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Morpho-Molecular and Phylogenetic Characterization of *Hyalomma dromedarii* Infesting Camels and Associated Protozoan Pathogens in Saudi Arabia

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ABSTRACT

***Background*:** Ticks are major blood-feeding ectoparasitic arthropod vectors of zoonotic and veterinary pathogens, posing serious threats to livestock production, as well as animal and public health worldwide. In tropical and subtropical regions such as Saudi Arabia, one-humped camels (*Camelus dromedarius*) are important livestock hosts and are frequently infested with hard ticks, particularly species of the genus *Hyalomma*. These ticks are known vectors of several protozoan and bacterial pathogens of veterinary importance. However, despite their epidemiological relevance, comprehensive studies that integrate morphological identification, molecular characterization, and phylogenetic analysis of camel-infesting ticks, along with screening for associated protozoan pathogens, remain scarce. This study aimed to characterize *Hyalomma dromedarii* infesting camels in western and northwestern Saudi Arabia using morphological, molecular, and phylogenetic approaches, and to investigate the presence of selected tick-borne bacterial and protozoan pathogens.

***Materials, Methods & Results*:** In this study, *Hyalomma* specimens were collected from one-humped camels between 2021 and 2022 from 6 localities in Saudi Arabia (Alkhumrah, Brayman, Asfan, Dahaban, Duba, and Tabuk). The collected tick specimens were initially identified morphologically using standard keys and subsequently confirmed at the molecular level through genomic DNA extraction, followed by amplification and sequencing of the mitochondrial *cytochrome c oxidase* subunit I (*cox1*) gene via polymerase chain reaction (PCR). The extracted genomic DNA was further screened for protozoan pathogens using PCR targeting the 18S rDNA genes. Phylogenetic analysis was performed using the maximum likelihood method on the Cyberinfrastructure for Phylogenetic Research (CIPRES) server. A total of 218 tick specimens were collected from 48 infested camels, comprising 116 males and 102 females. All collected specimens were morphologically identified as *Hyalomma dromedarii*, which was supported by cox1 sequence analysis. In BLAST results, the obtained *cox1* sequences showed maximum identity with *H. dromedarii* sequences reported from African, Middle Eastern, Asian, and Australian countries, and phylogenetically clustered with sequences of the same species. Notably, the extracted genomic DNA from *H. dromedarii* was screened for various pathogens, including *Rickettsia* spp., *Anaplasma* spp., *Coxiella* spp., *Babesia* spp., *Theileria* spp., and *Hepatozoon* spp. Among these, only protozoan species, specifically *Theileria annulata* and *Hepatozoon canis* DNA, were detected in the examined tick specimens. Co-detection of protozoan pathogens was observed in some samples, suggesting the circulation of multiple pathogens within camel-associated tick populations. In BLAST results, the obtained 18S rDNA sequences showed 100% identity with the corresponding species (*T. annulata* and *H. canis*) reported from various countries and phylogenetically clustered with the same species sequences.

***Discussion*:** This study provides integrated morphological, molecular, and phylogenetic data for *Hyalomma dromedarii* infesting one-humped camels in the western and northwestern regions of Saudi Arabia and confirms the dominance and genetic stability of this species across multiple study areas. The detection of *T. annulata* and *H. canis* highlights the potential epidemiological role of camel ticks in maintaining protozoan pathogens. These findings underscore the importance of continuous molecular surveillance to better understand the ecology, evolution, and pathogen transmission dynamics of camel-associated ticks in Saudi Arabia.

Keywords: camel, *cox1*, *Hyalomma dromedarii*, protozoan, Saudi Arabia.

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INTRODUCTION

Ticks are obligatory blood-feeding ectoparasites and are considered the most important arthropod vectors of pathogens transmitted to both animals and humans [7-9,27]. Among hard ticks, the genus *Hyalomma* is widely distributed across the Middle East, Africa, and Asia, and is strongly associated with camels. These ticks are of considerable medical and veterinary importance due to their role in pathogen transmission and their economic impact in tropical and subtropical regions [8,45,49]. *Hyalomma* ticks are known to harbor and transmit a wide range of pathogens, including *Theileria* spp., *Babesia* spp., *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Rickettsia* spp. [2,26,31,34,37,40,48].

