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MOLECULAR DETECTION OF *HYALOMMA* SPP. ISOLATED FROM SHEEP AND CAMEL IN THE AL-SAMAWAH DESERT OF AL-MUTHANNA PROVINCE, IRAQ

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ABSTRACT

Ticks are a common source for a variety of microorganisms especially those which are pathogenic or highly fatal. *Hyalomma* species are potential vectors for the CCHF virus. This study sought to identify *Hyalomma* spp. isolated form sheep and camel found in the Al-Samawah desert of Al-Muthanna Province. Sixty samples of ticks were used in this study, and each was subjected to PCR and partial gene sequencing using the *18S rRNA* gene. The results from the analysis showed that 45 of 60 samples (75%) were identified as *Hyalomma* spp. While 17 PCR-purified products were chosen for sequencing, and the aligned data were used to build the phylogenetic tree. Uphill climbing the roof of phylogenetic analysis, the result reigned these isolates in this study as *Hyalomma asiaticum* related to several isolates in GenBank. This research has shown that these ticks of sheep and camel in the Al-Samawah desert of Al-Muthanna Province are commonly a marker of many viruses, including the CCHF virus. Further in-depth work ought to be completed as a priority for disease control purposes.

***Keywords**:* Protozoa, tick-borne disease, viruses, 18S rRNA gene, PCR.

INTRODUCTION

Ticks are major arthropods of animals that are important as vectors of pathogens. They can transmit viral, bacterial, and protozoal diseases in ruminants, which negatively impact their health and significantly affect their productive and reproductive performance (Sadeddine *et al.,* 2020). Ticks require two or three blood meals to develop through their three host cycles, with the number of hosts varying depending on their developmental stage. Although several tick species are noteworthy, the genus *Hyalomma* attracts considerable attention from researchers worldwide due to its status as one of the most harmful tick genera to domestic and wild animals. The virus-infected ticks transmit the virus during a blood meal when they bite the animal (Ghafar *et al.,* 2020; Garcia *et al.,* 2022).

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Ticks (Acarina: Ixodida) are considered second only to mosquitoes as vectors of human and animal diseases. They affect animals either mechanically or by causing significant economic losses directly and through transmission of tick-borne diseases (TBDs). In desert climates, particularly in Iraq, various issues are associated with these ectoparasites (Aziz, 2022; Abed *et al.,* 2023). In hot climates such as Iraq, ticks widely impact livestock, especially sheep and goat flocks. Tick distribution in Iraq is linked to changes in environmental characteristics. Studies have shown that the most frequent genera are *Hyalomma* and *Rhipicephalus*, particularly in rural regions with abundant dense and dry grass. Sheep and goats, the second and third preferred hosts for ticks, are particularly affected by those parasites. The tick vector species that most commonly live on ruminants (sheep, goats and cattle) in Iraq are several species of the genus *Hyalomma* (Ismael and Omer, 2020; Makawi and Hadi, 2023).

Female ticks have two intermediate stages separated by a small gap from other ixodid sp. The ixodid tick stages include the larval (L), nymphal (N) and adult (A) stages. Notably, the nymphs are unusual among ixodid ticks on the same host during the same life stage (Schulz *et al.,* 2020; Chitimia-Dobler *et al.,* 2023). Adult females feed on vertebrates, for a few days to 1.5 to 2 weeks, displaying prominent sexual dimorphism of body size, on average measuring 10–33 mm long. While on host-feeding, larvae and/or nymphs detach to complete the final mount and next stage before hiding for 1 to 2 months in multiple off-host microhabitats (Leal *et al.,* 2020; Aiman *et al.,* 2022).

In areas with numerous hosts and concerning pathogens, it is advisable to treat both the tick hosts (e.g., livestock) and the tick vectors. Tick control typically involves altering the habitat of the pathogen. Where tick vectors thrive in both wild animals and livestock, control measures should include vegetation management, habitat alteration, and vaccination for wildlife (Eisen and Stafford, 2021; Rahman *et al.,* 2022; Eisen, 2021; De León *et al.,* 2021).

Ticks play crucial roles in pathogen transmission and cause extensive damage to animals. They use a variety of hosts, including farm animals, wildlife, and humans. The tick-host-pathogen relationships are numerous and complex. Large mobile ticks such as *Hyalomma* spp. can travel long distances and readily exploit various hosts. These ticks support a high diversity of pathogenic agents due to their consistent feeding behavior on different hosts (Bonnet *et al.,* 2022; Uiterwijk *et al.,* 2021; Sesmero-García *et al.,* 2023; Sharifah *et al.,* 2020).

