a minimum of 18 and a maximum of 31 camels inspected in each region (Table 1). Where present, about 1 to 5 ticks were collected from each animal. Ticks were placed into 24×100 mm vials containing 70% alcohol. Adult ticks were identified according to the taxonomic key of Estrada-Pena *et al.*[26].

Collection of blood samples: Blood samples were collected from 56 camels of both sexes and age groups; camels were selected at random. Blood samples were only collected from camels in Riyadh province and Ad-Dawaidmi district (Fig. 1). The camels ranged in age from 1-13 years. Of the 56 sampled camels, 36 were females and 20 were males. Blood samples were collected from the jugular vein into K3EDTA-tubes (HebeiXinleSci & Tech Co., Ltd., China) by qualified veterinary staff.

DNA extraction from ticks: After morphological identification and before DNA extraction, each individual tick was cut transversely and longitudinally. DNA extraction was carried out using a Nucleospin® 96 Tissue Core Kit (Macherey-Nagel, Germany) according to the manufacturer's guidelines. Fully fed ticks posed problems during extraction because of large volumes of clotted blood, which even after overnight digestion in double the recommended volume of Proteinase-K and tissue lysis buffer did not digest and clogged the silica column. For this reason, only the anterior two-thirds of fully engorged ticks (containing salivary glands) were used for DNA extraction and the protocol used: 40 µL of Proteinase-K (instead of 30 µL) and 400 µL of tissue lysis buffer (instead of 240 µL). An internal amplification control (IAC) was spiked into the tick samples before DNA extraction to monitor for successful extraction and the absence of PCR inhibitors by subsequent quantitative (q) PCR analysis of the IAC, as the procedures described by Davies *et al.*[27].

DNA extraction from camel blood samples: Total genomic DNA (gDNA) was isolated from each sample using the DNA easy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The samples were stored at -20°C for further analysis.

***Babesia* spp. qPCR:** *Babesia* spp. were detected in DNA extracts using a probe based generic Babesia qPCR targeting the 18S rRNA gene. The primer/probe combination used for detection of *Babesia* spp. was Babesia 944 for (5-GTTAACGAACGAGACCTTAACCTG-3), Babesia1315rev (5-CCGAATAATTCACCGGATCAC-3) and *Babesia* Taq Man probe (5-FAM-CGATCGGTAGGAGCGACGGGC-BHQ1-3) (Diagnostic Laboratories, Langford Vets, UK). Positive (*Babesia canis*, 12763 g DNA diluted at 10^{-1}) and negative (water) controls were included in each 96 well PCR plate. PCR conditions comprised an initial denaturation at 95°C for 2 min; 45 cycles of 95°C for 15 sec and 60°C for 30 sec (Agilent MX3005P qPCR, Agilent, UK). Fluorescence data were collected at 520 nm at the end of each annealing/extension step.

***Borrelia* spp. PCR:** For *B. burgdorferi* s.l detection in the DNA extract a conventional PCR was used with primers BSLF (5-AATAGGTCTAATAATAGCCTTAATAGC-3) and BSLR (5-CTAGTGTTTTGCCATCTTCTTTGAAAA-3) which amplify a 250-300 bp region of the ospA gene found in all *B. burgdorferi* s.l[28]. *Borrelia burgdorferi* sensu stricto PCR product diluted 10^{-10}. and water were used as positive and negative controls, respectively. The PCR protocol consisted of an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 20 sec, 56°C for 30 sec and 72°C for 30 sec. Agarose gel electrophoresis was used to visualise target amplicons. Positive samples were identified as having a defined band of 250–300 bp on the gel in comparison to the molecular weight marker (Bioline Easy ladder 1 BIO-33046).

***Theileria* sp. PCR:** For detection of *Theileria* spp. in the DNA extract a conventional PCR was used. A primer combination targeting the 18S rRNA gene of *Theileria/Babesia* apicomplexan parasites was selected using Bioedit (Version 7.2.5), Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) and unfold (http://unafold.rna.albany.edu/?q=mfold). The primers selected were the lBabF (5 -TGACACAGGGAGGTAGTGACAAG-3) and the BabR (5-CAAATCTAAGAATTTCACCTCTGACAGT-3), which amplify a 400-450 bp region of the 18S rRNA gene of both *Babesia* and *Theileria* species. *Babesia canis* (PCR product diluted at 10^{-1}) and water were used as positive and negative controls, respectively. The PCR protocol consisted of an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 20 sec, 58°C for 20 sec and 72°C for 30 sec. Agarose gel electrophoresis was used to visualise target amplicons. Positive samples were identified as having a defined band of 450 bp on the gelin comparison to the molecular weight marker (Bio line Easy ladder 1 BIO-33046)[28].