Two subgenera, *Hyalommina* and *Euhyalomma*, have been identified within the genus *Hyalomma*. Species of the subgenus *Hyalommina* have been classified into 2 groups based on their geographic distinctiveness, while the most diverse subgenus, *Euhyalomma*, is widespread across Africa, Asia, and Europe. Several *Hyalomma* species such as *Hyalomma punt*, *Hyalomma rhipicephaloides*, and *Hyalomma arabica* species are native to the Arabian Peninsula, northeastern Africa, and the Near East. In Saudi Arabia, *Hyalomma dromedarii*, commonly referred to as the camel tick, is the predominant species infesting camels [15,18]. To date, 27 *Hyalomma* species have been described across the Palearctic, Oriental, and African regions [18,19,27,49], with 7 of those species described in Saudi Arabia [15].

The one-humped camel (*Camelus dromedarius*) is a highly valuable livestock species in Saudi Arabia, with an estimated population of about 2 million animals [17,25,42]. *Hyalomma dromedarii* is the most common tick found on camels in Saudi Arabia [5]. This tick has been implicated in the transmission of various zoonotic and veterinary pathogens, including Crimean-Congo hemorrhagic fever (CCHF), Dhori virus, Sindbis virus, Kadam virus, *Coxiella burnetii*, *Rickettsia* spp., and agents of tropical theileriosis [2,12,21,31,42].

Advances in molecular techniques and the increasing availability of tick DNA sequences in databases have improved tick classification beyond traditional morphology-based strategies [23,44]. Mitochondrial genetic markers, particularly *cytochrome c oxidase* subunit I (*cox1*), are widely used due to their maternal inheritance, strong lineal transmission, and suitable evolutionary rates, making them effective for species identification at the genetic level [6,9-11,16,24,38,39]. Despite the veterinary importance of camel ticks in Saudi Arabia, integrated molecular and phylogenetic studies linking tick identity with associated protozoan pathogens across multiple regions remain scarce. Therefore, this study aimed to investigate the molecular and phylogenetic identification of camel ticks, with particular focus on *H. dromedarii*, and to assess their evolutionary relationships and associated pathogens in selected regions of Saudi Arabia. These findings provide baseline data that contribute to a better understanding of camel-associated tick diversity and ecology, as well as their potential role in the epidemiology of tick-borne bacteria in Saudi Arabia.

MATERIALS AND METHODS

Ethical statement

This study was conducted in compliance with the international guidelines for animals' research or healthcare. Oral consent was obtained from the owners of the camel for observation and tick collections.

Study area

This study was conducted in 4 regions in Jeddah such as Alkhumrah (21°21'26.4"N 39°15'10.3"E) at South, Brayman (21°37'59.2"N 39°14'54.8"E) at Eastern, Asfan (21°53'08.7"N 39°22'12.3"E) at Northeast and Dahaban (21°57'04.2"N 39°06'58.1"E) at North and in the cities of Duba (27°21'20.7"N 35°42'39.0"E) and Tabuk (28°21'19.6"N 36°29'42.6"E), that are located geographically western and northwestern Saudi Arabia.

Tick collections and preservation

Hyalomma ticks were collected during 2021-2022 from one-humped camels that were maintained under traditional and semi-intensive management systems in study regions. Tick specimens were carefully collected via tweezers from different body parts of the camel, such as ears, hump, tail, and legs. Specimens were washed with distilled water, followed by ethanol (70%), and then preserved in absolute ethanol (100%) in 1.5 mL microtubes. Then the collected samples were transferred to the Laboratory of Clinical Microbiology and Immunology, Faculty of Medicine at King Abdulaziz University, Saudi Arabia, for further morphological as well as molecular examinations.

Morphological examination

The collected tick specimens were morphologically identified at the species level via published morphological identification keys [22].

Genomic DNA extraction and PCR amplification

A total of 48 morphologically identified tick specimens (4 males and 4 females from each region) were selected for genomic DNA extraction using the QIAamp DNA Tissue Kit[1], following the manufacturer's instructions. Each specimen was homogenized with a sterile pestle and hygienic scissors in 1.5 mL microtubes. Twenty µL of "nuclease-free" water were used to hydrate the isolated DNA pellet. The quality and quantity of the extracted genomic DNA were assessed using a NanoDrop 2000 spectrophotometer[2]. The samples were stored at -20°C for subsequent molecular analysis.