Hyalomma spp. are vectors of several important livestock diseases in Iraq, including bovine, ovine, and camel theileriosis. Bovine, ovine, and equine anaplasmosis also occur in Iraq. Camels suffer significantly from dermatophilosis, and many protective measures used for other tick-borne diseases are ineffective against dermatophilosis. Tick treatment after infection with the bacterium responsible for the disease has minimal or no effect on the disease state (Olewi *et al.,* 2022; Omer *et al.,* 2023; Perveen *et al.,* 2021). *Hyalomma* spp. also damages the wool, hides, and skins of animals, increasing production costs and exploitation of the animals. Solvent residues in hides can cause skin irritation in humans. Acaricide-impregnated collars can reduce damage to hides and skins, but problems such as sublethal doses of acaricides or resistance to acaricides need to be addressed (Magzoub *et al.,* 2021).

Diseases transmitted by *Hyalomma* ticks have a serious economic impact on animal production. The economic effects of *Hyalomma* tick transmission include skin or mammary lesions and abscesses. *Hyalomma* spp. feed on blood and skin cells from many species of wild and domestic mammals, as well as humans, and serve as vectors and reservoirs for viral, bacterial, and rickettsial diseases. In Iraq, *Hyalomma* spp. are vectors of tick-borne piroplasmosis, a disease that remains absent in only a small number of locations outside the Kurdish region (Kumar *et al.,* 2020; Singh *et al.,* 2022; Dus2022).

Ticks, particularly *Hyalomma* species, are critical vectors for numerous dangerous microorganisms, including the Crimean-Congo hemorrhagic fever (CCHF) virus. The objectives of the present study were to identify and isolate *Hyalomma* spp. from sheep and camels in the Al-Samawah desert region of Al-Muthanna Province.

MATERIALS AND METHODS

Samples and DNA extraction

Animals and ticks

The current work was conducted to identify *Hyalomma* spp. collected from sheep and camels in the Al-Samawah desert of Al-Muthanna Province. The study involved the collection of 60 tick samples from different body parts, such as the front leg, neck, and ears of 60 animals exhibiting clinical abnormalities such as abdominal pain, severe fever, and depression. The study was conducted from November 2023 to February 2024. The tick samples were transported to the laboratory under sterile conditions.

PCR

Genomic DNA extraction

The DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Germany) following the kit instructions. In brief, the tick samples were placed in a lysis buffer and proteinase K. The obtained DNA was read for quality and quantity using a NanoDrop.

PCR Amplification

A gradient PCR technique was performed on all isolates following the kit instructions. The reaction was carried out in a total volume of 25 µl, which included 1.5 µl of each primer (Lv *et al.,* 2014) for the mitochondrial 18S rRNA gene: forward primer (F) CATTAAATCAGTTATGGTTCC and reverse primer (R) CGCCGCAATACGAATGC, targeting a 780 bp product. The reaction mix contained 5 µg of DNA and 12.5 µl of master mix. The thermocycler was programmed with the following settings: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. After PCR, a 1.5% agarose gel electrophoresis was performed, and the bands were visualized using a UV transilluminator.

Partial sequencing of 18S rRNA gene

Forty microliters of each purified PCR product by Qiagen QIAquick Gel Extraction Kit (Germany) were sent to Macrogen Inc. (Korea) for sequencing. The sequencing data were analyzed using the NCBI BLASTn engine to identify similarities with global isolates. The results were presented in a phylogenetic tree constructed using MEGA X software version 11.0.11.

RESULTS

The results showed that 45/60 (75%) of the samples belonged to *Hyalomma* spp. *18S rRNA* gene was amplified using the PCR method, resulting in the appearance of a 780 bp band on a 1.5% agarose gel under UV light (Fig.1). Sequencing of the purified PCR product confirmed that the detected species was *Hyalomma asiaticum.* The sequences were deposited under accession numbers PQ359502, PQ359503, PQ359504, and PQ359505 in GenBank (Fig. 2).