Ethical approval: This study was reviewed and approved by the Ethical Committee of the Department of Biological Science, Shaqra University, according to the ethical principles of animal research under the number SH010-2017.

RESULTS

Tick identification: Of the 116 camels inspected, 79 were infested by ticks. A total of 218 ticks were collected from these 79 camels and 5 different tick species were identified. Majority of the ticks were *Hyalomma dromedarii*, which were collected from all 5 districts in Saudia Arabia (Fig. 1). This was followed by *Hyalomma impeltatum*, which was again prevalent in all the districts. *Hyalomma anatolicum* were collected only from camels in Rumah, similarly ticks of the genus *Haemaphysalis* were only identified from Rumah, but these ticks were difficult to identify to the species level because the samples were damaged. *Rhipicephalus turanicus* were collected from Dawadimi and Rumah districts, but the number of these ticks was low. Male ticks were always more abundant than female ticks (Table 2).

Pathogen analysis: A total of 160 ticks collected from all 5 districts were analyzed for three pathogen species. The internal amplification control (IAC) was successfully amplified in all samples following qPCR and all samples gave similar threshold cycle values, indicating the extraction worked and the absence of PCR inhibitors.

***Babesia*/*Theileria*/*Borrelia* spp. distribution and prevalence:** All positive and negative controls for PCR assays were positive and negative, respectively. Only one *H. dromedarii* tick from Al-Majmaah was positive for *Theileria* sp. DNA by this PCR assay, the generic *Babesia* spp. qPCR indicated none of the ticks were positive for *Babesia* DNA, also the PCR indicated none of the ticks were positive for *Borrelia* DNA and needs further sequence analysis to confirmation (Fig. 2).

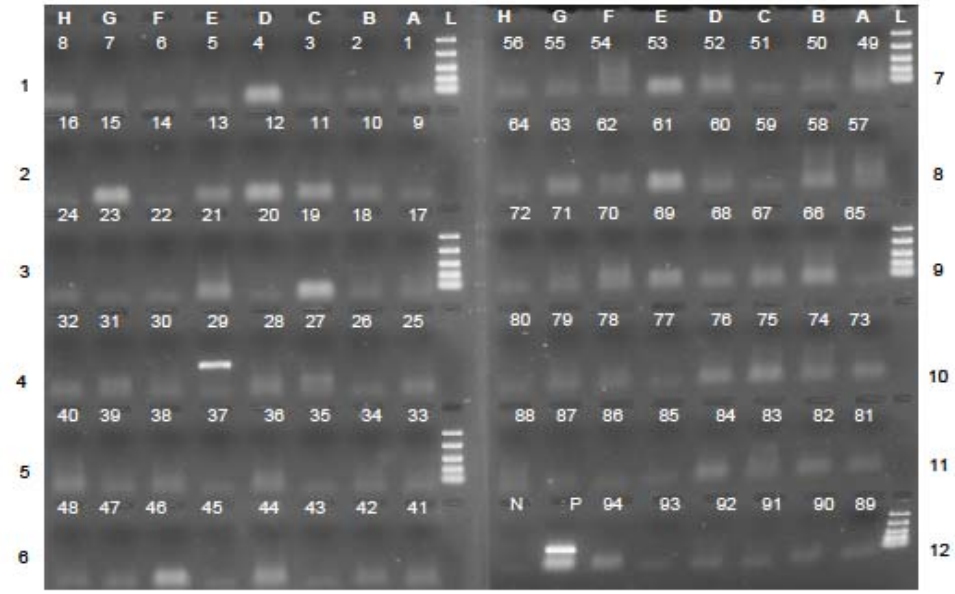

Fig. 2: 96 well gel electrophoresis set up for *Babesia/ Theileria* sp. DNA after cPCR. There are 8 columns and 12 rows (1-12) for the PCR samples and 1 column (L) for the ladder. Lane L is a 100-2000 bp molecular size marker (Bioline easy ladder 1 BIO-33046), P: Positive control DNA, N: Negative PCR control (water), Sample no. 29 showing *Theileria* sp. positive in the predictable band size