Genomic DNA that had been extracted was amplified via PCR[3] for the mitochondrial *cox1* gene (HC02198: TA AA CT TC AG GG TG AC CA AA AT CA, and LCO1490: GG TC AA CA AA TC AT AA AG AT AT TG G) [24] for tick identification. Whereas 18S rRNA gene (forward: GG CG GC GT TT AT TA GA CC and reverse: TC AA TT CC TT TA AG TT TC AG CC) was used for the detection of *Theileria* spp. [28] and 18S rRNA gene (HEP2_144-169-F: GG TA AT TC TA GA GC TA AT AC AT GA GC and HEP2_743-718-R: AC AA TA AA GT AA AA AA CA YT TC AA AG) for the detection of *Hepatozoon* spp. [13]. The volume of each PCR mixture was 25 µL and it contained the following components: 0.75 µL of each primer (10 µM for both forward and reverse), 4.5 µL of "nuclease-free" PCR water, 0.5 µL of thermostable DNA polymerase (1U/µL), 12.5 µL of PCR buffer 2X2, 5 µL of dNTP (2 mM) and 1 µL of template DNA (50-100 ng/µL). The thermocycler was operated under these conditions: initial denaturation at 95°C for 3 min; 35 cycles of 95°C for 30 s, followed by 55°C for 60 s (*cox1* for ticks); 58°C for 60 s (18S rRNA for *Theileria* spp.); 54°C for 45 s (18S rRNA for *Hepatozoon* spp.); and 72°C for 30 s of extension; and a final extension at 72°C for 10 min. The amplicons amplified by PCR were purified with a commercial PCR Clean-up Kit[4] and GeneClean II Kit[5] following the manufacturer's instructions. The PCR results from the 2% agarose gel electrophoresis were analyzed under ultraviolet (UV) light using a gel documentation system[6].

Sequence analysis

The Sanger sequencing method was used to sequence the amplified amplicons: *cox1* for ticks and 18S rDNA for pathogens. The sequencing was done in both directions. The sequences were then processed with SeqMan v. 5 software to trim and eliminate contaminated or poorly read sequences from the *cox1* and 18S rDNA sequences obtained. For every gene, identical sequences were merged into a single sequence, which represented one haplotype. The trimmed DNA sequences of *cox1* and 18S rDNA were obtained and uploaded to the National Center for Biotechnology Information (NCBI) for analysis using the Basic Local Alignment Search Tool (BLAST) [14]. Homologous sequences were sourced in FASTA format from NCBI, based on the maximum identity percentage for each gene sequence. Using ClustalW Multiple Sequences Alignment [46], these sequences were aligned with the obtained sequences in BioEdit V. 7.0.5 [29]. The aligned *cox1* and 18S rDNA sequences were uploaded in FASTA format to the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v. 3.3 [36]. Using the NCL Converter v. 2.1 tool [35], the file was later converted into RELAX-PHYLIP format. Using the IQ-Tree v. 2.3.2 software with default settings, a maximum-likelihood (ML) phylogenetic tree was generated under XSEDE in CIPRES [36], utilizing 1000 bootstrap replicates for ULTRAFAST [30] as support for estimation. The tree file was visualized and formatted in the FigTree v. 1.4.4 software [43].

RESULTS

Tick description and morphological identification

In this study, we examined a total of 240 camels at 6 locations in Saudi Arabia, of which 48 camels were found infested with ticks. From these infested animals, *Hyalomma* ticks were collected, including 116 males and 102 females out of 218 ticks. The highest numbers of *Hyalomma* ticks were recorded from Asfan and Duba, while relatively low numbers were obtained from Brayman and Alkhumrah (Table 1). These collected tick specimens from all the collection sites were morphologically identified as *H. dromedarii* by the following trait including the shape of the capitulum, scutum, eyes, festoons and hypostome, spiracle, genital groove, spur of coxa, adanal shield.