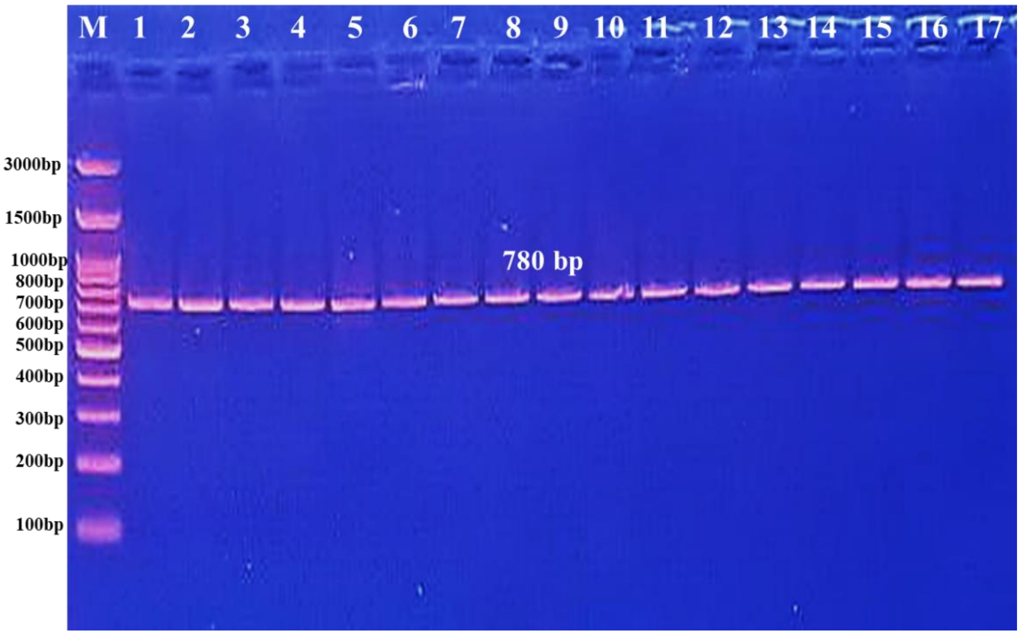

Figure 1: Image of agarose gel of *1٨S rRNA* gene in *Hyalomma* spp. M represents DNA marker, and 1- 17 lanes are positive PCR products. Lanes (1-9) refer to camel isolates while lanes (10-17) refer to sheep isolates.

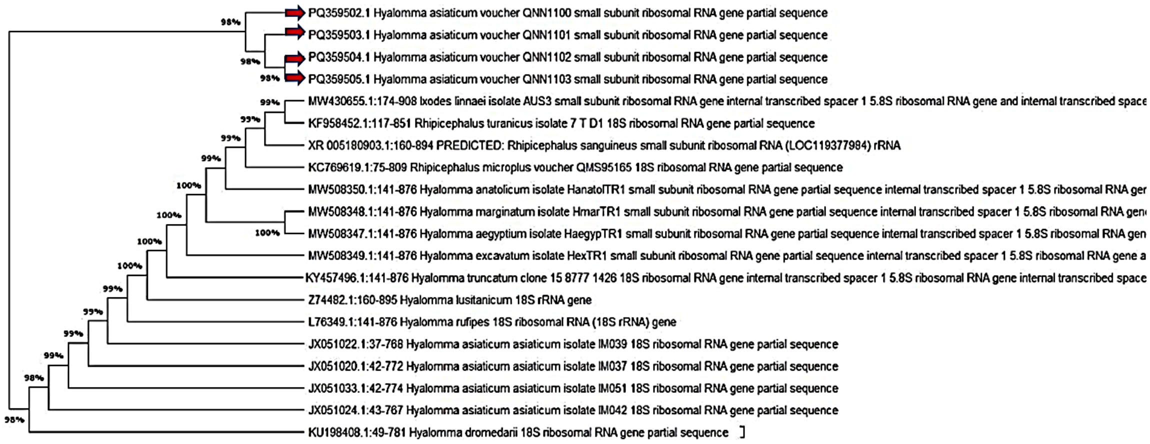

Figure 2: Phylogenetic tree of the *1٨S rRNA* gene in *Hyalomma asiaticum*

The tree was built using NCBI-related websites and MEGA X software version 11.0.11. Blue arrows represent the current study isolates.

DISCUSSION

Diagnosis of ticks of medical and veterinary significance is crucial for understanding disease pathogenesis, prevention, and treatment. It also plays a key role in describing both new and established tick species and in subsequent molecular studies of tick samples from Iraqi populations and other host animals across Iraq. Accurate identification of tick species and their associated pathogens is vital for implementing effective preventive measures. Our data contribute valuable information on tick population dynamics and pathogen status (Ali *et al.*, 2021; Aziz and AL-Barwary, 2020).