Table 2: Number of different tick species and sex, collected from 5 districts of Riyadh province

		H. dromedarii		*H. impeltatum*		*H. anatolicum*		*Hae.* sp.		*R. turanicus*	
Location	Total No. of ticks	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Riyadh	45	22	8	10	5	0	0	1	0	0	0
Majmaah	47	21	14	5	7	0	0	0	0	0	0
Al-Kharj	50	22	18	3	7	0	0	0	0	0	0
Dawadimi	56	27	18	7	3	0	0	0	0	1	0
Rumah	20	2	2	6	2	4	0	1	0	2	0
Total	218	94	60	31	24	4	0	2	0	3	0
Prevalence (%)	(43.1%)	(27.5%)	(14.2%)	(11%)	(1.8%)	(0.0%)	(0.92%)	(0.0%)	(1.3%)	(0.0%)	

Pathogen analysis of camel blood samples: The DNA extracted from a total of 56 camel blood samples were analyzed for the three pathogen species. The PCR indicated none of the blood samples were positive for *Borrelia* and *Bebesia* or *Theileria* sp. DNA.

DISCUSSION

In this study, five different tick species on camels from Saudi Arabia were detected, all of which have been previously reported[11,21,22]. Al-Khalifa *et al.*[29] and Al-Khalifa *et al.*[30] reported the tick species, *H. dromedarii* was the most prevalent (70.6%), which has also been shown by previous studies in Saudi Arabia[16,29] and other parts of the world[30-34]. *Hyalomma dromedarii,* along with *H. impeltatum* were widely distributed and were found in all 5 districts of Riyadh province with prevalence of 70.6 and 25.3%, respectively *Hyalomma anatolicum* and *R. turanicus* were less prevalent, representing less than 2% of the ticks found, which is similar to the study described by Diab *et al.*[11]. *Haemaphysalis* ticks were found on camels in Riyadh city and Rumah only. Similar distribution patterns were reported in Riyadh province by Al-Khalifa and Diab[35].

Polymerase chain reaction analysis of the tick and camel blood samples for *Borrelia* sp. DNA did not give any positive results, supporting the conclusion that the tick species examined in the current study do not carry this pathogen or have a low prevalence that could not be detected by the relatively low sample sizes of this study. Although the pectoral potential of these tick species for *Borrelia* has not been reported previously, they were analyzed for this pathogen in the current study because most were three-host ticks and their immature stages usually infest burrowing rodents, lizards and birds[36,37], which are the main reservoirs of this pathogen[38].

Analysis of tick and blood samples for *Babesia* sp. and *Theileria* sp. again indicated that the samples were free from the DNA of these pathogen species, except for one tick sample that was positive for *Theileria* sp. similar results have been reported in earlier studies by Alsarraf *et al.*[39], where all tick samples were negative for *Babesia* spp. Also, Mohammed *et al.*[19] reported lack of evidence of tick-borne pathogens in the blood samples of camels of Riyadh province. Al-Deeb *et al.*[40] reported the prevalence of these pathogens in ticks as low and found only 1.6% of ticks to be positive for *T. annulata*.

The inability to detect the DNA of the various pathogens in our tick and blood DNA samples does not confirm that the ticks in the Riyadh Provinces are free of the pathogens or the camels of this region are not carrying the pathogens. The obtained results and inferences are limited by the small sample size and the possible low prevalence of the pathogens in the ticks and/or the host.

CONCLUSION

This preliminary survey provides valuable baseline data on the tick species of importance, their distribution and the prevalence of tick-borne pathogens in camels in the Riyadh province of Saudi Arabia. Although the sample size and the area of tick collection are relatively restricted in this study, it nevertheless provides a foundation for a future country-wide study of prevalence and distribution patterns of ticks and tick-borne diseases in camels.

SIGNIFICANCE STATEMENT

This study can be beneficially to give a novel useful aspect dealing about preliminary data to inform the prevalence of tick-borne pathogen in camels in the Riyadh Province, Saudi Arabia. Although the sample size and the area of tick collection were limited the data showed for Riyadh region. This study will suggest the researcher to explore and need for future full-scale country-wide surveys.

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