cox1-*based molecular confirmation of* Hyalomma dromedarii

The mitochondrial *cox1* sequencing confirmed that the collected or morphologically identified tick was *Hyalomma dromedarii*. In the BLAST analysis, the obtained *cox1* sequence (650 bp) showed a maximum identity of 99.85% with *H. dromedarii* specimens reported from Iran (KT906107), Kenya (OQ540932), India (PV687563), and Pakistan (PV262400). Identities of 99.69% were observed with sequences reported from Kenya (OQ457674), Tunisia (PP453768), Saudi Arabia (MH590886 and MH590871), Ethiopia (AJ437061), and Israel (KT989618), while a 99.38% identity was found with sequences from Australia (AF132822) and India (GQ483461). Sequences from other countries, including Egypt, Kenya, Pakistan, India, Tunisia, Cameroon, and Saudi Arabia, showed identities ranging from 99.36% to 99.67%. The obtained *cox1* sequence from *H. dromedarii* in Saudi Arabia was phylogenetically clustered with the same species reported from various countries (Figure 1).

18S rDNA-based detection of pathogens in Hyalomma dromedarii

From these identified ticks, 48 ticks were selected for molecular analysis, of which 24 were males and 24 were females, and 8 ticks (4 males and 4 females) were molecularly analyzed from each location (Table 1). The molecular assay revealed an overall low prevalence of the pathogen. DNA of *Theileria* spp. and *Hepatozoon* spp. were detected, while no *Rickettsia* spp., *Anaplasma* spp., *Coxiella* spp., and *Babesia* spp., were detected. In this study, the distribution of *Theileria* spp. was detected in camel ticks *H. dromedarii*, found across Alkhumrah, Asfan, Dahaban, and Tabuk, while *Hepatozoon* spp. was consistently identified in positive samples from Alkhumrah, Asfan, Dahaban, and Duba. The consistent detection of *T. annulata* across geographically separated sites suggests stable circulation rather than sporadic introduction. Notably, co-detection of *Theileria* spp. and *Hepatozoon* spp. was observed, indicating the possibility of multiple pathogens within individual tick populations. No molecular evidence was found for *Rickettsia* spp., *Anaplasma* spp., *Coxiella* spp., and *Babesia* spp. in any of the specimens from the collection site. These results suggest a true absence or undetectable circulation of the pathogens in the samples.

Based on 18S rDNA sequencing, the obtained sequence showed the presence of 2 protozoan pathogens in *H. dromedarii* ticks from Saudi Arabia. The BLAST results for the 18S rDNA sequence (579 bp) of *Theileria* spp. showed a maximum identity of 100% with sequences of *Theileria annulata* reported from multiple countries, including Saudi Arabia, Italy, China, Pakistan, the USA, Japan, Azerbaijan, Egypt, and Iraq. Similarly, the obtained 18S rDNA sequence (527 bp) of *Hepatozoon* spp. showed 100% genetic sequence identity with *H. canis*. This identity matched sequences previously reported from various countries, including Taiwan, Romania, Zambia, Israel, Spain, and Italy. The obtained 18S rDNA *T. annulata* and *H. canis* sequences were clustered with the corresponding maximum identity sequences reported from multiple countries (Figures 2 & Figure 3, respectively).