Recent research has focused on the distribution of individual *Hyalomma* species. A study in Russia assessed 50 years of climate-driven geographic distribution changes in *Hyalomma*. Margos et al. (2021) mapped areas in central and eastern Germany and southwestern Lithuania, noting differences in biotopes, vegetation, and water bodies influenced by climate, which may have facilitated the northern expansion of *Hyalomma* ticks up to 240 km north of their theoretically optimal latitudinal niche (Song and Kim, 2020; Hao *et al.,* 2021; Li *et al.,* 2023). Guglielmone *et al.* (2010) suggested that the expansion of desert areas in Kazakhstan, the dry steppes of Kalmykia, and the central districts of Kobystan in Georgia has impacted local fauna. Initial estimates of 20–30 live ticks per animal led to the discovery of about 1,500 *Hyalomma* ticks, including nymphs, across various wild and domestic animals. Records showed 84% were from Atelidors cats, 9% from foxes, 4.5% from wolves, and 2.5% from omnivores. Bayisch and Hiechinger (2021) suggested that the expanding Asian raccoon population poses a potential threat to ticks and the pathogens they carry. In 2021, the European Food Safety Authority (EFSA) reviewed surveillance methods for *Hyalomma emarginatum* and *Hyalomma marginatum*, compiling 32 pages of literature records. However, most records pertained to the Middle East, Spanish North Africa, or the Mediterranean coastline, with limited information on European introductions from imported Arabian thoroughbred horses (Ma *et al.,* 2021). While interest in the geographical and species distributions of *Hyalomma* ticks is evident, there appears to be a lack of studies providing verifiable records of previous *Hyalomma* species distributions and prevalence rates post-1980 (*Hyalomma persicum* = 215, Hy. marginatum = 154; *Hyalomma persicum* = 1343, *Hy. marginatum* = 2184) (Liu and Shi, 2020).

For example, Rees and colleagues sequenced the mitochondrial cytochrome b gene from the *Hyalomma* ticks sampled from diverse geographical locations and found that there is a high level of genetic diversity both within and among species. They suggested that at least two species, the north-tropical species H. marginatum and the tropical species, *H. rufipes*, have two distinct genetic lineages corresponding to geographical distribution. More recently, Zheng and colleagues similarly applied multilocus sequence typing to investigate the population structure of *H. asiaticum* in China. They also reported significant genetic diversity among sampled populations from different provinces (Rees *et al.,* 2008).

These comparative analyses, together with some additional studies, helped to clarify evolutionary relationships among species of *Hyalomma*. Using molecular markers and mitochondrial DNA, for instance, Burger et al (2014) produced a large-scale phylogenetic analysis demonstrating that *Hyalomma* evolved in a monophyletic well-supported group of the *Rhipicephalinae* subfamily. Furthermore, they have demonstrated that several species of *Hyalomma* (e.g., *H. truncatum* and *H. lusitanicum*) are more related to the *Rhipicephalus* and *Dermacentor* genera.

At the same time, data from these studies may provide a clearer quantification of previous observations. For example, Rees *et al.* (2008) reported an interspecific sequence divergence of 7.2% in the mitochondrial cytochrome c oxidase I gene among Hyalomma species. Additionally, Zheng *et al.* (2015) identified up to 15 distinct sequence types in *H. asiaticum* populations across China.

CONCLUSION

The present study reveals a high prevalence of Hyalomma ticks in both desert sheep and camels in the Al-Samawah desert, Al-Muthanna Province. This may indicate the presence of certain pathogenic microorganisms, such as the Crimean-Congo hemorrhagic fever (CCHF) virus. Screening for these pathogens would provide valuable insights for the effective control of disease outbreaks.

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الكشف الجزيئي عن ***Hyalomma spp*** المعزولة من الأغنام والإبل في صحراء السماوة بمحافظة المثنى

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القراد، وخاصة *Hyalomma*، هي ناقلات مهمة لنقل الكائنات الحية الدقيقة القاتلة، مثل فيروس حمى القرم الكونغو النزفية (CCHF). أجريت الدراسة الحالية لتحديد طفيليات *Hyalomma* spp. التي تم عزل هذه السلالة من الأغنام والإبل في صحراء السماوة بمحافظة المثنى. أجريت الدراسة بجمع ٦٠ عينة من القراد. خضعت العينات لتقنية تفاعل البوليميراز المتسلسل والتسلسل الجزئي للجينات لهذه القراد باستخدام جين ١٨S rRNA. أظهرت النتائج أن ٦٠/٤٥ (٧٥%) من العينات تنتمي إلى *Hyalomma* spp. تم تسلسل العينات (١٧ منتجًا منقحًا بتقنية تفاعل البوليميراز المتسلسل) ووضع البيانات المنسقة في شجرة النشوء والتطور. كشف التحليل النشوئي لهذه البيانات أن عزلات الدراسة الحالية لهذه القراد تنتمي إلى *Hyalomma* spp وتقف بالقرب من عزلات مختلفة مودعة في بنك الجينات. وتكشف الدراسة الحالية عن وجود هذا القراد بكثافة في الأغنام والإبل في صحراء السماوة بمحافظة المثنى، وقد يكون ذلك مؤشرا مهما لوجود بعض الكائنات الحية الدقيقة المسببة للأمراض، مثل فيروس حمى القرم الكونغو النزفية (CCHF)، والذي يحتاج إلى تحقيقات أكثر عمقا لغرض السيطرة على المرض.