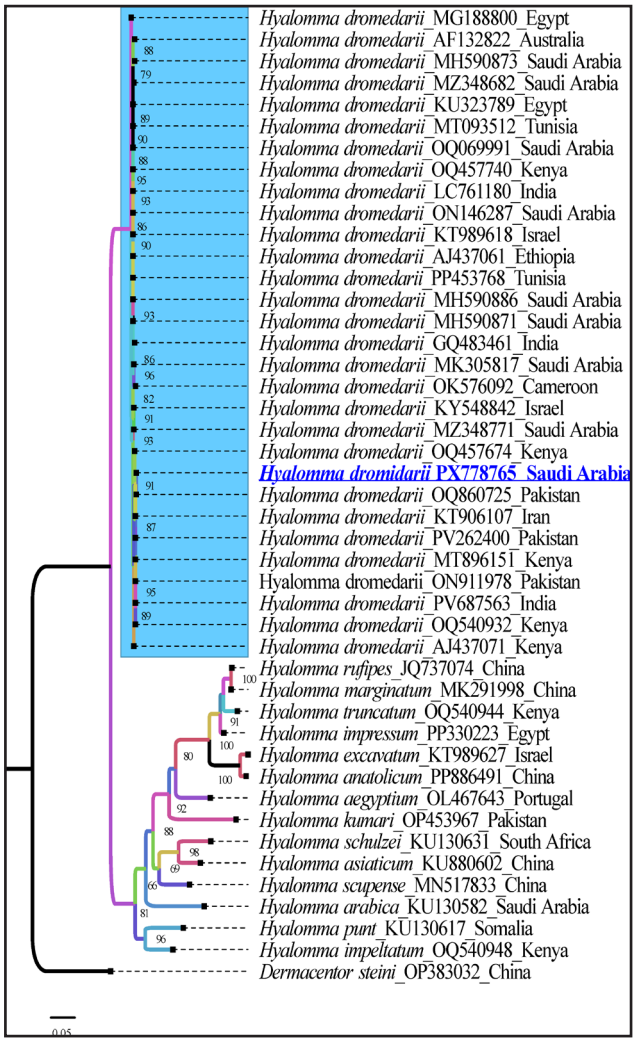

Figure 1. A phylogenetic tree was constructed using maximum likelihood based on the *cox1* sequences of *Hyalomma* spp. The *cox1* sequence of *Dermacentor steini* served as an outgroup, with supporting values (1000 replicons) at each node. The scale bar indicates the number of substitutions that occur per site. The acquired sequence is shown in blue (accession number: PX778765).

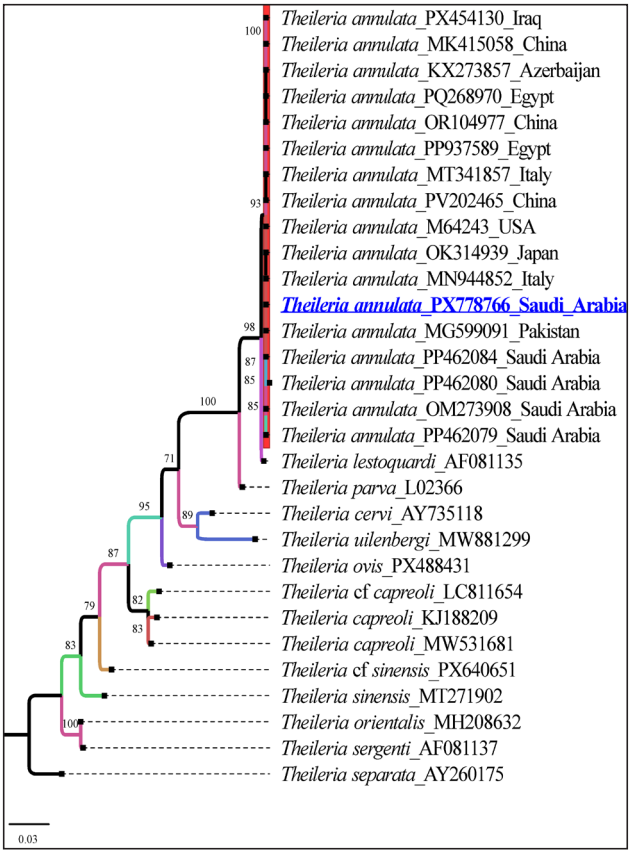

Figure 2. A phylogenetic tree was constructed using maximum likelihood based on the 18S rDNA sequences of *Theileria* spp. The 18S rDNA sequence of *Theileria* separata served as an outgroup, with supporting values (1000 replicons) at each node. The scale bar indicates the number of substitutions that occur per site. The acquired sequence is shown in blue (accession number: PX778766).

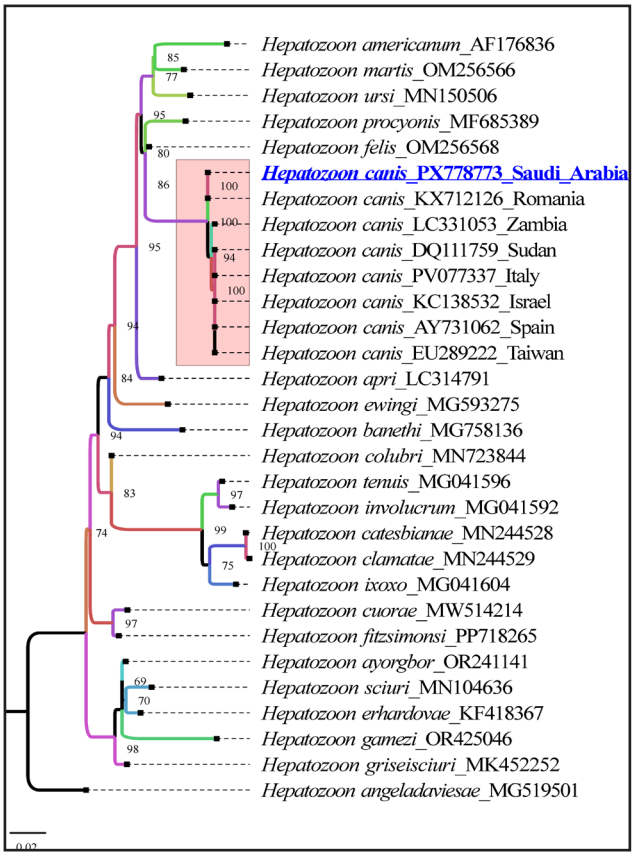

Figure 3. A phylogenetic tree was constructed using maximum likelihood based on the 18S rDNA sequences of *Hepatozoon* spp. The 18S rDNA sequence of *Hepatozoon angeladaviesae* served as an outgroup, with supporting values (1000 replicons) at each node. The scale bar indicates the number of substitutions that occur per site. The acquired sequence is shown in blue (accession number: PX778773).

Table 1. The collected and identified *Hyalomma* ticks, and their associated localities, hosts and pathogens in the study regions in Saudi Arabia.

Location	Observed/ Infested Camels	Collected Ticks (M/F)	Molecular Analysis (M/F)	*Theileria* spp. (M/F)	*Hepatozoon* spp. (M/F)	*Rickettsia* spp. (M/F)	*Coxiella* spp. (M/F)	*Anaplasma* spp. (M/F)	*Babesia* spp. (M/F)
Alkhumrah	40 / 8	18 / 16	4 / 4	0 / 1	1 / 0	0	0	0	0
Brayman	38 / 7	17 / 14	4 / 4	0	0	0	0	0	0
Asfan	42 / 9	21 / 18	4 / 4	1 / 0	1 / 1	0	0	0	0
Dahaban	39 / 8	20 / 17	4 / 4	1 / 1	0 / 1	0	0	0	0
Duba	41 / 8	22 / 19	4 / 4	0	1 / 0	0	0	0	0
Tabuk	40 / 8	18 / 20	4 / 4	1 / 1	0	0	0	0	0
TOTAL	240 / 48	116 / 102 (218)	24 / 24 (48)	3 / 3	3 / 2	0	0	0	0

M: males and F: females.

DISCUSSION

The genus *Hyalomma* includes some of the most important ticks infesting camels, yet accurate molecular identification remains challenging. This study provides an integrated morphological, molecular, and phylogenetic characterization of *H. dromedarii* on camels in the western and northwestern regions of Saudi Arabia, along with molecular screening for associated bacterial and protozoan pathogens. Importantly, this work represents regional comprehensive studies that combines morpho-molecular identification with phylogenetic analysis and pathogen detection in camel-associated ticks from Saudi Arabia. Combining classical morphology with mitochondrial *cox1* sequencing enabled reliable species identification and reduced the risk of misidentification, a known issue in the genus *Hyalomma* due to overlapping morphological features and intraspecific variation [15,23].

To date, 7 of *Hyalomma* species reported from multiple hosts including camels in Saudi Arabia such as *H. excavatum*, *H. dromedarii*, *H. anatolicum*, *H. impeltatum*, *H. marginatum*, *H. rufipes*, and *H. schulzei*. In this study, morphological examinations showed that all collected specimens were *H. dromedarii*, consistent with previous studies indicating this species is the dominant camel tick in Saudi Arabia [2,5,12,17,22,31,40,41]. The confirmation of a single dominant species across multiple geographically separated locations highlights the strong host specificity and ecological adaptation of *H. dromedarii* to camels in arid and semi-arid regions of the Kingdom. The biased sex ratio observed in the present study was similar to that found in previous studies and may be explained by the longer attachment periods and higher host-seeking behavior of male *Hyalomma* ticks [41,47].

The higher tick abundance recorded in Asfan and Duba is likely due to favorable ecological conditions, including an arid climate, open landscape, and traditional camel husbandry practices that support tick survival and transmission. These findings suggest that local environmental conditions and management systems may shape regional tick population dynamics, emphasizing the need for area-specific tick control strategies. Molecular confirmation using the cox1 sequence showed high genetic similarity (99.36-99.85%) between Saudi Arabian *H. dromedarii* sequences and those reported from Africa, the Middle East, South Asia, and Australia. This high degree of mitochondrial conservation across continents supports the hypothesis that historical livestock movement, camel trade routes, and nomadic pastoralism have contributed to gene flow and limited population structuring within *H. dromedarii* [1,3,4,9,15,20,33,38,39,45,47,48]. The phylogenetic grouping of the Saudi Arabian isolates with worldwide *H. dromedarii* lineages supports the taxonomic validity of the species, which lead to confirmed the global connectivity of camel associated tick diversity.

Pathogen screening revealed an overall low prevalence of protozoan infections, with only *T. annulata* and *H. canis* detected. *The detection of T. annulata DNA in H. dromedarii* collected from camels is epidemiologically significant, as it suggests that camel ticks may contribute to the maintenance of this pathogen in multi-host ecosystems, even if camels themselves are not primary reservoirs. *Theileria annulata* is the etiological agent of tropical theileriosis, a major constraint on livestock production in the Middle East and surrounding regions [25,32]. These findings raise the possibility of pathogen spillovers between camels and other livestock species in shared grazing environments.

The detection of *H. canis* in *H. dromedarii* is also noteworthy, as this protozoan traditionally been associated with canids and transmitted mainly by *Rhipicephalus sanguineus*. Its presence in camel-associated *Hyalomma* ticks supports emerging evidence that *H. canis* may circulate through a broader range of tick vectors than previously recognized, either through vector competence or incidental acquisition during blood feeding [13,47]. The co-detection of *Theileria annulata* and *H. canis* in individual ticks further indicates that *H. dromedarii* may harbor multiple pathogens simultaneously, highlighting its potential role as a bridge vector linking camels, livestock, wildlife, and possibly humans under natural conditions.

Notably, no molecular evidence was detected for *Rickettsia* spp., *Anaplasma* spp., *Coxiella* spp., or *Babesia* spp., although some of these have been reported in camel ticks in other regions of Saudi Arabia [13,20]. This absence may reflect genuine spatial or temporal variation in pathogen circulation rather than methodological limitations, underscoring the importance of region-specific surveillance rather than generalized assumptions of pathogen presence. Seasonal influences and host-related factors may also affect the prevalence and detection rates of pathogens. Overall, the findings of this study emphasize the need for continuous, integrated surveillance of camel-associated ticks to better understand their epidemiological role in pathogen maintenance and transmission in Saudi Arabia.

CONCLUSION

This study provides accurate morpho-molecular evidence of the presence of *Hyalomma dromedarii* as a major camel tick in the western and northwestern regions of Saudi Arabia. Mitochondrial *cox1*-based analysis revealed a high level of genetic conservation and phylogenetic relationships among different populations of *H. dromedarii* reported from various countries representing all regions of the globe. Molecular screening detected *Theileria annulata* and *Hepatozoon canis* in camel-associated ticks, indicating the potential epidemiological role of *H. dromedarii* in the maintenance and possible transmission of protozoan pathogens. By integrating morphology, mitochondrial genetics, and pathogen screening across multiple regions, this study establishes a foundational reference for camel-associated *H. dromedarii* populations in Saudi Arabia.

MANUFACTURERS

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***Ethical approval*.** This study was conducted in compliance with the international guidelines for animals' research or healthcare. Oral consent was obtained from the owners of the camel for observation and tick collections.

***Declaration of interest*.** The authors certify that they have no affiliations with, or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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